Regulatory Peptides, 44 (1993) 49–59

REGPEP 01273

The effects of transforming growth factor alpha and somatostatin on regenerating hepatocytes in the rat

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(Received 6 May 1992; revised version received 23 November 1992; accepted 24 November 1992)

Key words: Liver regeneration; Hepatocyte; Transforming growth factor alpha; Somatostatin; DNA synthesis

Summary

Transforming growth factor alpha (TGFα) stimulates DNA synthesis in adult rat hepatocytes, and plays a physiological role after partial hepatectomy by an autocrine mechanism. Somatostatin (SS-14) is a potent inhibitor of gastrointestinal function and inhibits proliferation in various cell types. We examined the proliferative effect of TGFα and the inhibitory effect of SS-14 on hepatocytes isolated at various times after partial hepatectomy. To study the mechanism of SS-14 further, we treated rats with the long acting SS-14 analog, octreotide, before or after 70% hepatectomy to determine whether or not a differential effect could be seen. We confirmed the proliferative effects of TGFα, and the inhibitory action of SS-14 in the early phase of liver regeneration in vitro. Regenerating hepatocytes isolated from hepatectomized livers respond to TGFα only at early time points (2 h) but do not respond to SS-14. In addition, the long acting SS-14 analog, octreotide, inhibited hepatic regeneration only when administered prior to hepatectomy. We conclude that exogenous peptide stimulation is effective only in the early phase of the hepatic proliferative response. After the initial changes brought about by hepatectomy, subsequent steps of the regenerative process appear refractory to external stimuli.

Introduction

The mechanisms responsible for liver regeneration after partial hepatectomy are still poorly characterized despite extensive study. The time course of liver

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regeneration after partial hepatectomy is well synchronized and permits a distinction between a prereplicative phase (G0-G1) and a replicative phase (S-M). Several peptides – or hepatotrophic factors – have been proposed to regulate liver regeneration [1]. Transforming growth factor alpha (TGFα), was originally detected in the culture media of retrovirally transformed cell lines [2]. TGFα is a potent growth factor and has been shown to stimulate DNA synthesis in cultured rat hepatocytes [3]. Increased levels of TGFα mRNA appear in hepatocytes during liver regeneration, suggesting an autocrine mechanism of action [3].

Somatostatin is known to inhibit cell growth of gastric and intestinal mucosa, the exocrine pancreas, lymphocytes, and the well-differentiated hepatoma cell line, Hep3B [4–7]. The inhibitory effects of somatostatin on DNA synthesis in cultured hepatocytes of rats stimulated by various factors, insulin, cyclic AMP (cAMP), hepatocyte growth factor (HGF), epidermal growth factor (EGF), and TGFα have also been reported [8–11].

There are few reports documenting the effect of various hepatotrophic factors in vivo or any correlation between data in cultured hepatocytes and in in vivo studies [12,13]. We investigated the time course of the proliferative effects of TGFα and the antiproliferative effects of SS-14 on normal or regenerating hepatocytes at various intervals after sham or 70% hepatectomy (HTX) in rats. These results were compared to the anti-proliferative action of the long acting analog of somatostatin, octreotide, in vivo by measuring [^{3}H]thymidine incorporation into DNA.

Materials and Methods

Animals and surgical procedures

Male Sprague-Dawley rats (200–275 g) were purchased from Charles River Laboratories (Wilmington, MA). Prior to surgery, the animals were kept in a temperature and light controlled room, and received food and water ad libitum. Rats were anesthetized with isoflurane (Forane, Anaquest, Madison, WI) and supplemental oxygen [14]. 70% hepatectomy (median and left lateral lobes) was performed after the method of Higgins and Anderson [15]. Postoperatively, no unplanned mortality was noted. Rats were allowed water ad libitum but were otherwise fasted until hepatocyte isolation or killing.

Chemicals

Collagenase (type I), *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes), TGFα and rat serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO). Somatostatin-14 (SS-14) was obtained from Peninsula Laboratories (Belmont, CA). Octreotide, a long-acting analogue of somatostatin, was obtained from Sandoz Pharmaceutical (East Hanover, NJ). All reagents used for polyacrylamide gel electrophoresis and immunoblot analysis were obtained from Bio-Rad (Richmond, CA). [^{3}H]Thymidine was obtained from Amersham (Arlington Heights, IL). Nuclear track emulsion (NTB 3) and all other autoradiographic chemicals were from Eastman Kodak (Rochester, NY).

Hepatocyte isolation

Hepatocyte cultures were prepared from the liver of normal rats subjected to either sham (0 h) or 70% hepatectomy. Hepatocytes were obtained during regeneration at 0, 2, 6, 24 or 72 h following partial hepatectomy, using a two step collagenase perfusion technique described previously [8–10,16]. Briefly, anesthetized rats were subjected to midline laparotomy and the liver was perfused via the portal vein for about 10 minutes at a flow rate of 10 ml/min with Ca^{2+}/Mg^{2+}-free Hank's buffer (Gibco, Grand Island, NY) containing 476.6 mg of Hepes at 37°C. The perfusate was then changed to Eagle's minimum essential medium (MEM, Gibco, Grand Island, NY) containing 0.05% collagenase (type I), for 10–12 min. The liver was then excised and placed in a sterile Petri dish containing fresh MEM with collagenase. The liver capsule was stripped, and cells were released by gentle manipulation. The resulting crude

preparation was filtered through four layers of cheesecloth and the collagenase was inactivated with 0.5 ml fetal calf serum (Gibco, Grand Island, NY)/10 ml cell suspension. After two low-speed centrifugal separations in fresh media to minimize contamination by nonparenchymal cells, cell viability was assessed by Trypan blue exclusion (greater than 90%). Hepatocytes were plated at a cell density of $1 \cdot 10^5$ per 16-mm Falcon Primaria tissue culture dish (Becton Dickson Co., Lincoln Park, NJ) in 1.0 ml of MEM, maintained at 37°C and examined under the microscope daily.

Immunoblot analysis

To ascertain that the inhibitory effects of SS-14 on thymidine incorporation into DNA were not due to toxicity, and to check the liver-specific functional capability of the cells, immunoblot analysis of the media from hepatocyte cultures for albumin was performed. Media (100 μl) from hepatocyte cultures after treatment with TGFα and/or SS-14 was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with purified goat anti-rat albumin (Organon Teknika Corp., Westchester, PA). Gels were electrophoretically transferred to nitrocellulose membranes, incubated with goat anti-rat albumin antibody at a 1:250 dilution [17], and then detected with an alkaline phosphatase reagent (Fig. 1).

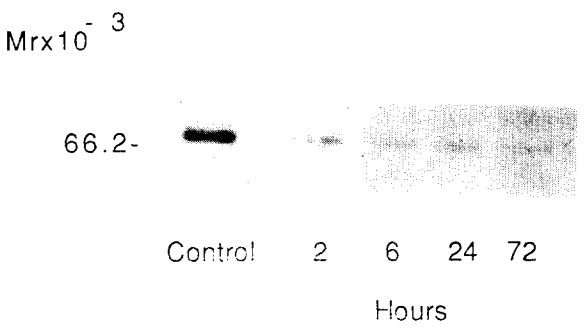

Fig. 1. Immunoblot analysis of albumin secreted into media by rat hepatocytes. Media (100 μl) from hepatocyte culture dishes treated with TGFα (50 ng/ml) plus SS-14 (10^{-8} mol/l) for 24 h were subjected to SDS-PAGE and immunoblot analysis using anti-rat albumin antibodies (Organon Teknika Corp., Westchester, PA).

Estimation of DNA synthesis

DNA synthesis was estimated by the rate of [^{3}H]thymidine incorporation into DNA and by autoradiographic measurement of the number of labeled nuclei [8–11]. Briefly, hepatocytes were plated for 24 h in MEM (serum free). After 24 h of attachment, fresh media was added containing the appropriate added reagents. To establish experimental conditions for a maximal mitotic effect of TGFα alone, preliminary experiments were carried out by incubating cells with TGFα for various time intervals. [^{3}H]Thymidine was added 6 h before harvesting in this preliminary experiment. Half of the harvested cells were processed for [^{3}H]thymidine counting, and the other half of the cells were used to determine DNA content. The former half was filtered on to glass microfiber filters (Whatman International, Maidstone, UK), rinsed with 1 ml of ice-cold 15% trichloroacetic acid (TCA) twice and with 1 ml of 70% ethanol once. Filters were dried and placed in 10 ml of scintillation cocktail (EcoLite, ICN Biochemicals Inc., Irvine, CA) and were counted in a scintillation counter (Beckman LS 6000LL, Beckman Instruments Inc., Fullerton, CA). The measurement of DNA was performed with a modified diphenylamine reaction for deoxypentose

TABLE I

Time course of [^{3}H]thymidine incorporation into hepatocytes after change to media containing TGFα (10 ng/ml)

Hours[a]	[^{3}H]Thymidine incorporation into DNA (cpm/μg DNA)
6	7679 ± 1778
12	36,083 ± 7170
18	8296 ± 3718
24	989 ± 140
30	782 ± 258

Mean ± S.E.M. ($n = 5$).

[a] Harvesting hours after change of media.

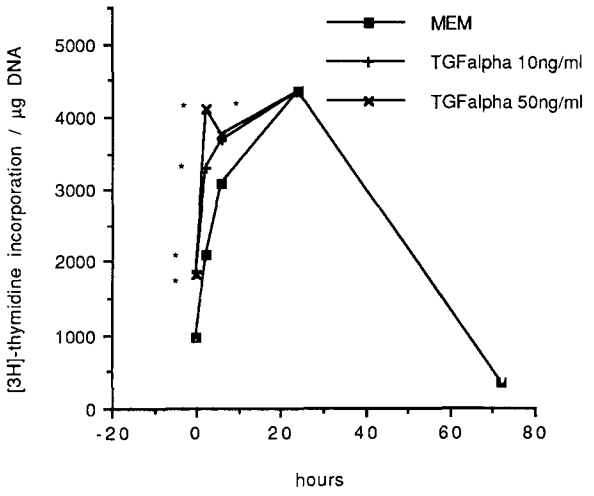

Fig. 2. [^{3}H]Thymidine incorporation into DNA of normal hepatocytes or regenerating hepatocytes obtained 2, 6, 24 and 72 h after partial hepatectomy. Freshly isolated hepatocytes were plated as described in text. After a 24 h attachment period, media was changed, and [^{3}H]thymidine incorporation into DNA was measured 18 h after exposure to [^{3}H]thymidine (3 μCi/well). The vertical bar indicates mean ± S.E.M. ($n = 6$–8, $^*P < 0.05$).

[18]. [^{3}H]Thymidine incorporation into DNA was expressed as counts per min (cpm)/μg DNA. [^{3}H]Thymidine incorporation into DNA stimulated by TGFα increased 6 h after addition of TGFα, reached maximum 12 h and ceased at 24 h (Table I). The results of this time course of DNA synthesis in culture lead us to use a period of 24 h to evaluate the effect of TGFα and SS-14.

For measurement of the rate of [^{3}H]thymidine incorporation into DNA, media was changed and appropriate reagents were added – MEM, TGFα (10 ng/ml or 50 ng/ml), TGFα (10 ng/ml or 50 ng/ml) plus SS-14 (10 nM), or SS-14 (10 nM) alone, were added after a 24 h attachment period. 6 h later [^{3}H]thymidine (3 μCi/well) was added. Cells were harvested 24 h after change of media. [^{3}H]Thymidine incorporation into DNA was measured as above.

In order to evaluate the proliferative effect of TGFα (10 ng/ml or 50 ng/ml) on hepatocytes during liver regeneration, [^{3}H]thymidine incorporation stimulated by TGFα was expressed as % matched control with media containing MEM only during the same isolation period. To evaluate the inhibitory effects of SS-14 on [^{3}H]thymidine incorporation stimulated by TGFα,% inhibition of [^{3}H]thymidine incorporation was calculated according to the formula below using matched TGFα or TGFα + SS-14:

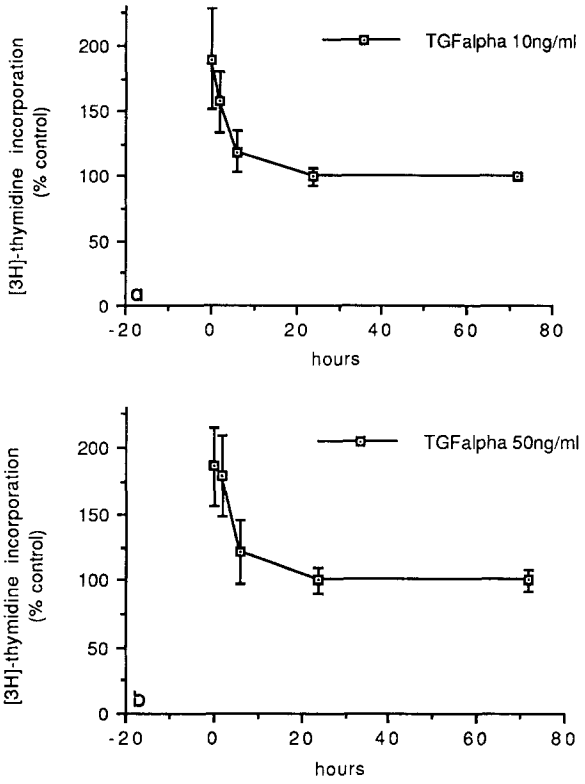

Fig. 3. (a,b) The hepatotrophic effects of TGFα on hepatocytes were expressed as % matched control (MEM only). TGFα (a: 10 ng/ml, or b: 50 ng/ml) stimulated [^{3}H]thymidine incorporation into DNA in hepatocytes obtained from sham operated rats and rats 2 h after hepatectomy. TGFα did not enhance growth in hepatocytes at 6 h or later after 70% hepatectomy. The ordinate is obtained by dividing [^{3}H]thymidine incorporation with that of matched controls x 100. The vertical bar indicates mean ± S.E.M. ($n = 6$–8, $^*P < 0.05$).

% Inhibition =
(([^{3}H]thymidine TGFα) – ([^{3}H]thymidine TGFα + SS-14))/
([^{3}H]thymidine TGFα)

An autoradiographic labelling index of the number of nuclei labeled with [^{3}H]thymidine was measured. Briefly, after 18 h exposure to [^{3}H]thymidine (3 μCi/well), hepatocytes were washed with phosphate-buffered saline and fixed in 4% paraformaldehyde for 10 min, coated with NTB-3 emulsion, and exposed for 14 days. After developing with Dektol for 2 min at 24°C, the percentage of morphologically identifiable hepatocytes with labelled nuclei was determined by phase contrast microscopy. The labeling index was expressed as the percentage of total nuclei labelled with [^{3}H]thymidine.

In vivo DNA analysis

To confirm the growth inhibitory effect of somatostatin in the early phase of liver regeneration, octreotide (30 nmol/kg), was injected subcutaneously 1 h before or after 70% hepatectomy. [^{3}H]Thymidine incorporation was compared for sham operated rats and hepatectomized rats with or without octreotide. Liver regeneration was evaluated by hepatic DNA synthesis at 24 h after hepatectomy using [^{3}H]thymidine incorporation into DNA (cpm/μg DNA) over a 1 h period before killing (24 h after hepatectomy) [12]. A 0.5 ml aliquot of the remnant liver homogenate was precipitated twice with ice-cold 10% TCA and once with 70% ethanol. The [^{3}H]thymidine radioactivity in the precipitate was the measured in a scintillation counter as described above. Another 0.1 ml aliquot was analyzed to measure DNA using the modified diphenylamine reaction for desoxypentose [18]. This [^{3}H]thymidine incorporation into DNA (cpm/μg DNA) was expressed as % of [^{3}H]thymidine incorporation into DNA of sham hepatectomy animal at each experiment.

Statistical analysis

All values shown represent the mean ± S.E.M. The Wilcoxon signed rank test or Student's *t*-test were used to compare group means. The level of significance was accepted at $P<0.05$.

Results

DNA synthesis in isolated hepatocytes at various intervals after hepatectomy

With MEM alone, [^{3}H]thymidine incorporation into DNA was 978 ± 139 cpm/μg DNA in normal hepatocytes, followed by a gradual increase up to 24 h after hepatectomy (Fig. 2). Hepatocytes isolated at 24 h after hepatectomy showed a more than 4-fold

TABLE II

Effect of TGFα and SS-14 on normal and regenerating hepatocytes

Reagents	Labeling index (%)				
	normal	2 h	6 h	24 h	72 h
MEM	33.4 ± 1.7	52.1 ± 1.2	52.5 ± 2.3	78.1 ± 2.2	8.8 ± 3.4
TGFα (10 ng/ml)	65.8 ± 3.2*	60.1 ± 1.7*	57.0 ± 2.1	75.3 ± 2.8	10.5 ± 0.6
+ SS-14 (10 nM)	29.3 ± 1.6#	62.5 ± 2.4	56.1 ± 2.6	75.5 ± 1.1	8.3 ± 3.1
TGFα (50 ng/ml)	75.1 ± 3.1*	63.3 ± 1.9*	60.5 ± 2.9	78.1 ± 2.5	9.1 ± 2.9
+ SS-14 (10 nM)	37.1 ± 5.9#	58.7 ± 3.4	56.2 ± 2.2	81.5 ± 1.9	11.7 ± 3.6
SS-14 (10 nM)	26.3 ± 2.1	51.8 ± 2.6	49.6 ± 2.1	81.3 ± 2.9	9.0 ± 3.2

The labeling index; the percentage of labeled nuclei with [^{3}H]thymidine was measured as described in Materials and Methods. Data was the mean ± S.E.M. for 6 to 12 determinations from two or three different experiments.

* $P<0.05$ versus MEM.

\# $P<0.05$ versus TGFα (10 ng/ml) or TGFα (50 ng/ml) stimulated labeling index.

increase in DNA synthesis (4341 ± 679 cpm/μg DNA), but at 72 h after hepatectomy, DNA synthesis was lower than in normal hepatocytes (344 ± 29 cpm/μg DNA).

Effect of TGFα on hepatocyte DNA synthesis at various intervals after hepatectomy

In hepatocytes from sham or regenerating liver, there were no differences in DNA synthesis between TGFα (10 ng/ml) and TGFα (50 ng/ml). In hepatocytes obtained from sham operated livers, TGFα (10 ng/ml or 50 ng/ml) stimulated [^{3}H]thymidine incorporation into DNA almost 2-fold (from 978 ± 139 cpm/μg DNA to 1857 ± 371, or 1818 ± 284 cpm/μg DNA). In regenerating hepatocytes, however, DNA synthesis was stimulated by TGFα only in hepatocytes isolated at 2 h after hepatectomy (from 2091 ± 426 cpm/μg DNA to 3291 ± 495, or 4095 ± 637 cpm/μg DNA). At 6 h after hepatectomy, hepatocyte DNA synthesis was stimulated by TGFα (10 ng/ml or 50 ng/ml) but was not statistically different from MEM alone (from 3086 ± 511 cpm/μg DNA to 3680 ± 494, or 3748 ± 755 cpm/μg DNA) (Fig. 2). In order to evaluate whether TGFα has any effect during liver regeneration, we compared [^{3}H]thymidine incorporation with matched controls containing MEM only. The stimulatory effect of TGFα decreased further during regeneration so that there were no effects 24 h after hepatectomy (Fig. 3a,b). In order to ascertain the observed results of limited effects of TGFα during liver regeneration, we performed autoradiographic analysis. Autoradiographic analysis of the number of nuclei labelled with [^{3}H]thymidine showed similar results (Table II).

Effects of SS-14 on DNA synthesis in TGFα stimulated hepatocytes

In sham and regenerating hepatocytes, there were no significant effects of SS-14 alone on DNA synthesis. The effects of SS-14 on DNA synthesis stimulated by TGFα (10 ng/ml) or TGFα (50 ng/ml) were the same (Fig. 4a,b). SS-14 inhibited [^{3}H]thymidine incorporation into DNA in normal hepatocytes stim-

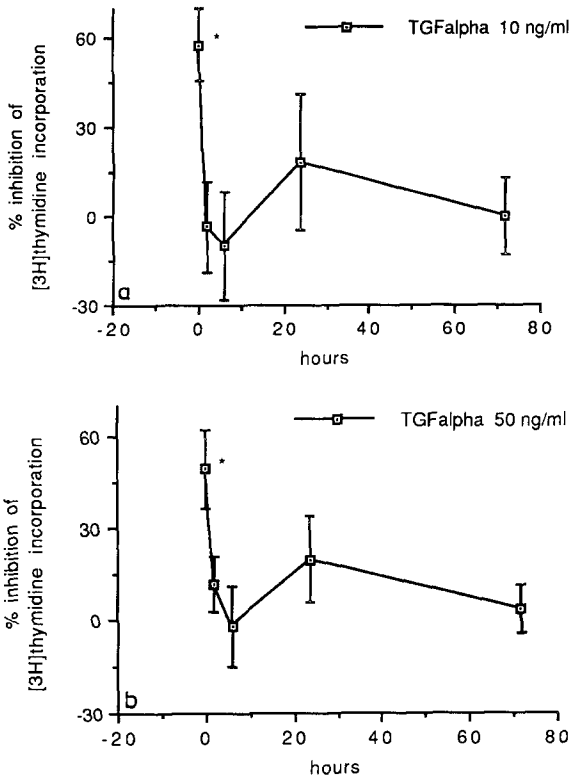

Fig. 4. (a,b) Inhibitory effects of SS-14 on hepatocytes stimulated by TGFα (a: 10 ng/ml, or b: 50 ng/ml) were expressed as % inhibition of [^{3}H]thymidine incorporation (see formula below) using matched TGFα alone and TGFα + SS-14 stimulated [^{3}H]thymidine incorporation; %inhibition = (([^{3}H]thymidine TGFα)−([^{3}H]thymidine TGFα + SS-14))/([^{3}H]thymidine TGFα). Vertical bar indicates mean ± S.E.M. (n = 6–8, *P < 0.05). For details, see Materials and Methods.

ulated by TGFα (from 1857 ± 371 or 1818 ± 284 cpm/μg DNA to 781 ± 221 or 918 ± 234 cpm/μg DNA respectively). % inhibition of SS-14 was 57.9% and 49.5%, respectively. In hepatocytes isolated at 2 h after hepatectomy, DNA synthesis was stimulated by TGFα, but [^{3}H]thymidine incorporation into DNA was not inhibited by SS-14 (from 3291 ± 495 or 4095 ± 637 cpm/μg DNA to 3404 ± 505 or 3608 ± 377 cpm/μg DNA respectively). After hepatectomy, SS-14 had no effect on hepatocyte proliferation in presence of TGFα (Fig. 4a,b). Autoradio-

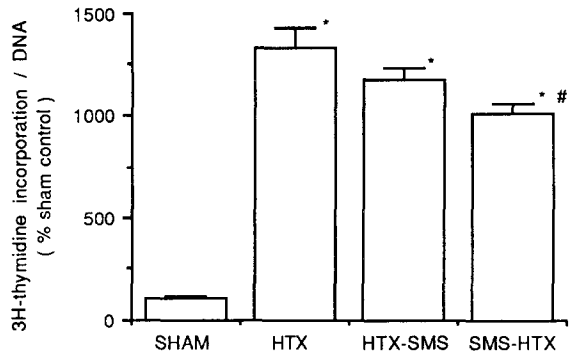

Fig. 5. [^{3}H]Thymidine incorporation into DNA 24 h after partial hepatectomy or sham operation in vivo: [^{3}H]thymidine incorporation into DNA was measured 24 h after partial hepatectomy as described in text and expressed % of [^{3}H]thymidine incorporation into DNA of sham hepatectomy animal (* = $P < 0.05$ vs. sham hepatectomized rats, # = $P < 0.05$ without octreotide or octreotide injected 1 h after hepatectomy).

graphic counts of the number of nuclei labelled with [^{3}H]thymidine again showed similar results (Table II).

Analysis of the effect of octreotide on liver regeneration in vivo

In hepatectomized rats, [^{3}H]thymidine incorporation into DNA at 24 h after hepatectomy was significantly increased from $98 \pm 5\%$ (sham operated rats, $n = 12$) to $1330 \pm 106\%$ (hepatectomy without octreotide, $n = 11$). Subcutaneous injection of octreotide 1 h after hepatectomy had limited effect on [^{3}H]thymidine incorporation ($1181 \pm 54\%$, $n = 12$). Octreotide treatment 1 h before hepatectomy showed a significant, but modest decrease in [^{3}H]thymidine incorporation ($1005 \pm 54\%$, $n = 12$, $P < 0.05$, Fig. 5).

Discussion

Liver regeneration is subject to tight regulation. In the rat model of 70% hepatectomy, there exists a prereplicative phase (G0-G1) of about 12 h, followed in 12 h by a replicative period (S-M). DNA synthesis reaches a maximum 24 h after hepatectomy and is followed by a wave of mitosis, the peak of which is about 8 h later. Regeneration ceases when the liver regains its original cell mass [19].

We have previously reported the effects of the hepatotrophic factors, insulin, cAMP, HGF, EGF and TGFα on cultured hepatocytes obtained from sham operated rats, and on the inhibition of hepatotrophic factor-mediated proliferation by somatostatin [8–11]. In the present study, we have shown attenuation in the hepatotrophic effects of TGFα, and also the lack of inhibitory effects of somatostatin on hepatocytes from liver subjected to hepatectomy. These effects are presumably due to specific stimulatory or inhibitory actions, as the cells in culture appear viable and healthy under the microscope, and continue to secrete albumin over the time course of the study. At longer times after plating in culture, hepatocytes are known to cease synthesis of messenger RNA coding for differentiated hepatocyte function, and are less useful markers [20]. We used a 24 h attachment period of cell culture, as in our hands hepatocytes of a 24 h attachment period to Falcon Primaria tissue culture dishes were more adherent than at 2 h and appeared healthier.

To confirm our visual observations, as a preliminary study to assess the effect of attachment period on DNA synthesis, we carried out thymidine incorporation experiments under 2 h and 24 h attachment period and compared the effect of TGFα. We did not find any difference between 2 and 24 h (TGFα 10 mg/ml $195 \pm 44\%$; $190 \pm 16\%$, TGFα 50 mg/ml $207 \pm 46\%$; $186 \pm 29\%$ of media control).

To study the effects of an individual mitogen on DNA synthesis, we prepared TGFα in minimal media. This gave us less stimulation than that observed in other studies but demonstrated a pure effect of TGFα, free from serum or other culture conditions. When studied in vitro with more permissive media, TGFα mRNA increased, suggesting that the predominant mode of action is autocrine [3]. Two doses of TGFα were chosen for these studies are based on our previous study [11] and the literature

[3], and are somewhat arbitrary. Given the postulated autocrine mechanism of action of endogenous TGFα, the question as to whether the chosen doses constitute physiological levels is not answered with these experiments. Despite this potential drawback, TGFα in the doses chosen stimulated increases in [^{3}H]thymidine incorporation in a quantifiable way, and clear differences in hepatocytes isolated at early times after hepatectomy are demonstrated when compared to later time points. Analysis of Fig. 2 shows that hepatocytes isolated at progressively increasing times after hepatectomy are able to incorporate [^{3}H]thymidine at higher rates than cells obtained from livers immediately after hepatectomy. The addition of TGFα converts the [^{3}H]thymidine incorporation curve from a peak at 24 h, as seen with media treated cells, to a plateau. This pattern suggests that the early addition of TGFα can enhance the proliferative process by promoting the rate of DNA synthesis, rather than causing an additional incremental increase in the maximal level of incorporation.

The use of individual growth factors, such as TGFα, to study the mechanism(s) of hepatic regeneration have advantages and drawbacks. Although the data clearly show a decrease in the time to maximum [^{3}H]thymidine incorporation, and reflect a 'pure' response to TGFα, the level of incorporation is less than that seen in hepatocytes cultured with other growth factors added [3]. TGFα might be able to induce greater DNA synthesis under less stringent culture conditions. Although several different signals must act in an appropriate sequence to initiate a proliferative response, studies such as the ones described here are designed to analyze the effect of individual agents, and are also important in dissecting out the role of putative hepatotrophic factors.

A second important consideration is the effect of starvation on hepatic regeneration. A great deal of data exist to document the profound effect of adequate nutrition on hepatocyte proliferation [21,22]. The insensitivity of hepatocytes to TGFα at 72 h may reflect a lack of adequate nutrition as a source for energy or essential cofactors for cell growth. The studies reported here do not allow a separation of the effects of TGFα from those of nutritional privation, and warrant experiments designed to assess, among other conditions, the effects of pair feeding, sham operation and dietary composition (high fat, protein or carbohydrate).

One potential mechanism for the observed effects of TGFα seen here may be the ability of exogenous TGFα to move cells more rapidly through the G1 phase of the cell cycle, and into the S, or DNA synthetic phase. To further study the differential effects of TGFα on regenerating hepatocytes, the change in [^{3}H]thymidine incorporation was expressed as a percentage of media treated controls (Fig. 3a,b). Regardless of whether 10 ng/ml or 50 ng/ml were used, significant increases in [^{3}H]thymidine incorporation were seen only in hepatocytes isolated at 0 or 2 h after hepatectomy. It is important to note that the return to baseline seen in Fig. 3a and b is due predominantly to the increase in [^{3}H]thymidine incorporation seen in the media treated controls, as documented in Fig. 2.

The biologically active form of TGFα is a 50 amino acid peptide, and shares a 30–40% structural homology with EGF. TGFα and EGF bind to the same receptor in the plasma membrane [23]. TGFα/EGF receptor number decreases during liver regeneration by 25, 40 and 55% at 12, 24 and 72 h, respectively [24,25]. TGFα messenger RNA increases after partial hepatectomy and reaches a peak corresponding to the time of maximal DNA synthesis. TGFα is considered to be one of the physiological effectors of hepatic regeneration as a promoter, not an initiator of liver regeneration, and which has an effect only on hepatocytes in the G1 phase of the cell cycle [19]. TGFα may also act on hepatocytes in transition from G0 to G1, and which subsequently enter the cell cycle [26,27].

Once the effect of TGFα was documented, experiments were undertaken to determine whether or not somatostatin could inhibit cell proliferation. Fig. 4a,b

represents the effect of SS-14 (10 nM) on hepatocytes isolated at increasing times after hepatectomy. Inhibition could only be seen in hepatocytes isolated after sham operation. Octreotide, the long acting somatostatin analogue, has been shown to reach a maximum circulating blood level around 1 h after subcutaneous injection [28]. Octreotide inhibited DNA synthesis only when injected prior to hepatectomy (Fig. 5). The unresponsiveness to octreotide during the early phase of liver regeneration is compatible with results reported in regenerating hepatocytes [29].

Elevation of cAMP is one of the early events in liver regeneration [30,31]. We have shown that somatostatin inhibits normal hepatocyte proliferation stimulated by insulin via cAMP dependent pathways and by HGF, EGF and TGFα via cAMP-independent pathways [8–11]. Somatostatin does not inhibit DNA synthesis generated by non-mitogen induced growth stimulation, and may not inhibit DNA synthesis generated by other mitogens not studied here. Initial stimulatory events in liver regeneration might be multiple and some of these factors may not be affected by somatostatin. In the present study, SS-14 had an inhibitory effect on DNA synthesis of regenerating hepatocytes isolated 2 h after hepatectomy, and stimulated by TGFα. In hepatocytes isolated at times greater than 2 h after hepatectomy, TGFα promoted DNA synthesis but SS-14 could not inhibit this stimulation. These disparate effects may be explained by a decrease in number of SS-14 membrane receptors (which goes down least 2 h after hepatectomy [11]), or because SS-14 inhibits only some signals regulating G0-G1 transition.

To confirm the [^{3}H]thymidine incorporation data, autoradiographic analysis of the proportion of labelled nuclei, or nuclear labelling index, was calculated. Again, TGFα promoted DNA synthesis only in hepatocytes isolated from rats subjected to sham hepatectomy or 2 h after hepatectomy (Table II). In this study, the hepatotrophic effects of TGFα, as measured by thymidine incorporation have been shown to decrease after hepatectomy. One possible mechanism for the decrease in TGFα activity is endogenous TGFα. Endogenous TGFα may maximally stimulate proliferation in regenerating hepatocytes by an autocrine mechanism. Increases in TGFα mRNA reach maximum value 24 h after partial hepatectomy in vivo [3]. Under the conditions imposed in our experiments, endogenous TGFα secretion might occur prior to the addition of exogenous TGFα in regenerating hepatocytes. TGFα mRNA in cultured hepatocytes has not been reported, nor has TGFα been reported to be secreted into culture media in regenerating hepatocytes. Among the possible mechanisms for decreased TGFα activity is either a decrease in the number or affinity of the TGFα receptor. A third possibility is inactivation of postreceptor pathways regulating signal transduction.

Continuous infusion of somatostatin, or injection of the somatostatin analog, octreotide, have been reported to inhibit liver regeneration in vivo [33,34]. cAMP in regenerating liver begins to rise around 2 h after hepatectomy [30]. The studies reported here suggest that octreotide inhibits DNA synthesis when injected 1 h before but not 1 h after 70% hepatectomy. This result is compatible with the absence of an effect of SS-14 on isolated regenerating hepatocytes and suggests that octreotide has an inhibitory effect in the immediate early period. This lack of effect may be explained by a decrease in the number or affinity of somatostatin membrane receptors [11], or because somatostatin inhibits only some signals regulating the G0-G1 transition.

In conclusion, TGFα stimulates DNA synthesis in normal hepatocytes and in regenerating hepatocytes early after hepatectomy. Somatostatin has been shown to inhibit TGFα a stimulated normal hepatocyte proliferation and in vivo DNA synthesis only when administered prior to hepatectomy. Although the physiological relevance of these findings has yet to be elucidated, the combination of in vitro and in vivo studies, as reported here, provide useful models for examining the phenomenon of hepatic growth.

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