A growth factor for hepatocytes is inactivated by sub-lethal γ-irradiation in vivo or in vitro

George G. Skouteris, Margery G. Ord and Lloyd A. Stocken

Department of Biochemistry, South Parks Road, Oxford OX1 3QU, England

Received 11 January 1989

Serum from partially hepatectomised rats promoted DNA synthesis in primary adult rat hepatocyte cultures. If the rats had been exposed to sub-lethal γ-irradiation immediately following operation or if their serum, collected at 3 h, was exposed to irradiation in vitro, the growth-promoting activity was destroyed. Prostaglandin E$_2$ also stimulated DNA synthesis in the cultures; if PGE$_2$ was irradiated in serum from intact or partially hepatectomised rats its growth-promoting activity was markedly diminished.

Liver growth factor; Prostaglandin E$_2$; Liver regeneration; Ionizing irradiation

1. INTRODUCTION

Primary adult rat hepatocyte cultures [1] can be induced to grow by various factors including EGF, serum from partially hepatectomised rats and eicosanoids [1–5]. When liver growth is promoted in vivo by partial hepatectomy [6], regeneration is delayed if rats are exposed to ionizing irradiation immediately before or up to 3 h after operation [7,8]. The atypical sensitivity of liver in G$_0$ phase to ionizing irradiation [7,8] is unexplained; many of the biochemical events associated with hepatocyte transition from G$_1$ to S phase, such as the induction of thymidine kinase, are affected. Recent experiments [8] indicated that levels of cyclic AMP, which are markedly elevated 4–6 h after partial hepatectomy [9] are lower if the animals had received sub-lethal γ-irradiation 0–2 h after operation. Adenylate cyclase activity in plasma membranes isolated from the livers of the partially hepatectomised, irradiated rats, was low but responded normally to stimulation by β-agonists, suggesting ionizing irradiation might be affecting the signal for regeneration, rather than the response of the hepatocytes to the signal. The growth-promoting effects of serum collected from partially hepatectomised rats 3 h after operation were therefore compared with those of serum from rats which had been exposed to sub-lethal γ-irradiation.

2. MATERIALS AND METHODS

2.1. Hepatocyte cultures

Hepatocytes from adult male Wistar rats (130–150 g) were prepared by the two-step collagenase method [10]. Viability of the cells was checked by trypan blue exclusion and was always greater than 85%. The hepatocytes were plated in triplicate at 5 × 10$^5$ cells/2 ml on 60 mm collagen-coated dishes [1] in Dulbecco's modified Eagle's medium (MEM), arginine-free, supplemented with 10% MEM essential amino acid solution, 10% MEM non-essential amino acid solution, 1.63 μM glutathione, 0.5 mM L-ornithine, 10 mM Na-pyruvate and 50 μg·ml$^{-1}$ gentamycin. All culture media contained insulin (0.5 μg·ml$^{-1}$). The cells were allowed to attach for 2 h at 37°C in humidified 5% CO$_2$/95% air; the medium was changed and the growth factors were added. Culture medium was changed at 24 h and fresh additions were made. Appropriate assays were made on each dish.

2.2. Serum collection

Serum was collected under ether anaesthesia from sham-operated or partially hepatectomised [6] rats 3 h after operation [5]. Where indicated the animals were exposed to 4 Gy $^{60}$Co γ-
Fig. 1. The effect of 4 Gy γ-irradiation in vivo on the growth-promoting properties of rat serum on adult liver hepatocyte cultures. Hepatocytes were plated at $5 \times 10^6$ cells/dish. Culture medium was changed at 24 h and fresh additions made. Serum (10% v/v) was obtained and incorporation into DNA measured over 2 h periods as in section 2. [3H]Tdr uptake into DNA is expressed as cpm pg DNA$^{-1} \pm$ SEM. (Δ--Δ) Serum collected 3 h after partial hepatectomy; (Δ--Δ) serum collected 3 h after partial hepatectomy, from rats given 4 Gy in vivo; (○--○) serum from sham-operated animals; (●--●) serum from sham-operated animals, from rats given 4 Gy in vivo; (●--●) control cultures (insulin only).

irradiation (dose rate, 4 Gy/5 min) immediately after operation. Serum was stored for up to 10 days at $-15^\circ$C. Serum was irradiated in vitro in air at $0^\circ$C in closed, plastic tubes (dose 4 Gy in 5 min).

2.3. [3H]TdrR incorporation into DNA

Cultures were incubated with 4 μCi [3H]methylthymidine (spec. act. 92 Ci·mmol$^{-1}$, Amersham International) for 2 h periods. At the end of the labelling period the cells were washed 3 times with ice-cold phosphate-buffered saline and frozen in liquid N$_2$ until radioactivity in the DNA was determined [1].

Protein was estimated by the method of Lowry et al. [11]; DNA was determined fluorimetrically [12]. Calf thymus DNA was used as a standard.

3. RESULTS AND DISCUSSION

Serum taken at 3 h from animals which were exposed to 4 Gy immediately after laparotomy was indistinguishable from that obtained from non-exposed animals in its growth promotion of the hepatocytes (fig.1). Serum obtained from rats 3 h

| Table 1 |
|-----------------|-----------------|-----------------|
| **Prostaglandin release into medium** | **Prostaglandin release into medium** |
| Time in culture (h): | 6 | 0 | 1 | 6 | 24 | 0 | 1 | 6 | 24 |
| cAMP levels | PGE$_2$ | PGF$_{2\alpha}$ | PGE$_2$ | PGF$_{2\alpha}$ |
| Serum from: | | | | | | | | | |
| Sham-operated rats | | | | | | | | | |
| Collected 3 h after partial hepatectomy | 27 ± 0.66 | 9 ± 0.5 | 90 ± 2.5 | 165 ± 5 | 140 ± 3.5 | 14 ± 1 | 230 ± 5 | 215 ± 2.5 | 187 ± 5 |
| Collected 3 h after partial hepatectomy + 4 Gy | 44 ± 0.80 | 29 ± 0.5 | 104 ± 5 | 237 ± 7.5 | 208 ± 5 | 39 ± 1 | 310 ± 5 | 332 ± 5 | 195 ± 2.5 |
| Cultures were set up with $5 \times 10^6$ cells/dish. Serum was collected from sham-operated or partially-hepatectomised rats 3 h after operation. Where indicated, the animals were exposed to 4 Gy $^{60}$Co γ-irradiation immediately after operation. The serum was present at 10% (v/v). Cyclic AMP levels [16] are expressed as pmol·mg protein$^{-1} \pm$ SEM. PGE$_2$ and PGF$_{2\alpha}$ in the culture medium were determined by radioimmune assay [17] and are given as pg·ml$^{-1}$. |
after partial hepatectomy enhanced DNA synthesis especially by 60 h (fig.1). If the partially hepatectomised rats had been exposed to 4 Gy immediately after operation, the enhanced growth-promoting activity of the serum was lost. Irradiating the hepatocyte cultures or using hepatocytes obtained from irradiated rats did not affect their response to serum or EGF (not shown).

Other growth-associated parameters were examined. Cyclic AMP levels in the cells supplemented with serum from irradiated, partially hepatectomised rats were diminished (table 1) and prostaglandin release into the culture medium [5] after 1, 6 and 24 h was also lowered (table 1).

Whilst the decreased cyclic AMP levels and lowered DNA synthesis found with the hepatocyte cultures resembled the results following irradiation of partially hepatectomised rats, effects of total body exposure are complex, so that the changes in growth promoting activity of the serum are difficult to interpret. Serum from sham-operated animals or which had been collected from rats 3 h after partial hepatectomy was therefore irradiated with 4 Gy in vitro. No effect was observed in the growth-promoting activity of serum from the laparotomised animals (table 2B) but the expected stimulation produced by serum from the partially hepatectomised rats was diminished (table 2D).

The period of sensitivity of liver regeneration to inhibition by the cyclooxygenase inhibitor indomethacin [13], coincides with that found for the sensitivity of partially hepatectomised rats to sub-lethal irradiation in vivo (-0.5 to +3 h after operation) [8]. Eicosanoids stimulate DNA synthesis in hepatocyte cultures [4,5]; the growth-promoting effects of PGE2 were therefore tested in combinations with serum and irradiation.

PGE2 is available commercially in solutions sterilised by irradiation; predictably therefore its effects on hepatocyte growth were not diminished by exposure to 4 Gy (table 2A). In other experiments where PGE2 or serum from partially hepatectomised rats was present in the culture medium for only 2-6 h after attachment, both PGE2 and serum caused DNA contents of the hepatocytes to be doubled by 67 h [5]; lower uptake of [3H]Tdr into DNA was observed if the growth factors were continuously present. In the experiments presented here, where the growth factors were present continuously, the increments in [3H]Tdr incorporation into DNA when PGE2 and serum from laparotomised rats were present together, over that caused by insulin alone (fig.1), were approximately the sums of those found when the growth factors were present separately (table 2A,B,C). A similar result was obtained when serum from partially hepatectomised rats was used, but even in the presence of added PGE2 [3H]Tdr uptake into DNA was lower if serum from the partially hepatectomised rats had been exposed to 4 Gy in vitro (table 2E). When, however, PGE2 was added to either serum and the mixture irradiated, growth-
promoting activities were greatly diminished (table 2F,G).

About 80% of PGE₂ in serum is bound to protein in an alkali-labile linkage which has to be dissociated before radioimmune assay [14,15]. PGE₂ levels in serum from irradiated partially hepatectomised rats were not different from those in the unirradiated groups (table 1 and [7]) indicating that the alkali-labile linkage was not irreversibly affected by exposure to γ-rays. However, as the stimulatory activity of (serum + PGE₂) is so markedly lowered when the two are exposed together, irradiation may alter a protein/PGE₂ complex thus diminishing its growth-promoting potential.

The relative concentrations and potencies of different growth-promoting factors in serum from partially hepatectomised rats are unknown. The similarity in behaviour of serum + PGE₂, when irradiated together, to that observed after irradiation in vivo or after exposing serum from partially hepatectomised animals suggests that prostaglandins are involved in the promotion of liver growth.

Acknowledgements: G.G.S. was a research fellow of the Commission of the European Communities, Dir. Gen. XII for Science, Research and Development, Brussels, Belgium. We are very grateful to Dr G.Z. Panos for performing the radioimmune assays for prostaglandins. The PGE₂ was in part a generous gift from Dr J.E. Pike, Upjohn Co., Kalamazoo, Michigan, USA.

REFERENCES