

# JOURNAL SYMPOSIUM

## A novel role of activin in inflammation and repair

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### Introduction

Activins are members of the transforming growth factor  $\beta$  (TGF- $\beta$ ) superfamily of dimeric proteins, consisting of  $\beta$ A and  $\beta$ B subunits which are connected by disulfide linkages. Three different forms of activin, the homodimeric activin A ( $\beta$ A $\beta$ A), and activin B ( $\beta$ B $\beta$ B), as well as the heterodimeric activin AB ( $\beta$ A $\beta$ B) have been described, and just recently  $\beta$ C,  $\beta$ D and  $\beta$ E chains have been discovered (for review see Yu & Dolter 1997). In most assays, activin A, AB and B have similar activities, although differences between the three isoforms have also been described. Thus, the specific functions of each variant have yet to be defined.

The biological activity of activin is mediated by heteromeric receptor complexes consisting of two different types of receptor, the type I (ARI and ARIb) and the type II receptors (ARII and ARIIb), which are all characterized by an intracellular serine/threonine kinase domain (for review see Mathews & Vale 1993). Besides these transmembrane receptors, a soluble activin-binding protein, follistatin, has been discovered which binds to activin and thereby inhibits its biological effects (for review see Mather 1996).

Activin has been shown to affect growth and differentiation of many different target cells of various origins (for review see Yu & Dolter 1997). During early embryonic development it can act as a mesoderm-inducing factor.

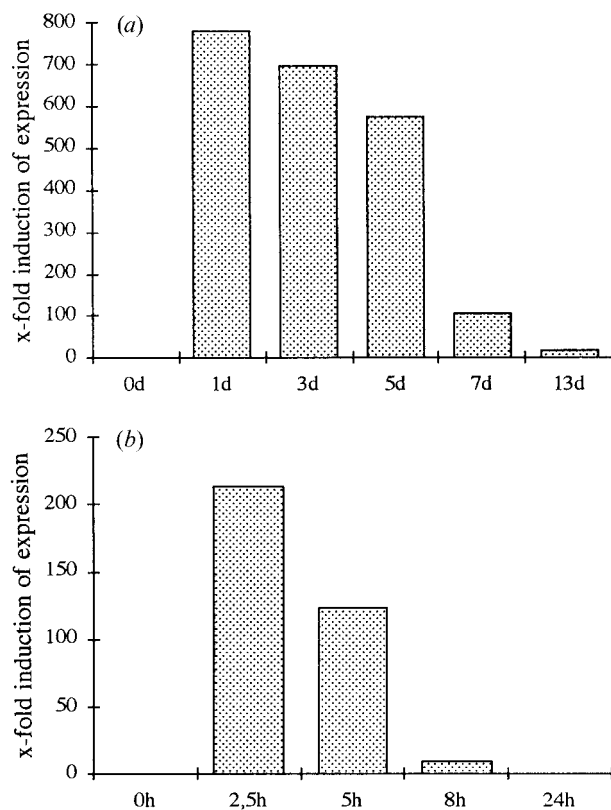
Furthermore, recent results obtained with mice lacking activin or its receptors revealed a role of activin in organogenesis (for review see Mather *et al.* 1997). During the past few years, we and others have provided evidence for a novel and important role of activin in inflammatory processes (Table 1).

### Expression of Activin is Strongly Induced after Skin Injury

In a first set of experiments we determined the expression and possible function of activin during cutaneous wound repair. For this purpose, we analyzed the expression of activin and its receptors in normal adult mouse skin and during the wound healing process. Surprisingly, we found a large induction of activin  $\beta$ A and  $\beta$ B mRNA expression within 1 day after skin injury, and high levels of activin mRNA persisted during the first week after wounding (Hübner *et al.* 1996b) (Fig. 1a). *In situ* hybridization demonstrated the presence of  $\beta$ B mRNA in suprabasal keratinocytes at the wound edge which undergo re-differentiation (Hübner *et al.* 1996b), whereas highest levels of activin  $\beta$ A mRNA were found in the granulation tissue below the scab. These cells are likely to represent fibroblasts and activated macrophages, which also express activin *in vitro* (Erämaa *et al.* 1992, Hübner & Werner 1996). The expression pattern of activin in the wound

**Table 1** Increased expression of activin in inflammatory processes

Process	Reference
Cutaneous wound repair	Hübner & Werner (1996), Hübner <i>et al.</i> (1996b)
Inflammatory bowel disease	Hübner <i>et al.</i> (1997)
Liver injury and cirrhosis	Yasuda <i>et al.</i> (1993), De Bleser <i>et al.</i> (1997), Sugiyama <i>et al.</i> (1998)
Pulmonary fibrosis	Matsuse <i>et al.</i> (1995, 1996)
Repair of the vasculature, arteriosclerosis	Inoue <i>et al.</i> (1994), Pawlowski <i>et al.</i> (1997)
Inflammatory arthropathies	Yu <i>et al.</i> (1998)
Brain repair	Lai <i>et al.</i> (1996, 1997), Tretter <i>et al.</i> (1996)



**Figure 1** Induction of activin expression after skin injury *in vivo* and by serum in cultured Balb/c fibroblasts. (a) Full-thickness excisional wounds were generated on the back of Balb/c mice. At different time points after injury, the complete wounds were isolated, immediately frozen in liquid nitrogen and used for RNA isolation. Expression of activin  $\beta$ A mRNA was analyzed by RNase protection assay. The degree of induction which is shown in the figure was assessed by laser scanning densitometry of the autoradiograms. (b) Mouse Balb/c 3T3 embryonic fibroblasts were grown to confluence and rendered quiescent by serum starvation. They were subsequently treated with 10% fetal calf serum. At different time points after serum addition, RNA was isolated from these cells and analyzed by RNase protection assay for activin  $\beta$ A mRNA expression. The degree of induction was determined by laser scanning densitometry of the autoradiograms and is shown schematically in the figure.

tissue was confirmed at the protein level by immunostaining with an activin-specific antiserum. In contrast to the  $\beta$  chains, expression of the  $\alpha$  chain was hardly detectable in normal and wounded skin, indicating that activins but not inhibins are produced in the wound. In addition to activin, all known activin receptors were expressed in normal and wounded skin, although their expression was not induced after injury (Hübner *et al.* 1996a).

To identify potential inducers of activin expression in the wound tissue, we analyzed the effect of various growth factors and cytokines on the expression of activin in cultured fibroblasts and keratinocytes (Hübner & Werner 1996). Interestingly, a strong induction of activin  $\beta$ A

expression was observed in both cell types upon addition of serum (Fig. 1b), TGF- $\beta$ 1, epidermal growth factor or platelet-derived growth factor. Since these factors are released from platelets upon hemorrhage, they might be responsible for the early increase in activin expression after wounding. Furthermore, the pro-inflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) were identified as potent positive regulators of activin expression *in vitro*. This might be important for the *in vivo* situation, since we recently demonstrated a strikingly increased expression of these cytokines after skin injury, whereby the time-course of expression correlated with the expression of activin in the wound (Hübner *et al.* 1996a). *In situ* hybridization studies and immunohistochemistry revealed the presence of highest levels of IL-1 and TNF- $\alpha$  mRNAs and proteins in polymorphonuclear leukocytes and macrophages adjacent to the activin-producing fibroblasts and keratinocytes. The strong temporal and spatial correlation of the expression of activin and the pro-inflammatory cytokines suggests a possible role of these factors in activin induction during wound healing. From these findings we propose a model for activin regulation during wound healing, which is shown schematically in Fig. 2.

The function of activin in normal and wounded skin is presently unknown. However, preliminary *in vitro* and *in vivo* studies from our laboratory suggest a role of activin in the regulation of keratinocyte proliferation and/or differentiation. Surprisingly, no inhibitory effect of activin on keratinocyte proliferation was observed *in vitro* (Shimizu *et al.* 1998), and expression of activin in the epidermis of transgenic mice even stimulated proliferation of keratinocytes and modulated their differentiation (B Munz, unpublished data). This is in contrast to TGF- $\beta$ , which strongly inhibits proliferation of these cells *in vitro* and *in vivo*. In the mesenchymal compartment, activin could act as an inducer of extracellular matrix molecules, since a stimulatory effect of activin on fibronectin expression was observed in embryonic 3T3 fibroblasts (G Hübner, unpublished data). In addition, activin was recently shown to increase the mRNA levels of collagen type I mRNA in normal rat kidney fibroblasts (Sugiyama *et al.* 1998). These findings suggest a role of activin in the repair of the injured dermis and epidermis. Furthermore, activin might be involved in cutaneous scar formation.

### Overexpression of Activin in Inflammatory Bowel Disease

The strong induction of activin expression by pro-inflammatory cytokines suggested a role of activin in other types of inflammatory processes. Therefore, we determined the expression of this molecule in affected and nonaffected areas of patients suffering from inflammatory bowel disease. This comprises two major forms of

intestinal inflammation: Crohn's disease (CD) and ulcerative colitis. Both chronic disorders are characterized by an unpredictable course, with acute phases of inflammation followed by remission. Histologically, severe damage of the epithelium and the underlying mesenchyme can be observed, accompanied by massive infiltration with inflammatory cells. Furthermore, fibrosis, leading to stenosis and obstruction, is a major complication, especially in CD (for review see Podolsky 1991). Our studies revealed a strikingly increased expression of the activin  $\beta$ A subunit in surgical specimens from the gut of both types of patients, whereas no activin  $\beta$ A expression was detected in the normal human gastrointestinal tract (Hübner *et al.* 1997). The levels of activin expression showed an outstanding correlation with the degree of inflammation. Using *in situ* hybridization, the mucosa and submucosa of highly inflamed areas were identified as the major sites of activin  $\beta$ A expression. In contrast to the  $\beta$ A chain, activin  $\beta$ B mRNA levels in most specimens from inflamed areas were only slightly higher compared with control tissue (Hübner *et al.* 1997). These data suggest a novel role of activin in the pathogenesis of inflammatory bowel disease, although the precise function is, at present, unclear. Similar to the skin, activin might stimulate deposition of connective tissue and thus might be involved in fibrotic processes. In addition, activin could affect differentiation processes of the epithelial cells as recently demonstrated for the gastric epithelium (Li *et al.* 1998).

### Increased Expression of Activin in Cirrhotic and Fibrotic Rat Livers and in Pulmonary Fibrosis

A role of activin in normal and pathological repair processes of the liver was first suggested by Yasuda *et al.* (1993), who found increased expression of activin  $\beta$ A mRNA in rat livers within 24 h after partial hepatectomy. Subsequently, De Bleser *et al.* (1997) analyzed the expression of activin in rat livers after treatment with chloroform. This induces liver injury leading to fibrosis. Interestingly, high levels of the activin  $\beta$ B chain and especially of the  $\beta$ A chain were found in stellate cells of the fibrotic liver. The potential role of activin in fibrotic processes of the liver was supported by results of Sugiyama *et al.* (1998). In these experiments, liver cirrhosis or fibrosis was induced by intraperitoneal injections of dimethylnitrosamine or porcine serum into rats. Interestingly, enhanced activin  $\beta$ A mRNA expression was observed in fibrotic livers as assessed by Northern blot analysis, and immunoreactive activin was detected in hepatocytes, especially around the fibrotic areas. Although the cellular source of activin in fibrotic livers remains controversial, these studies suggest a role of activin in the pathogenesis of liver fibrosis. *In vitro* studies demonstrated a stimulatory effect of activin A on the expression of collagen type I mRNA in both Ito/fat storing cells and fibroblasts which was further enhanced by

co-treatment of the cells with TGF- $\beta$ 1 (Sugiyama *et al.* 1998). Since both members of the TGF- $\beta$  superfamily are highly expressed in fibrotic livers, they might act synergistically to stimulate fibrotic processes in this organ. In addition, activin was shown to inhibit proliferation of hepatocytes and to induce apoptosis in these cells (Schwall *et al.* 1993, Yasuda *et al.* 1993), suggesting that activin contributes to the replacement of functional epithelial cells by mesenchymal cells.

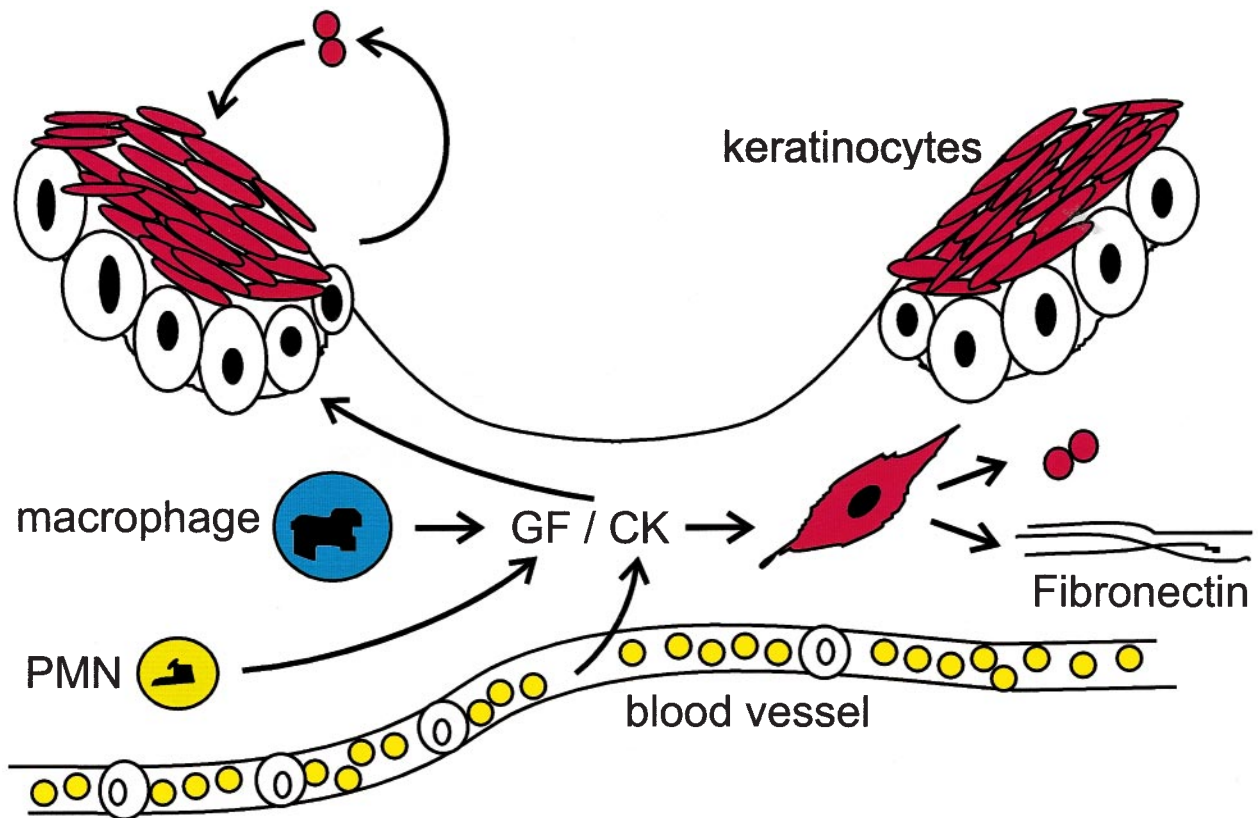
Increased expression of activin A was also detected in the fibrotic lung. In a first study, Matsuse *et al.* (1995) demonstrated elevated levels of activin A after bleomycin treatment of mice, whereby the upregulation of activin expression correlated with the appearance of fibrotic changes. Immunohistochemistry identified bronchiolar epithelial cells, smooth muscle cells and particularly alveolar macrophages as the cellular sources of activin. These findings were confirmed by the same group in the human system, where increased activin A expression was found in various types of pulmonary conditions associated with interstitial pulmonary fibrosis (Matsuse *et al.* 1996). Since activin A was shown to stimulate proliferation of lung fibroblasts and their differentiation into myofibroblasts (Ohga *et al.* 1996), activin might contribute to the structural remodeling of the lung tissue observed in pulmonary fibrosis.

### Activin Expression is Upregulated in the Injured Vessel Wall

A role of activin in the pathogenesis of arteriosclerosis was first suggested by Inoue *et al.* (1994). In this study, increased levels of activin were seen in arteriosclerotic lesions of Watanabe heritable hyperlipidemic rabbits. Subsequently, Pawlowski *et al.* (1997) demonstrated a striking upregulation of activin mRNA expression within a few hours after balloon injury of rat carotid arteries, and immunoreactive activin A protein was detected in the expanding neointima 7 and 14 days later. Thereby, both neointimal and medial smooth muscle cells were stained with the antibody. Since activin has been shown to stimulate proliferation of these cells *in vitro* (Kojima *et al.* 1993, Pawlowski *et al.* 1997), a role of this factor in the pathogenesis of arteriosclerosis seems likely. However, activin might also have a beneficial effect, since it has been shown to inhibit foam cell formation *in vitro* – a characteristic event in the early stage of arteriosclerosis (Kozaki *et al.* 1997). Thus the precise role of activin in physiological and pathological repair processes of the vessel wall has yet to be defined.

### A Possible Role of Activin in the Pathogenesis of Inflammatory Arthropathies

A role of activin in the pathogenesis of inflammatory arthropathies has been suggested by Yu *et al.* (1998). The

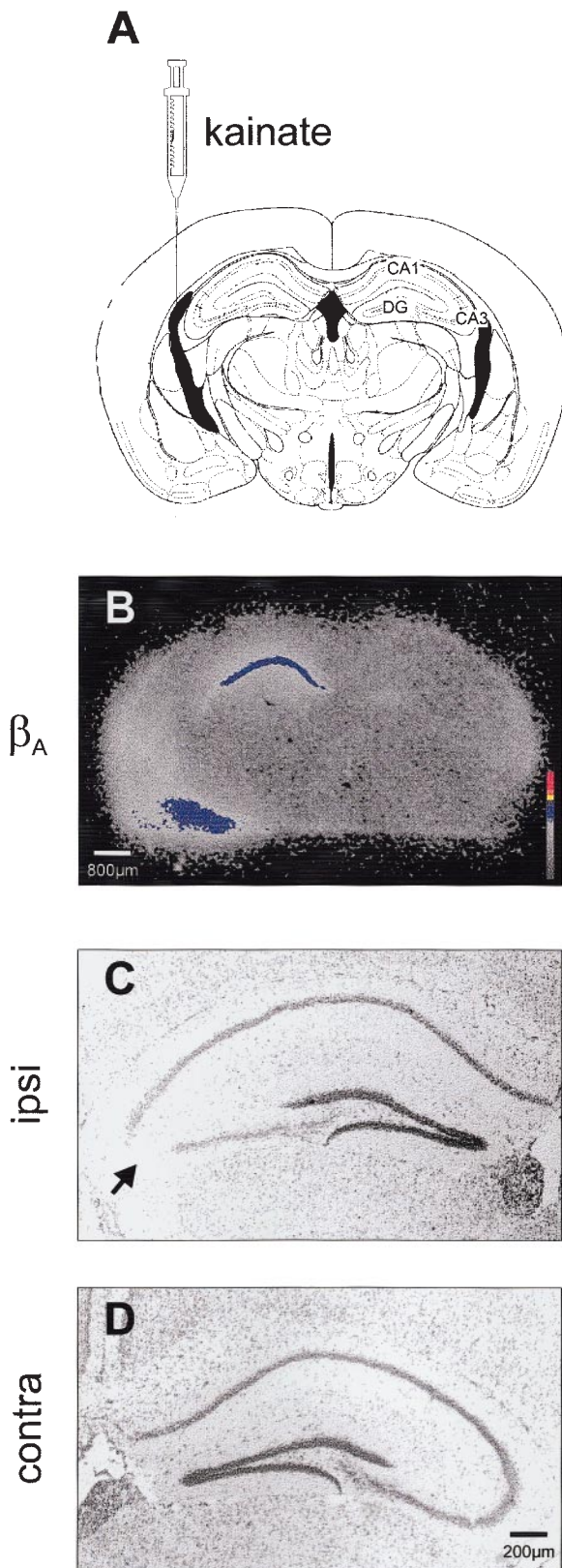


**Figure 2** Hypothetical model illustrating the regulation of activin expression during wound repair. A wound during the proliferative phase (3–5 days after injury) is shown schematically. The activin-producing cells (suprabasal keratinocytes of the epidermis as well as dermal fibroblasts) are shown in red. The activin protein is shown in red as a dimer. Growth factors (GF) and cytokines (CK) derived from the blood, from polymorphonuclear leukocytes (PMN), and from macrophages induce activin expression in fibroblasts and keratinocytes. In addition, activated macrophages themselves might contribute to the high activin levels in the wound. The most likely functions of activin in the wound are the induction of extracellular matrix production (especially of fibronectin) in the dermis and the regulation of keratinocyte differentiation.

authors demonstrated significantly elevated levels of activin A in the synovial fluid of patients with rheumatoid arthritis and gout relative to those with osteoarthritis. This finding is significant, in that rheumatoid arthritis and gout are both inflammatory diseases, while osteoarthritis is primarily a degenerative process. Similar to other tissues, expression of activin might be stimulated by pro-inflammatory cytokines, since activin A production by synoviocytes and chondrocytes in culture was stimulated by cytokines such as IL-1, TGF- $\beta$ , interferon- $\gamma$  and IL-8 (Yu *et al.* 1998). Interestingly, activin was shown to suppress major biological activities of IL-6 on various cell types, suggesting that activin could dampen inflammatory responses. This hypothesis was supported by a recent study where activin A was shown to inhibit the production of mature IL-1 $\beta$  in human monocytic cells (Ohguchi *et al.* 1998). Thus activin could have a novel anti-inflammatory activity.

### Activin and Brain Injury

Does activin also play a role in lesions of brain tissue, and if so, is its action beneficial to neuronal recovery? Two observations had been particularly stimulating for the experimental studies that addressed these questions and that we shall summarize here. First, activin A displays a neuroprotective profile *in vitro*, enhancing survival of cultured central nervous system neurons and offering protection against neurotoxic damage in the cultures (Kriegelstein *et al.* 1995, Iwahori *et al.* 1997). Secondly, activin A has been implicated in neuronal development, where it is especially important for neurotransmitter phenotype expression (Fann & Patterson 1994, Andreasson & Worley 1995, Darland *et al.* 1995). Since various developmentally relevant growth and differentiation factors are upregulated after lesions of adult brain tissue, we first wanted to determine whether this also holds for activin.



Using a well-established animal model of local excitotoxic brain damage, where intracerebroventricular injection of kainic acid (Fig. 3A) leads to neuronal death in the ipsilateral hippocampal CA3 region (Fig. 3C), we found indeed a striking induction of the activin  $\beta$ A subunit (Fig. 3B) (Tretter *et al.* 1996). RNase protection assays performed at different times post lesion served to determine the time window of enhanced  $\beta$ A mRNA expression. Whereas  $\beta$ A mRNA is virtually absent in control hippocampus of adult mouse, strong upregulation occurs within 6 h of injury. Expression levels then stay elevated for approximately 24 h post lesion before declining to baseline. Because the expression of  $\beta$ B and  $\alpha$  chains is not induced by the lesion, the  $\beta$ A transcripts most likely give rise to activin A, but not to other members of the activin/inhibin family.

One important question relates to the source of activin A. Our data suggest that activin A is of neuronal rather than of glial origin, because both  $\beta$ A mRNA and activin protein were detected almost exclusively in neurons adjacent to the site of the lesion (Tretter *et al.* 1996). Given that all known activin receptors are present in the hippocampus and that the endogenous activin inhibitor, follistatin, is expressed at very low levels in normal and lesioned hippocampus (Tretter *et al.* 1996), activin A is likely to be functionally active after a brain lesion. Recent studies employing other models of acute brain injury strongly favor the notion that enhanced activin A expression represents a common response to acute neuronal damage of various origins. Both hypoxic/ischemic injury and mechanical irritation produced patterns of activin A induction very similar to the one observed after excitotoxic lesion (Lai *et al.* 1996, 1997).

Although these studies identify activin A as a putative new player in the early neuronal response to brain injury, the functional implications of enhanced activin A expression are not known at present. In particular, it remains to be determined whether activin A affects solely neuronal survival and growth, or whether it also promotes glial scar formation, like TGF- $\beta$ <sub>1</sub> (Logan *et al.* 1994), thereby impeding neuronal regeneration. Further studies on the role of activin in experimental brain lesions should thus not only provide new insights into basic mechanisms of injury and repair, but might also open new venues for therapy.

**Figure 3** Activin  $\beta$ A induction 24 h after intracerebroventricular kainate injection. (A) Schematic coronal section through rat brain, showing site of injection (lateral ventricle, black-filled) and the hippocampal subfields (CA1, CA3, DG: dentate gyrus). (B) Pseudo-colored *in situ* hybridization revealing strong activin  $\beta$ A induction in the ipsilateral CA1 region of the hippocampus (additional signals are obtained in the amygdala, lower left). (C and D) Nissl-stained cryostat sections of hippocampus (same animal), depicting site of lesion in the ipsilateral hippocampus (in (C), arrow points towards lesioned CA3 region), whereas contralateral hippocampus remained unaffected (D).

## Summary and Conclusions

In summary, a series of studies suggests a novel role of activin in inflammation and repair processes of various tissues and organs. Although the precise function of activin in these processes is as yet unknown, *in vitro* studies suggest a role of this factor in the repair of the mesenchyme and possibly also the epithelium. Furthermore, activin could exert neuroprotective and anti-inflammatory activities. Thus upregulation of activin expression could be beneficial for the repair of damaged tissues. However, prolonged and/or significantly increased expression of this factor might also be involved in the development of fibrosis. Thus a tight control of the levels of activin is likely to be important for normal repair.

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