RESEARCH ARTICLE

Rat activin-βE mRNA expression during development and in acute and chronic liver injury

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ABSTRACT

Activin-βE mRNA expression was investigated in male and female rats using gel-based and quantitative RT-PCR, in fetal and post-natal liver during development and in a variety of tissues from 200 gm adult animals. Activin-βE expression was also assessed in rat liver after partial hepatectomy, and after repeated toxic insult. Male Sprague Dawley rats were subjected to partial hepatectomy or sham operations. Samples were collected from the caudate liver lobe during regeneration, from 12 to 240 hr after surgery. Three groups of 5 male rats were treated with CCl_4 for 0 (control), 5 or 10 weeks, to induce liver fibrosis and cirrhosis. Activin-βE mRNA was predominantly expressed in liver, with much lower amounts of mRNA observed in pituitary, adrenal gland and spleen, in both males and females. Low activin-βE expression was observed in liver at fetal day 16, with higher levels seen between post-natal days 3 and 35 and a further increase noted by day 47, in both males and females. Liver activin-βE mRNA concentrations did not change from control values 12-72 hr after PHx, but significantly increased over six fold, 168 hr post-hepatectomy, when liver mass was restored. Activin-βE mRNA was up-regulated after 5 weeks of CCl_4 treatment, but not after 10 weeks. The changes in activin-βE mRNA concentrations after liver insult did not always parallel those reported for the activin-βC subunit, suggesting activin-βE may have an independent role in liver under certain conditions.

KEYWORDS: *Activin-βE, liver, cirrhosis, hepatectomy, development*

INTRODUCTION

The activins belong to the transforming growth factor-β (TGF-β) gene family. Members of this family share structural similarities, but are diverse in their biological activities (Kingsley, 1994). Activins are hetero- or homodimers of β-subunits held together by disulphide bonds (Ying, 1988). Five activin subunits (βA-βE) have been identified to date (Ying, 1988; Hotten et al, 1995; Fang et al, 1996).

Activin-βC and activin-βE mRNAs appear to be predominantly expressed in the liver (Hotten et al, 1995; Fang et al, 1996; Lau et al, 1996; Schmitt et al, 1996; Fang et al, 1997; Lau et al, 2000), but have also been reported in a variety of other tissues (Mellor et al, 2000; O'Bryan et al, 2000; Rosendahl et al, 2001; Ball et al, 2002; Hashimoto et al, 2002; Vejda et al, 2002; Mellor et al, 2003; Gold et al, 2004). The predicted mature region of the activin-βE subunit gene shows > 60% identity with activin-βC (Fang et al, 1996) and both genes are on closely linked loci, separated by only 5.5 kb, suggesting tandem duplication of an ancestral gene (Fang et al, 1997). The expression of the activin-βC subunit is increased in the earlier stages of cirrhosis development in rat liver when many hepatocytes are undergoing apoptosis (Gold et al, 2003) and is down regulated in the early stages of liver regeneration after PHx in the rat, suggesting that it may be a negative

regulator of liver cell growth (Esquela et al, 1997; Zhang et al, 1997). Similarly, *in vivo* treatment of rats with lipopolysaccharide, as a means of activating the immune system and the hepatic acute phase response, results in increased activin-βE mRNA levels (O'Bryan et al, 2000). It has, therefore, been proposed that activin-βE has a role in the hepatic inflammatory response. Rosendahl and colleagues demonstrated the presence of activin-βE mRNA in mouse lung using RT-PCR: Activin-βE mRNA was up-regulated five-fold during chronic airways inflammation, suggesting this activin subunit may be involved in acute allergic inflammation and may thus be important in respiratory pathophysiology (Rosendahl et al, 2001). Despite these observations, studies of transgenic mice deficient in activin-βC, activin-βE or both genes showed no deficiencies in embryonic development, or adult female liver function or liver regeneration (Lau et al, 2000). These observations suggest redundancy of function amongst some of the members of the TGFβ gene family, but are hard to reconcile with clear changes in expression in response to injury or toxic insult.

In order to investigate this apparent discrepancy, we examined activin-βE mRNA expression during post-natal development and in a variety of adult tissues in male and female rats. In addition, we have investigated activin-βE expression in adult male rat liver in two models of liver injury, partial hepatectomy and repeated toxic insult.

MATERIALS AND METHODS

Experimental animals

All experiments were performed in accordance with the New Zealand Animal Welfare Act 1999, after approval was granted by the University of Otago Medical School Animal Ethics Committee. Rats were housed under constant room temperature (22°C ± 1°C), with free access to food and water, 12 hr light and 12 hr dark cycles and were fed rat laboratory pellets.

Developmental studies

Liver samples were obtained from 11 fetal rats of undetermined sex on fetal day 16, from new-born pups at postnatal day (D)3 and from growing animals at D25 (prepubertal), D35 (peripubertal) and D47 (adult) (n=2-3 males and females at each time point).

Adult tissue distribution

The heart, spleen, kidney, lung and adrenal glands were collected from 5 male and 5 female 200 gm Sprague Dawley rats. The ovaries and uterus were taken in females and both testes were collected from males. The top of the skull was removed and samples of frontal cortex and the whole pituitary were collected. All tissues were immediately snap-frozen and stored at -80°C for subsequent RNA extraction.

Partial hepatectomy

Sprague Dawley rats (220gm, n = 35 males) were randomly allocated to PHx or control sham operations. PHx was carried out according to the method of Higgins and Anderson (Higgins et al, 1932). Control operations consisted of opening the abdominal cavity and briefly handling the liver. Two control and 3 PHx rats were sacrificed at 12 hr, 24 hr, 48 hr, 72 hr, 96 hr, 168 hr (7 days) and 240 hr (10 days) post surgery, in three independent experiments (Gold et al, 2005).

Repeated toxic insult

Fifteen 150 gm male rats were included in the study. Micronodular hepatic cirrhosis was induced according to the protocol of Proctor and Chatamra (1982) as described previously (Gold et al, 2003). After 14 days of sodium phenobarbitone treatment (35mg/100ml in drinking water), to increase the sensitivity of the liver to CCl_4, rats were randomly allocated to one of three groups and treated with CCl_4 for 0 weeks (controls), 5 weeks or 10 weeks. Changes in liver architecture were studied histologically as described (Gold et al, 2003).

Liver collection in PHx and CCl4-treated animals

Samples were collected at all time points after PHx and in sham operated controls, from the caudate liver lobe, which is left undisturbed by the surgery, but does contribute to the restoration of liver mass. Tissue was also collected from the site of liver lobe removal, 12 hr and 24 hr after PHx, to determine changes in the concentrations of mRNA for activins-βA, -βC and -βE, activin receptor subunits ActRIIA and ActRIIB and follistatin at the wound site. The site of lobe removal had completely regressed by 24 hr, therefore, no further time points were collected from this site. A portion of the left lateral lobe was collected from control and CCl_4-treated animals. Liver samples were removed and immediately stored at -80°C for subsequent RNA extraction.

RNA extraction

Total RNA was extracted from 30 mg of tissue using the RNeasy Mini Kit (Qiagen Pty. Ltd., Victoria, Australia) according to manufacturer's directions. Whole ovary and pituitary (anterior and posterior) were extracted for RNA. An equal amount of RNA from each tissue was used for generation of first strand cDNA.

Reverse transcription

Total RNA (1 µg) and 25 mM oligo-dT primer (Roche Molecular Biochemicals, Auckland, New Zealand) were heated a total volume of 14.5 µl to 65°C for 10 min, to disrupt possible secondary structures and then snap cooled on ice. 12.5 U MMuLV Reverse Transcriptase (ABgene, Epsom, Surrey, UK), 1x reverse transcription buffer (ABgene) and 1 mM of each deoxy-NTP (ABgene), in a final volume of 20 µl, was incubated at 37°C for 50 min, heated to 95°C for 2 min and then stored at -20°C. The cDNA was used as a template for PCR.

Gel-based PCR

Primers for activin-βE PCR were designed based on published rat and mouse sequences (Fang et al, 1996; O'Bryan et al, 2000; Vejda et al, 2002) and GenBank sequences (GenBank Accession numbers NM008382, NM031815, AF140032, AF89825). The activin-βE primers (βEF: 5' CTG TGA CAC TGG TTT GCT 3'; βER: 5' CAG CTC CTG GAA ATC GAC AT 3')

corresponded to rat mRNA nucleotides 76-93 and 949-968 (NM031815) and were designed so that the amplicon was derived from two exons. Preliminary PCR assays were optimised for annealing temperature, number of cycles of amplification and Mg^{++} concentration as previously described (Gold et al, 2003; Gold et al, 2005) and the major liver amplicon (895 bp) was sequenced in both directions to verify sequence identity, as previously described for activin-βC RT-PCR (Gold et al, 2003).

The β-actin PCR reaction was continued for 20 cycles, as described previously (Gold et al, 2003; Gold et al, 2005). The activin-βE PCR reaction contained 1 µl of cDNA, 20 pmol of each primer, 2 µl 10x reaction buffer IV (ABgene), 2 mM $MgCl_2$, 200 µM of each deoxy-NTP (ABgene), 1 U Red Hot Taq polymerase (ABgene) and sterile water to 20 µl. PCR reactions were amplified in a Corbett PC-960 air-cooled thermocycler (Corbett Research, NSW, Australia) for 25 cycles. An initial denaturation step at 95°C for 2 min was followed by 25 cycles of 30 sec at 95°C, 30 sec of annealing at 58°C and elongation for 40 sec at 72°C. Controls containing no template, or with gDNA or RNA rather than cDNA, were run alongside all experimental samples. PCR products (15 µl) were separated by agarose gel electrophoresis and quantified by densitometry. Sample densities were normalised with respect to the density of β-actin mRNA as previously described (Gold et al, 2003). Comparison of the results obtained from gel-based PCR with quantitative PCR showed similar amounts of activin-βE mRNA were measured by the two methods (data not shown), as reported for activin-βC mRNA (Gold et al, 2003).

Gel-based PCR assays to semi-quantify activin-βA, activin-βC, activin receptor type II subunits ActRIIA and ActRIIB and follistatin expression at the site of lobe removal, were performed exactly as described previously (Gold et al, 2005).

Real time RT-PCR

A liver cDNA of known concentration was used to construct a standard curve and the same standard cDNA was used as a reference in all assays. To ensure amplification was cDNA-specific, an initial experiment was performed where genomic DNA, RT-negative and RT-positive samples were run for each primer pair. No product was detected in the genomic DNA or the RT-negative samples (data not shown). Experimental samples were assessed in triplicate. Levels of cDNA were quantified relative to the standard curve and normalised to β-actin expression.

The cDNA template was amplified in a total reaction volume of 20 µl, with 300nM target-specific primers and 200 nM fluorescent probe (Table 1) using the Taqman Universal PCR mix and the ABI7700 (PE Applied Biosystems, Perkin Elmer Corporation, CA, USA) sequence detection system according to manufacturer's directions. Data were analysed using ABI7700 software (PE Applied Biosystems, Perkin Elmer Corporation, CA, USA).

Analysis and statistics

Two independent RNA extractions, duplicate cDNA and duplicate gel-based PCR reactions were performed on liver samples from male and female animals at different developmental ages. Results are presented as the mean ± range of the band densities. Gel-based PCR data from developmental studies were not compared statistically. Tissue distribution results from quantitative RT-PCR are presented as mean ± standard error of 3 independent PCR reactions with different cDNAs from 5 male and 5 female rats.

PHx data were log transformed and time differences assessed by analysis of variance (ANOVA, SPSS for Windows 6.1). Normalised densities are expressed as a percentage of sham operated controls. Data from the cirrhosis studies are presented similarly. The tissue distribution of activin-βE mRNA, the data on activin subunit, activin receptor subunit and follistatin expression at the site of lobe removal (n = 3 animals per time point) and cirrhotic liver injury data were analysed by Mann-Whitney U Test (SPSS for Windows 6.1).

RESULTS

Developmental expression of activin-βE mRNA

Figure 1A shows a representative optimised gel-based RT-PCR assay for activin-βE and β-actin expression through post-natal development. A major amplicon at approximately 895 bp was observed after 20 PCR cycles. To confirm amplicon identity, PCR products were sequenced in both directions using the direct-cycle method, by the University of Otago Centre for Gene Research sequencing facility. The 895 bp band showed 99% homology with mouse inhibin C on chromosome 7. A minor amplicon of 1100 bp was present in some, but not all cDNA samples from adult rat liver. This band was not included in analysis as sequencing indicated there was a small degree of homology with mouse chromosome 3 and thus it could be non-specific for activin-βE. Figure 1B shows the levels of activin-βE mRNA relative to β-actin

Table 1. Quantitative RT-PCR probes and primers. All sequences are depicted as 5' to 3'. F: forward; R: reverse

activin-βE probe	Fam -CAAAACTCCAACTGGAATTCAGACCT-Tamra
activin-βE primer F	TTCCTAGCTGATTACCAAACCACTT
activin-βE primer R	CTCCGCAAGCCGCTAGAG
β-actin probe	Fam-TTTGAGACCTTCAACACCCCAGCCA –Tamra
β-actin primer F	CGTGAAAAGATGACCCAGATCA
β-actin primer R	CACAGCCTGGATGGCTACGT

mRNA in fetal rat liver and in post-natal liver during development. Low amounts of activin-βE mRNA were measured in the livers of fetal rats at F16. An approximately 7-fold increase in band density was seen by post-natal D3 and densities remained constant between D3 and D35 for both males and females. A further increase in expression was observed at D47. No attempt was made to compare activin-βE concentrations statistically in this experiment.

Tissue distribution of activin-βE mRNA

Activin-βE mRNA was predominantly expressed in rat liver (Figure 2). There were low amounts of mRNA in most other tissues assessed, particularly in the pituitary, adrenal and spleen. There were no significant differences in the amounts of liver and pituitary activin-βE mRNA observed in males and females (liver: $p = 0.072$; pituitary: $p = 0.084$; Mann-Whitney U Test).

Activin-βE expression after PHx

PHx rats lost 4% of their body weight post-PHx, and were back to pre-operation weight after 60 hours. By 168 hours post-PHx, rat body weight was steadily increasing (14% compared to pre-operation weight). In comparison, the sham operated controls did not lose weight post-sham operation and body weight increased throughout the study (25% increase by 168 hr). Normal liver cellular architecture was restored in sections of liver after 168 hr (data not shown).

Activin-βE and β-actin concentrations were measured by quantitative RT-PCR in tissue extracts from the site of liver lobe removal and from the caudate liver lobe. A significant fall ($p < 0.005$) in the amounts of activin-βE mRNA, relative to β-actin mRNA concentrations, was measured at the site of lobe removal 12 hr and 24 hr post-PHx (Figure 3A). Activin-βA, activin-βC, ActRIIA, ActRIIB and follistatin mRNA concentrations were measured only at the site of lobe removal by gel-based RT-PCR. Changes in the expression of these mRNAs relative to β-actin levels at the wound site, 12 hr and 24 hr post-PHx, are also shown in Figure 3A. This site had completely regressed by 24 hr; therefore, no further samples were collected from this site. Significant decreases in the relative amount of mRNA were also observed for activin-βC ($p<0.001$), ActRIIA ($p<0.05$) and ActRIIB ($p<0.001$) at the wound site, whereas a significant increase in the amount of activin-βA mRNA was observed 12 hr after PHx ($p<0.03$) and in follistatin mRNA 24 hr after PHx ($p<0.01$) at this site.

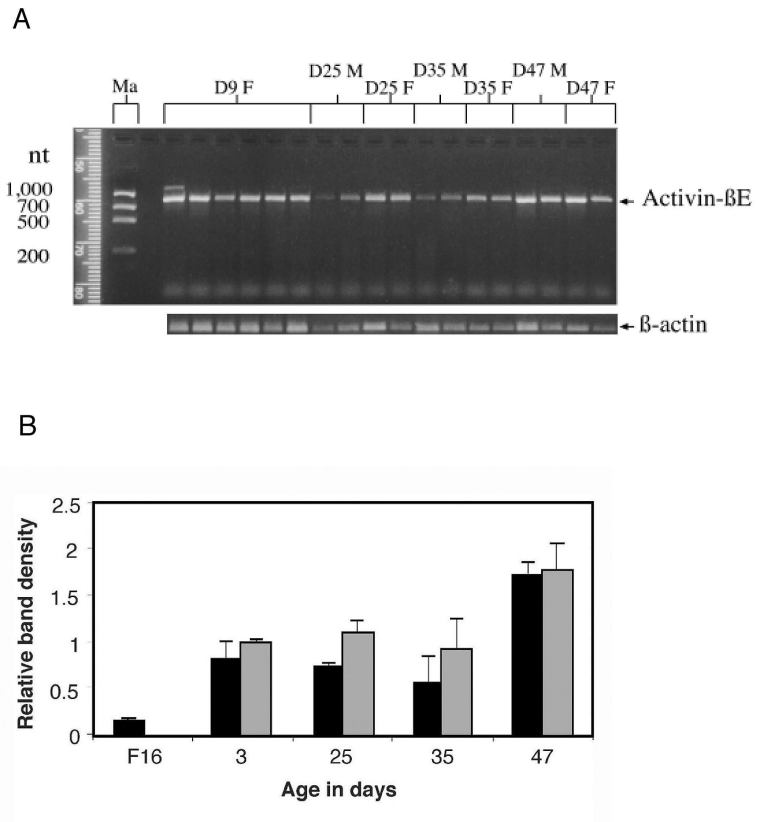

Figure 1. (**A**) A representative gel-based RT-PCR for activin-βE and β-actin. A major amplicon at approximately 895 bp was observed in adult rat liver extracts, with a minor amplicon at 1100 bp observed in a small proportion of extracts. The 1100 bp amplicon was not included in the densitometric analysis. Samples of liver from male (M) and female (F) rats collected on post-natal days (D) 9, 25, 35 and 47 are shown on this gel. Ma; size marker DNA (**B**) Mean normalised levels of activin-βE mRNA relative to β-actin mRNA in fetal (n = 11, sex not determined) and post-natal male (black bars) and female (grey bars) rat liver during development, assessed by gel-based RT-PCR. Results are mean ± SD of 3 independent PCR reactions with cDNAs from 2-3 animals per time point.

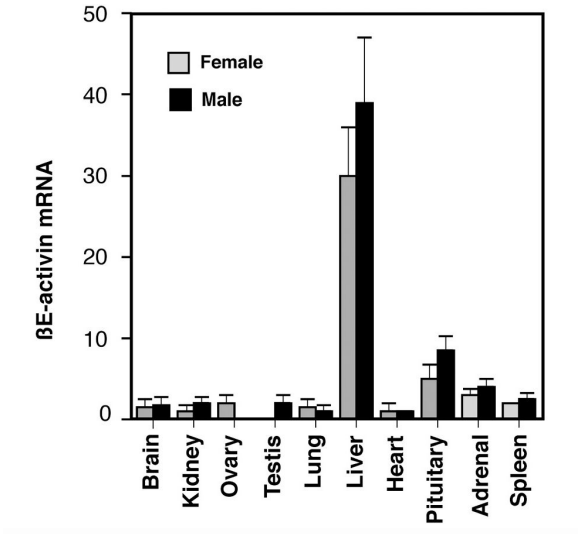

Figure 2. Distribution of activin-βE mRNA relative to β-actin mRNA concentrations in a range of male (n = 5) and female (n = 5) rat tissues, assessed by quantitative RT-PCR (Taqman). Results are mean ± standard error of 3 independent PCR reactions with different cDNAs.

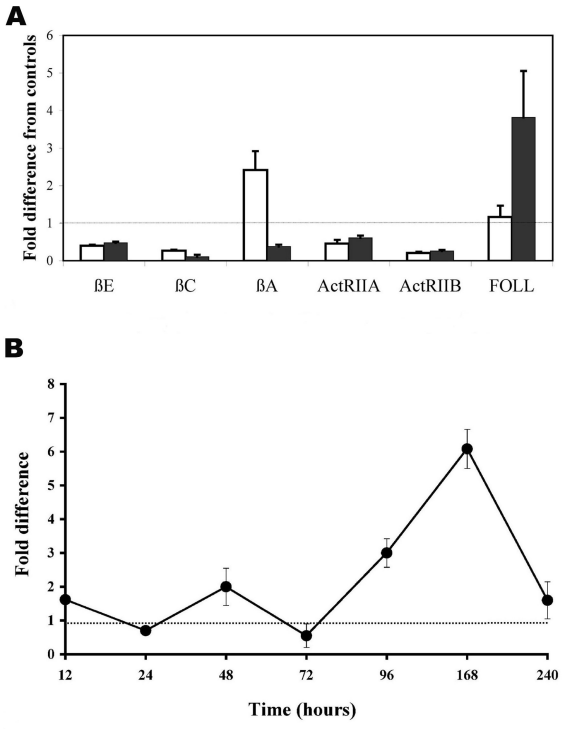

Figure 3. (**A**) Relative amounts of activin-βA, -βC and βE, activin receptor subunits ActRIIA and ActRIIB and follistatin (FOLL) mRNAs expressed at the site of lobe removal, around the suture, at 12 hr (white bars) and 24 hr post-PHx, measured using gel-based RT-PCR. Significant increases in activin-βA mRNA were seen at 12 hr ($p<0.03$) and in follistatin mRNA at 24 hr after PHx ($p<0.01$). The amounts of all other mRNAs were significantly less than those in control liver at both 12 and 24 hr post-PHx. (**B**) Activin-βE mRNA, assessed by quantitative RT-PCR, in regenerating caudate lobe of the liver, after PHx in male Sprague Dawley rats (n = 3 PHx at each time point). Data were normalised to β-actin expression and are presented relative to mean values in sham-operated control rat livers (n = 2 at each time point). A significant ($p = 0.04$), six fold increase in relative activin-βE concentrations was observed 168 hr after PHx, with concentrations returning to control values 240 hr post-PHx.

No significant changes in β-actin mRNA expression over time were measured in the caudate lobe of regenerating male rat liver (ANOVA; $F = 0.68$; $p = 0.543$). Changes in activin-βE expression after PHx, expressed, therefore, as amount of mRNA relative to expression in sham control livers, are presented in Figure 3B. The mean concentration of activin-βE measured in sham-operated control caudate liver lobe was 11.3±2.3 µg cDNA/ml extract or 0.56±0.12 ng cDNA/µg RNA (n = 14 rats). Activin-βE expression in the regenerating caudate lobe was not significantly changed from sham-operated control values 12-48 hr after PHx, but increased 6 fold at 168 hr ($p = 0.040$), and fell back to control values at 240 hr ($p = 0.015$) (Figure 3B). In a previous study we found lower amounts of βA-activin mRNA in the caudate lobe of regenerating liver at 12 and 24 hr, and observed a marked increase in βA-activin mRNA at 168 hr when liver mass appeared fully restored (Gold et al, 2005).

Activin-βE expression in the development of liver cirrhosis

Activin-βE mRNA expression in controls and during the development of CCl4–induced cirrhosis is shown in Figure 4. Activin-βE mRNA increased significantly after 5 weeks of CCl_4 treatment ($p < 0.02$, by Mann-Whitney U Test), returning to control levels after 10 weeks.

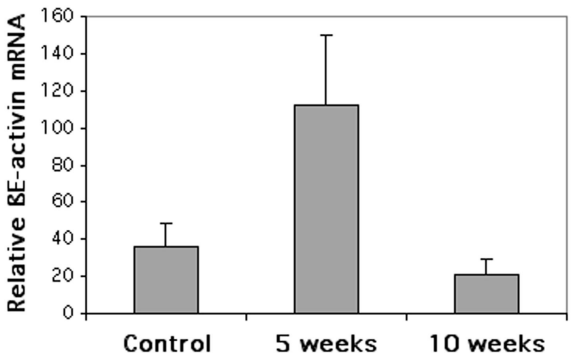

Figure 4. Amounts of activin-βE mRNA, relative to β-actin mRNA, during the development of CCl_4-induced liver cirrhosis. Results are mean ± SEM, of n = 5 animals for each experimental group. Activin-βE mRNA levels were significantly higher at 5 weeks than in control livers or after 10 weeks of CCl_4 treatment ($p<0.02$).

DISCUSSION

Low concentrations of activin-βE were observed by gel-based RT-PCR in fetal liver. In contrast to postnatal tissues, the rat fetal liver goes through dynamic changes during its 21-day gestation period. Vassy et al (1988) found that at day 12 of gestation, the hepatocytes, which are the cellular source of activin-βE secretion (O'Bryan et al, 2000) comprised only 35% of the liver volume, and between days 18 and 20, this volumetric fraction increased significantly to approximately 70% (Vassy et al, 1988). Since the fetal rats used in the present study were obtained on day 16 of gestation, a portion of the increase in activin-βE expression between fetal and postnatal tissues could be accounted for by the fact that there were fewer hepatocytes in the fetal tissue samples compared with the postnatal samples.

βC-activin has been shown to be growth inhibitory in liver (Esquela et al, 1997; Zhang et al, 1997; Chabicovsky et al, 2003) or growth promoting (Kobayashi et al, 2002; Wada et al, 2004). Mellor et al has shown βC-activin subunit over-expression antagonises activin A by forming intracellular heterodimers (Mellor et al, 2000, Mellor et al, 2003). Activin-βE, like activin-βC, may in some instances be a potent inhibitor of DNA synthesis (Chabicovsky et al, 2003), meaning low levels of activin-βE expression in fetal tissues would allow liver growth to proceed at a rapid rate and up-regulation at the end stages of restoration of liver mass (168 hr) may terminate liver regeneration. Wada et al (2004) propose that activin C homodimer, rather than activin A, is formed in normal liver and partial hepatectomy may lead to increased activin A, terminating liver regeneration (Wada et al, 2004). In an earlier study we showed activin homodimers (βAβA, βCβC) are present at low to moderate levels in control liver while increased activin AC was evident during liver regeneration. Therefore the formation of the activin heterodimer AE may also antagonise the formation of the activin A homodimer and allow restoration of liver mass.

Despite reports of liver-specific localisation of activin-βE mRNA (Fang et al, 1996; Lau et al, 1996; Fang et al, 1997; Lau et al, 2000), some groups have demonstrated a wider tissue distribution (O'Bryan et al, 2000; Rosendahl et al, 2001; Hashimoto et al, 2002; Vejda et al, 2002). The current study found activin-βE mRNA was predominantly expressed in the liver, but low amounts of activin-βE mRNA were evident in other tissues, notably the pituitary, spleen and adrenal gland. Vejda et al reported different tissue expression patterns in male and female rats, with male skeletal muscle and heart and female kidney showing higher amounts of activin-βE mRNA (Vejda et al, 2002). In agreement with the data presented by Vejda et al (2002), male rat liver expressed slightly higher amounts of activin-βE mRNA compared with liver from females, but the differences were not statistically significant. The differences in tissue distribution between this study and those previously reported may be attributable to the age or strain of the rats used in the different investigations or may reflect differences in the primer sets used.

Activin-βE expression remained relatively constant from post-natal day 3 until day 35 and increased at day 47, around the time of initiation of steroidogenesis in both male and female rats (Okatani et al, 1997; Gomez et al, 2002). This peri-pubertal increase suggests hepatic activin-βE expression may be regulated by gonadal steroids. This hypothesis should be investigated further using isolated liver cells in primary culture, to identify the cell type that is responsive to male and female gonadal steroids. Lau et al (2000) showed that the livers of mice deficient in activin-βC, activin-βE, or both, developed normally (Lau et al, 2000), highlighting the possibility of either functional substitution by other members of the TGFβ family, or lack of a regulatory role during normal growth for these activin subunits. The post-natal increase in activin-βE expression in rat liver observed in the current investigation may be merely the result of an increase in the volumetric fraction of hepatocytes in growing liver, rather than suggestive of a regulatory role for this peptide in liver growth.

In contrast, mRNA amounts in the sham-operated adult controls were high compared with fetal or immature values and did not change significantly in the caudate lobe in response to PHx, until liver mass was restored, when mRNA concentrations increased over six fold (Figure 3B). This suggests a "chalone"-like response, to inhibit cell division or induce apoptosis, as liver mass reaches an optimal size, as has been previously reported (Esquela et al, 1997; Zhang et al, 1997). We have previously reported a peak of cell division 48-72 hr and an increase in apoptosis 96-240 hr post-PHx in this model (Gold et al, 2005). However the response of activin-βE to PHx was subtly different from those of activin-βC and -βA, which both decreased significantly in the caudate lobe, 12-96 hr after PHx (Gold et al, 2005). Takamura et al (2005) studied the expression of activin βA, βC and βE mRNA from 0-120 hr in response to portal vein ligation (PBL) or PHx (Takamura et al, 2005). Expression of activin subunit mRNA was stronger in response to PBL compared to PHx at the early time points (0-6 hr). Interestingly fold increase of all activin subunits in both experimental models was less when there was increased evidence of proliferating cells (24-72 hr) perhaps indicating reduced activin subunit expression was associated with liver re-growth after injury (Takamura et al, 2005). Our study confirms supports and extends this work as we assessed mRNA expression post hepatectomy until liver mass was completely restored (240 hr).

If the relative abundance of mRNA determines which activin dimers form in vivo, (Mason et al, 1996; Mellor et al, 2003) the large peak of activin-βE mRNA at 168 hr post-PHx may indicate a change in the relative proportion of activins E, AE or CE since both βA and βC mRNA has also been shown to be significantly increased at this time point (Gold et al, 2005). While it was not possible to determine which proteins were present in the tissue in this study, the possibility of an activin CE dimer has been reported (Vejda et al, 2002).

The PHx study examined activin-βE gene expression in the wound site and the caudal lobe of rat liver after PHx. Activin-βE expression decreased significantly at the site of

liver lobe removal, suggesting this activin subunit is down-regulated in the inflammatory response at this site. This implies that activin-βE has a different role to activin-βA, expression of which is up-regulated in inflammation and wound healing (Yu et al, 1997; de Kretser et al, 1999; Munz et al, 1999; Gribi et al, 2001; Munz et al, 2001; Phillips et al, 2001). We also observed increased activin-βA mRNA at the site of lobe removal 12 hr after PHx. Reaction at the site of lobe removal was local and separate from that occurring in the regenerating caudate liver lobe, implying that activin-βE may be differentially regulated or have a different function in the two sites, depending on the local cytokine environment.

Activin-βE mRNA was significantly increased after five weeks of CCl4 treatment, at a time when hepatocyte apoptosis is significantly increased and proliferation minimal (Gold et al, 2003). It has been proposed that activin-βE has a role in liver response to inflammation (O'Bryan et al, 2000), as well as allergic-airway inflammation (Rosendahl et al, 2001). However activin-βA and activin-βC mRNA concentrations are also raised after 5 weeks of CCl4 treatment (Gold et al, 2003), so in the liver this response appears not to be subunit specific. Furthermore, activin-βE mRNA concentration decreased at the site of lobe removal at PHx, suggesting expression is not related to increased inflammatory response. If we consider relative abundance of mRNA as an indicator of which dimers may form; βA was up-regulated 3 fold, βC 1.8 fold (Gold et al, 2003) and βE 3 fold, after 5 weeks of CCl4 treatment perhaps indicating an activin AE dimer is more likely to form leading to hepatocyte apoptosis and early stage liver disease, rather than the end stage cirrhosis observed at 10 weeks.

CONCLUSIONS

In summary our study demonstrates activin-βE mRNA is predominantly expressed in rat liver, with small amounts of mRNA being observed in the pituitary, spleen and adrenal glands. Increasing amounts of activin-βE mRNA can be seen through post-natal development, in parallel with an increase in hepatocyte numbers. Activin-βE expression appears to be up-regulated in the early stages of chronic liver disease, rather than in end stage cirrhosis, but expression in the caudate lobe is unchanged in the early response to PHx and increases markedly just as liver mass is restored. These data suggest activin-βE may form different dimeric activin species with specific roles in response to acute and chronic liver insult. While the response of the activin-βE gene parallels that of activin-βC in some circumstances, in other situations the response is different. Comparison of the results from the two liver insult models suggests the relative abundance of activin subunit mRNA may dictate which activin dimer species are able to form, which in turn may determine cellular fate, but final conclusions on the functions of the two liver-specific activin subunits will have to await further functional biology experiments.

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STATEMENT OF COMPETING INTERESTS

The authors declared no competing interests.

LIST OF ABBREVIATIONS

PHx; Partial hepatectomy
CCl_4; Carbon tetrachloride
ActRIIA; Activin receptor IIA
ActRIIB; Activin receptor IIB

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