Cloning and regulation of the rat activin β_E subunit

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ABSTRACT

Using a combination of polymerase chain reaction (PCR) procedures, we have cloned and sequenced the rat activin β_E subunit cDNA. The putative protein corresponding to the prepro-activin β_E subunit was predicted to comprise 350 amino acids which, when cleaved between amino acid residues 236 and 237, would yield a mature polypeptide of approximately Mr 12 500 with a predicted pI of 5.1. Two cDNA transcripts for activin β_E were identified; these differed by 738 bp in the 3' untranslated region. Activin β_E mRNA transcripts were expressed only in rat liver and lung tissue as assessed by Northern blotting and PCR analysis. Relatively higher levels of both transcripts were found in the liver, whereas the lung contained lower levels that were detectable by PCR only. In situ hybridisation data showed that, within the liver, activin β_E mRNA was localised to hepatocytes. In vivo treatment with lipopolysaccharide as a means of activating the immune system and the hepatic acute-phase response resulted in stimulated activin β_E mRNA levels, compared with untreated, control rats. This increased expression was accompanied by a preferential increase in the amount of the long activin β_E transcript over the shorter transcript. These findings suggested that the two activin β_E mRNA transcripts may be products of alternative splicing events or use alternative polyadenylation sites which are differentially regulated during inflammation. These data provide evidence of a role for activin β_E in liver function and inflammation in the rat. Journal of Molecular Endocrinology (2000) 24, 409–418

INTRODUCTION

Activins and inhibins are homo- and heterodimeric proteins with regulatory functions in many areas of biology, including spermatogenesis, ovarian function, erythropoiesis, tumorigenesis, bone growth, cardiac morphogenesis and immune function (Vale et al. 1994, de Paolo 1997, Yu & Dolter 1997). Activins are composed of two β subunits (β_β), covalently linked by a single disulphide bond, whereas the inhibins are composed of the same β subunit disulphide bonded to an α subunit (αβ). To date, a single α subunit and two β subunit proteins (β_A and β_B) have been purified. However, within highly differentiated cells, the presence of three additional β subunits (β_C, β_D and β_E) have been postulated on the basis of mRNA data (Hotten et al. 1995, Oda et al. 1995, Fang et al. 1996). Biological functions have not been delineated for these three novel β subunits. Microinjection of synthetic activin β_D mRNA transcribed from cDNA into Xenopus embryo blastomeres, however, led to secondary body axis formation, whilst microinjection into pole caps led to mesoderm induction. This finding suggested that the activin β_D subunit might be involved in embryogenic development and more specifically in mesoderm induction (Oda et al. 1995). Analysis of the predicted amino acid sequence homologies between the known activin β subunits showed a 50% overall homology, and suggests that the β_C, β_D and β_E subunits form a separate subset within the activin β subunit ‘family’ of the transforming growth factor-β superfamily (Fang et al. 1996).
Amongst other functions, activin A (βAβA) appears to act as an autocrine regulator of hepatic parenchymal cell function. Activin A potently inhibits hepatocyte proliferation and acute-phase protein secretion and induces cell apoptosis (Schwall et al. 1993, Yasuda et al. 1993, Hully et al. 1994, Russell et al. 1999). These effects can be antagonised by co-administration of the activin-binding protein, follistatin (Schwall et al. 1993, Russell et al. 1999). Activin A also appears to be a modifier of the immune response, acting at the signal transduction level to antagonise many of the effects induced by interleukin-6 (IL-6) (Brosh et al. 1995, Yu et al. 1998, Russell et al. 1999). In addition, the βH and βC subunits of activin/inhibin have also been found in liver tissue, but their functions remain unknown (Hotten et al. 1995, de Bleser et al. 1997, Zhang et al. 1997a). The cDNA sequence of the activin βE subunit has been reported for the mouse only, where its expression, as determined by Northern analysis, was restricted to the liver (Fang et al. 1997). The function and regulation of activin βE within the liver, however, remains completely unknown. In this study, we describe the cloning strategy used for the determination of the cDNA sequence of the rat homologue of activin βE and provide preliminary data on the role of activin βE in hepatic function.

MATERIALS AND METHODS

Chemicals and enzymes

Unless stated otherwise, chemicals were obtained from Sigma Chemical Company (St Louis, MO, USA). Enzymes and buffers used for molecular biology were purchased from Promega Corporation (Madison, WI, USA). All primers were obtained from Geneworks (Adelaide, Australia) with the exception of those used for 3' rapid amplification of cDNA ends (RACE) experiments which were purchased from Integrated DNA Technologies Inc. (Coralville, IA, USA).

Experimental animals and tissue processing

Outbred adult male Sprague-Dawley rats (80–100 days) were obtained from the Central Animal House, Monash University. Experimental procedures were approved by the Monash University Standing Committee on Ethics in Animal Experimentation and conformed to the NH&MRC/CSIRO/AAC Code of Practice for the Care and Use of Animals for Experimental Purposes. For in situ hybridisation studies, rats were anaesthetised using ether and then fixed by perfusion with Bouin’s fluid via the descending aorta (O’Bryan et al. 2000). Excised tissues were immersion-fixed in Bouin’s fixative for 3 h prior to being processed into paraffin using standard embedding procedures.

For the determination of the normal tissue distribution of activin βE mRNA, rats were anaesthetised with ether, tissues were removed and immediately snap frozen and stored at −70 °C until required. In the experiment on the effect of acute inflammation on activin βE mRNA levels, rats were treated and tissues collected as described previously (O’Bryan et al. 2000). Briefly, rats were given a single intraperitoneal injection of 0·1 mg/kg E. coli lipopolysaccharide (LPS, serotype 0127:B8) in saline, or endotoxin-free saline alone. LPS has been extensively used as an activator of the immune response (Cybulsky et al. 1988) and the dose was chosen as representative of a minor bacterial infection (O’Bryan et al. 2000). The body temperature and level of physical activity of the rats receiving this dose of LPS returned to control values within 12–18 h. Animals (three per group) were killed at 0, 3, 6, 12, 18, 24 and 72 h after the LPS injection and the livers and lungs were removed and stored at −70 °C for subsequent RNA extraction. The activin βE mRNA content was also assessed in the human prostate cell lines DU145 and LNCaP (AATCC, Rockville, MD, USA). Cells were grown as previously described (McPherson et al. 1997).

Cloning of rat activin βE cDNA

The rat βE cDNA was cloned using a combination of reverse transcriptase–polymerase chain reaction (RT-PCR) protocols. Primers used in these procedures are given in Table 1. Total RNA was purified using the method of Chomczynski & Sacchi (1987) from normal adult rat liver and reverse-transcribed to cDNA using avian myeloblastosis virus (AMV) RT as outlined by the manufacturer. The equivalent of 0·25 μg hepatic RNA was used for the PCR amplification of a 974 bp activin βE cDNA product using the activin βE specific primers, βE1 and βE2, designed from the published mouse sequence (Fang et al. 1996) and a DNA polymerase with proof-reading capability (Stratagene, La Jolla, CA, USA) in a touch-down PCR protocol. Cycling conditions were 30 cycles of denaturation at 94 °C for 30 s, followed by annealing at 60–45 °C (at 0·5 °C increments) for 70 s, followed by extension at 72 °C for 3 min (Katsahambas & Hearn 1997). Following the touch-down protocol, the signal was further amplified by ten rounds of cycling: denaturation at 94 °C for 30 s, annealing at 45 °C for 70 s and extension at 72 °C for 3 min followed by a
final extension of 5 min at 72 °C. The 974 bp product was gel purified using the Wizard PCR Prep DNA Purification System and subcloned into pCR-Script Amp SK(+) as outlined by the manufacturer (Stratagene), and designated as the activin βE1 clone.

The sequence of the 5′-region of the rat activin βE mRNA/cDNA was obtained by RT-PCR using a forward primer taken directly from the mouse βE sequence (Fang et al. 1996) and a reverse primer based on the sequence obtained from the activin βE1 clone. A rat activin βE fragment was obtained from hepatic cDNA using primers βE5 and βE6 in a touch-down PCR protocol. Cycling conditions were as outlined above with the exception that a 2-min extension time was employed. The resultant 369 bp product was gel purified using a Wizard column and subcloned into pCR-Script Amp SK(+) as outlined by the manufacturer (Stratagene), and designated as the activin βE5,6 clone.

The sequence of the 3′-region of rat activin βE mRNA/cDNA was obtained using the RACE method essentially as described elsewhere (Troutt et al. 1992). Rat hepatic total RNA was reverse transcribed using the primer RTP and AMV reverse transcriptase (Table 1). The equivalent of 0·15 µg reverse transcribed RNA was then amplified in a touch-down PCR protocol. An oligonucleotide designed to the 3′-region of known internal rat activin βE sequence (βE11) was used as the forward primer. The reverse primer was the anchor primer 3 (AP3). The cycling conditions were as outlined above with the exception that a 6-min extension time was used. The resultant PCR products were purified using Wizard purification columns and subcloned into pCR-Script Amp SK(+).

The inserts of all plasmids were sequenced at the Monash University Microbiology Department DNA Sequencing Facility (Clayton, Australia) from dye-terminator PCR reactions using an ABI Model 330 gene sequencer (Perkin Elmer, Warrington, Cheshire, UK). In order to ensure the veracity of sequences, both DNA strands and inserts from multiple colonies of each plasmid were sequenced. The Blast-N and Blast-P programs (Altschul et al. 1990) were used to compare the nucleotide and deduced amino acid sequences obtained for clones with the information stored in the genomic and protein sequence databases. Signal peptide prediction was carried out using the SignalP program (Nielsen et al. 1997) and the predicted pI and Mr were calculated using the Compute pI/MW program (Bjellqvist et al. 1993, 1994).

**Rat activin βE expression studies**

**RT-PCR analysis of the tissue distribution of activin βE in the rat**

The tissue expression pattern of βE mRNA in the rat was determined using an RT-PCR approach. Tissue and total RNA were purified as outlined above from rat prostate, testis, liver, epididymis, kidney, uterus, small intestine, lung, ovary, brain, spleen and seminal vesicles and from the human prostate cell lines DU145 and LNCaP. The mRNA was reverse transcribed and the activin βE cDNA was specifically amplified using the primers βE1 and βE2 as described above. Products were visualised on 1% agarose gels and bands of approximately the predicted size of 974 bp were subcloned into pCR-Script Amp SK(+) and sequenced. The presence of cDNA in each RT reaction was confirmed by amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA using the primers GAP-F and GAP-B using the conditions that are outlined above. The

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**Table 1. PCR primer sequences used in the cloning and analysis of the rat βE activin cDNA**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Length</th>
<th>Nucleotide position</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>βE1</td>
<td>21</td>
<td>323–343</td>
<td>GGAGCTAGCCAAAGCAGAAAT</td>
</tr>
<tr>
<td>βE2</td>
<td>21</td>
<td>1276–1295</td>
<td>GGAACCCCCAACCTGAACCTT</td>
</tr>
<tr>
<td>βE3</td>
<td>21</td>
<td>669–689</td>
<td>GAGCACCACAACCACCTCCTTCT</td>
</tr>
<tr>
<td>βE4</td>
<td>21</td>
<td>918–938</td>
<td>CCTACAACATAAGGGGTCTCT</td>
</tr>
<tr>
<td>βE5</td>
<td>20</td>
<td>349–368</td>
<td>GCGATCTACTCTCAGTCTTCT</td>
</tr>
<tr>
<td>βE6</td>
<td>21</td>
<td>349–368</td>
<td>GCTGTGTTAGGTGCAGTCCTCCT</td>
</tr>
<tr>
<td>βE11</td>
<td>20</td>
<td>1088–1107</td>
<td>TACGCTCCTCAAGGCCACAA</td>
</tr>
<tr>
<td>AP3</td>
<td>25</td>
<td>RACE anchor</td>
<td>CTCCTGAAAGTTCCAGAATCAGCTTAAGT(18)VN</td>
</tr>
<tr>
<td>GAP-F</td>
<td>21</td>
<td>GAPDH sequence</td>
<td>ATCACTGCCCACCAGAGACT</td>
</tr>
<tr>
<td>GAP-B</td>
<td>21</td>
<td>GAPDH sequence</td>
<td>CATGCCAGTGAGCTTCGCCGT</td>
</tr>
</tbody>
</table>

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tissue distribution was confirmed in three separate experiments using RNA from two different preparations.

As described in the results, the sequencing of activin β_E rat clones indicated that two different length activin β_E transcripts were present, the longer of which completely contained the shorter transcript plus an additional 738 bp at the 3′ end. The existence and identity of the longer transcript was further investigated in the rat liver and lung using an activin β_E long transcript specific PCR. Any long activin β_E transcript was specifically amplified from liver or lung with the PCR primers β_E12 and β_E17 using the touch-down protocol outlined above with a 4-min extension time. Specific amplification gave a product of 1320 bp that was subcloned into pCR-Script Amp SK(+) (clone β_E12,17) and the sequence was confirmed to be activin β_E by automated sequencing. The presence of the short form of activin β_E in the lung was determined using the 3′ RACE method as described above. Results were obtained from three separate experiments.

**Digoxigenin (DIG)-labelled riboprobe preparation and in situ hybridisation**

The expression of activin β_E mRNA within the rat livers (n=2) was determined by in situ hybridisation with two different DIG-labelled antisense cRNA probes using a previously described method (O’Bryan et al. 1998). The plasmids used for the generation of riboprobes were β_E3,4 and β_E5,6. The PCR β_E3,4 product corresponded to a 270 bp fragment of the protein coding region, and the β_E5,6 product corresponded to the 5′-untranslated region of the β_E cDNA. Plasmid inserts were prepared using the primers listed in Table 1. Labelled sense and antisense cRNAs were synthesised by incubating either the NotI (antisense) or HindIII (sense) linearised template (1 µg) with DIG-labelled UTP (3·5 µmol/l) in the presence of T3 or T7 RNA polymerase, appropriate for the respective restriction enzyme employed, for 2 h at 37 °C. Hybridisation was performed overnight at 50 °C. This step was followed by sequential 15-min washes in SSC solutions to a maximum stringency of 0·1 × SSC at 50 °C.

**Northern blotting**

Approximately 20 µg liver and lung RNA from saline-treated control and LPS-treated rats were fractionated by electrophoresis on a 1% agarose formaldehyde gel and transferred to Nytran+ membrane (Schleicher & Schuell, Keene, NH, USA) in 10 × SSC. RNA was fixed onto membranes by baking at 80 °C for 2 h. α32P-Labelled cDNA probes, corresponding to either the β_E1,2 or β_E5,6 clone inserts, were prepared using the Strip-EZ DNA probe synthesis kit (Ambion Inc., Austin, TX, USA). Unincorporated nucleotides were removed using Probe Quant micro-columns (Pharmacia Biotech, Uppsala, Sweden). Membranes were prehybridised in Ultrahyb (Ambion Inc.) at 42 °C for 4 h prior to hybridisation overnight at 42 °C in fresh prehybridisation buffer containing 1 × 106 c.p.m./ml α32P-labelled cDNA. Membranes were washed to a maximum stringency of 0·1 × SSC, 0·1% SDS at 65 °C prior to autoradiography. The amount of RNA loaded in each lane was equalised by comparison with GAPDH expression. GAPDH mRNA expression was determined by Northern blotting as outlined above using a 650 bp cDNA fragment of the rat GAPDH sequence for each treatment group. Northern analysis for the activin β_E mRNA was repeated for three rats per time-point. Signals were detected using a phosphoimager screen and read in a Fuji BAS1000 MacBAS Bio-Imaging Analyzer (Berthold, Sydney, Australia). Phosphoimager results were generated using MacBAS version 2·4 software supplied by the manufacturer. After background subtraction, the luminescence intensity of each rat activin β_E mRNA band was corrected for sample loading differences against the GAPDH mRNA band for the corresponding sample. The corrected data were normalised against the samples collected from untreated rats, which were assigned an arbitrary value of 1·0. Values for each β_E mRNA transcript, as well as the ratio between the band intensity of long and short mRNA transcripts for each animal, were analysed by ANOVA, followed by Dunnett’s test for comparison of multiple means using Sigmastat software version 1·0 (Jandel Corp., San Rafael, CA, USA).

**RESULTS**

**Cloning of rat activin β_E cDNA and sequence analysis**

Rat activin β_E cDNA was cloned using a combination of PCR techniques (Fig. 1). The cDNA sequence of the rat activin β_E encodes a putative prepro-protein of 350 amino acids which, when proteolytically cleaved between amino acids 236 and 237, would yield a mature polypeptide of approximately M₀ 12 500 with a pI of ~5·1. Comparison with the published mouse activin β_E sequence (Fang et al. 1997) showed 93% homology at the nucleotide level, 93% at the level of the predicted prepro-protein and 96% homology at the level of the predicted mature polypeptide. The rat β_E gene was found to encode for two cDNAs with lengths of
The cDNA sequence of the rat activin $\beta_E$ subunit. The numbers on the left-hand side indicate the relative position of nucleotides. The numbers on the right-hand side indicate the relative position of predicted amino acids. Two $\beta_E$ transcripts were isolated which were found to vary in the 3'- untranslated region. The shorter transcript is an orthologue of that reported for the mouse and was completely contained within the longer transcript. The 3'- most nucleotide of the shorter transcript is indicated by $\perp$. The highlighted areas indicate potential polyadenylation consensus sites. ** Indicates a predicted proteolytic cleavage location which would, on the basis of data from other activin subunits, give rise to the mature activin $\beta_E$ subunit. The boxed amino acids indicate a potential N-linked glycosylation site.

Figure 1. The cDNA sequence of the rat activin $\beta_E$ subunit. The numbers on the left-hand side indicate the relative position of nucleotides. The numbers on the right-hand side indicate the relative position of predicted amino acids. Two $\beta_E$ transcripts were isolated which were found to vary in the 3'-untranslated region. The shorter transcript is an orthologue of that reported for the mouse and was completely contained within the longer transcript. The 3'-most nucleotide of the shorter transcript is indicated by $\perp$. The highlighted areas indicate potential polyadenylation consensus sites. ** Indicates a predicted proteolytic cleavage location which would, on the basis of data from other activin subunits, give rise to the mature activin $\beta_E$ subunit. The boxed amino acids indicate a potential N-linked glycosylation site.
protein coding region was made by W Rossmanith, B Peter & R Schulle-Hermann (accession number AF40032). The identity between the two sequences was 99·6% at the nucleotide level and 99·4% at the protein level.) The longer transcript contained the smaller transcript plus an additional 738 bp at the 3'-end. Four putative poly-adenylation consensus sequences were detected in rat activin subunit sequence, two of which were in the appropriate position to generate the short and long transcript, i.e. approximately 10–30 nucleotide 5' to the start of the poly-A tail (Sachs & Wahle 1993) (Fig. 1). Similar to other activin subunits, the amino acid sequence of the putative rat activin prepro-protein contained a predicted signal peptide of 24 amino acids, one potential N-linked glycosylation site, and 13 cysteine residues. Homology alignment of the cysteine residues of the C-terminal region of the rat activin prepro-protein with the amino acid sequences of other activin subunits is consistent with the presence of a canonical cystine knot for the mature rat activin polypeptide and a single cysteine available for disulphide bond formation with other subunits (McDonald & Hendrickson 1993). A proteolytic cleavage site was predicted between amino acids 236 and 237. By analogy with the known activin subunit sequences, cleavage at this point would give rise to the mature rat activin subunit (Fig. 1).

Rat activin βE expression studies

The activin βE transcript content was assessed by RT-PCR in 12 rat tissues and two human prostate cell lines. The only tissues that were found to contain activin βE mRNA using this protocol were rat liver and lung (Fig. 2). cDNA sequence data from 3' RACE indicated that two different activin βE transcripts were present in rat liver and lung. This result was confirmed using the long transcript specific PCR primer and sequencing (PCR product βE12,17) and Northern blot analysis (Figs 3 and 5). In situ hybridisation on liver sections from normal adult, male rats indicated that the activin βE transcript was found within hepatocytes and tended to be more concentrated (as indicated by staining intensity) in hepatocytes that were in closer proximity to the central vein of the hepatic lobules (Fig. 4). Parenchymal cells further from the central vein contained less or no activin βE mRNA. Under these in situ hybridisation conditions, Kupffer cells, hepatic stellate cells and endothelial cells did not contain detectable activin βE mRNA. In situ hybridisation using a sense cRNA did not result in any staining of rat liver sections, thus indicating the specificity of the antisense cRNA hybridisation. With rat lung, activin βE mRNA was not detected by in situ hybridisation or Northern blot analysis, presumably due to low mRNA abundance.

Within the liver, the longer activin βE mRNA transcript appeared to be present at slightly higher abundance than the shorter transcript (Fig. 5, time 0). The injection of 0·1 mg/kg LPS into adult male rats resulted in a significant increase in hepatic activin βE mRNA expression (Fig. 5A) following normalisation of loading using GAPDH mRNA expression levels (Fig. 5B). Levels of the long

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**FIGURE 2.** An RT-PCR analysis of the activin βE expression in adult rat tissues and in two human prostate cell lines. Specific βE cDNA was amplified using the βE1 and βE2 primers and yielded products of 974 bp (upper panel). The identities of bands were confirmed by sequencing. The presence of cDNA in each RT mix was confirmed by specific amplification of GAPDH (lower panel). Lanes contain RT-PCR products from: (1) prostate, (2) testis, (3) liver, (4) epididymis, (5) DU145, (6) LNCaP, (7) kidney, (8) uterus, (9) small intestine, (10) lung, (11) ovary, (12) brain, (13) spleen and (14) seminal vesicle.
DISCUSSION

The rat activin $\beta_E$ transcript has been cloned, sequenced and found to be expressed only in liver and lung tissue. Consistent with the species relationships for activin $\beta_A$ and $\beta_B$, rat activin $\beta_E$ was found to be highly homologous to the mouse sequence at both the nucleotide and the predicted amino acid level. Both lung and liver possessed two transcripts, which differed by virtue of a 738 bp extension in the 3′-untranslated region of the longer transcript. These data are suggestive of either the involvement of alternative polyadenylation consensus sites or an alternative splicing event in response to different stimuli. Although the significance of this 3′-extension remains to be determined, it could, however, contribute to changes in mRNA stability, translational delay or modulation of the 20S ribosomal assembly. The observed tissue distribution of activin $\beta_E$ in the rat is consistent with the results of Fang et al. (1996) who found activin $\beta_E$ only in the liver of the mouse using the less sensitive method of Northern analysis. Injection of the inflammatory mediator LPS into rats resulted in a significant elevation in $\beta_E$ transcript levels in the liver 6 h post-injection. These significant differences in relative abundances of the two different length transcripts following LPS injection suggest that a degree of differential regulation occurs in response to this inflammatory stimulus.

The increase in activin $\beta_E$ mRNA levels at 6 h post-LPS injection correlates with a pattern of an acute-phase response in the liver (Moshage 1997). The stimulation may arise directly through the action of LPS on hepatocytes or via the mediation of proinflammatory cytokines, such as tumor necrosis factor-$\alpha$, IL-1$\beta$ or IL-6.

As yet the function and biological actions of the activin $\beta_E$ subunit remains unknown. Moreover, its potential to heterodimerise with other members of the activin family, to produce either heterodimeric activins or inhibit, or to interact with the binding proteins such as follistatin and $\alpha_2$-macroglobulin which are also expressed within the liver (Lorent et al. 1994, Zhang et al. 1997b) remains to be clarified. The activin $\beta_A$, $\beta_B$ and $\beta_C$ subunits and follistatin are known to be expressed in the liver of various species (Yasuda et al. 1993, Fang et al. 1996, de Bleser et al. 1997). $\beta_B$ has not been found in mammals. If all forms of the activin $\beta$ subunits known to be present in mammalian tissues were able to undergo homo- and heterodimerisation with each other, this process would potentially allow the formation of ten different members of the activin sub-family, some or all of which may have different biological actions. The restriction in expression of

transcript increased by more than twofold at 6 h post-injection (Fig. 5C). A similar profile was also observed for the $\beta_E$ short transcript (Fig. 5D, time 6); however, there was a preferential stimulation of the short transcript, so that the short transcript was slightly more abundant than the longer transcript after LPS treatment (Fig. 5E). Although the levels of both transcripts appeared to return to normal thereafter, the increased ratio of long-to-short transcript persisted for at least a further 72 h. Similar Northern analysis results were obtained using the rat activin $\beta_{E1,2}$ and the $\beta_E5,6$ plasmid inserts as probes (data not shown).

Northern blotting of LPS-treated rats also revealed the size of the full-length $\beta_E$ transcripts as approximately 3·1 kb and 2·25 kb. The difference of approximately 850 bp between the short and long transcripts is consistent, within the resolution of agarose gel electrophoresis, with that predicted from RT-PCR sequencing data. The possibility remains however, of further transcripts with alternatively processed 5′-untranslated regions.

FIGURE 3. An RT-PCR analysis of rat liver (H) and lung (L) tissue for the rat activin $\beta_E$ long transcripts run on an ethidium bromide-stained 1% agarose gel. Easily detectable levels of the long ($\beta_E$L) activin $\beta_E$ transcripts were found within the liver (lane 3), whereas lung tissue contained lesser amounts of the longer $\beta_E$ transcript (lane 2). Lane 1 contains DNA size markers with their respective sizes indicated.

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The expression of the activin $\beta_E$ mRNA in the adult rat liver. The activin $\beta_E$ mRNA was detected by in situ hybridisation using two antisense cRNA probes generated from plasmids $\beta_E$3,4 (C and D) and $\beta_E$5,6 (E). The specificity of the in situ hybridisation technique was confirmed by hybridising sections with sense cRNA probes generated from the $\beta_E$3,4 plasmid (A) and the $\beta_E$5,6 plasmid (B). The activin $\beta_E$ mRNA was found within hepatocytes in close proximity to the central veins (arrows). Those further away from the central vein contained less or no $\beta_E$ transcript. Endothelial cell, Kupffer cells and hepatic stellate cells did not appear to contain $\beta_E$ mRNA. Panel C, 40× magnification. Panels A, B, D and E, 100× magnification.
activin βE to the rat liver, with lower levels of expression in the lung, would suggest that activin βE may be important in hepatic function. The present investigation indicates a role for activin βE in inflammation or the acute-phase response. Further clarification of this role will be of vital importance in order to unravel the function of activin βE given the known expression of other activin subunits in the liver and the large number of processes in which activins have been implicated (Vale et al. 1994, de Paolo 1997).

Activins are known to have biological actions within the liver, for example, activin A (βAβA) is an autocrine regulator of parenchymal cell apoptosis (Yasuda et al. 1993). It can be proposed that activin A acts in concert with the mitogen hepatic growth factor (Zarnegar & Michalopoulos 1989) to maintain hepatic mass (Yasuda et al. 1993). This conclusion was supported by the relatively lower levels of activin A in areas of rapid cell division in livers from treated rats compared with control rats, following a reduction in hepatic mass due to carbon tetrachloride (CCl4) exposure (de Bleser et al. 1997). In this previous study, the most rapidly dividing hepatocytes were those in contact with hepatic stellate cells which were producing hepatocyte growth factor. In terms of sequence homology, however, the activin βE subunit shares the highest degree of similarity with the activin βC subunit. Following partial hepatectomy, a different mRNA expression response occurs for the βC subunit compared with that of activin βA. This observation was suggestive of different biological roles for activin A and activin C in hepatic function (Zhang et al. 1997a). The results from the present study indicate that activin βE may also have a different function from that of βA in the liver.

In summary, we have cloned and sequenced the rat homologue/orthologue of the activin βE subunit and found its expression to be largely restricted to the liver. Sequence data confirm that two transcripts exist for rat activin βE, putatively encoding for the same protein, which differ by a 3′ extension in the longer form.

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