Histol Histopathol (2005) 20: 843-850

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**Histology and Histopathology**

*Cellular and Molecular Biology*

# Decreased density of ß$_1$-adrenergic receptors in preneoplastic and neoplastic liver lesions of F344 rats

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**Summary.** There is some evidence that rodent hepatocarcinogenesis is accompanied by changes in the adrenergic responsiveness of liver cells to catecholamines. In this study, immunohistochemical expression of ß$_1$-adrenergic receptors (ß$_1$-ARs) has been examined in spontaneous and chemically induced preneoplastic and neoplastic liver lesions of female and male Fischer 344 rats. An antibody specific for ß$_1$-AR subtype was used. The study was carried out on archival formalin-fixed and paraffin-embedded livers from rats used in a previous study of hepatocarcinogenesis. One control group given distilled water by gavage, and two experimental groups, one initiated with a single dose of diethylnitrosamine (DEN) and one initiated with DEN and continuously treated with phenobarbital (PB) were examined. Rats were sacrificed after 2, 4, 8 and 21 months of experimentation. All types of liver putative preneoplastic lesions examined (basophilic, glycogen-retaining, or mixed cell foci) show a lower density of ß$_1$-ARs than the surrounding normal liver parenchyma, either in control and in DEN-treated or DEN+PB-treated rats. No immunostaining is detectable in several altered cell foci. Hepatocellular adenomas and hepatocellular carcinomas also show a very low density of ß$_1$-ARs, extensive areas completely devoid of ß$_1$-ARs being mingled with areas showing a weak immunostaining.

**Key words:** ß$_1$-adrenoreceptor, Immunohistochemistry, Rat liver, Preneoplastic lesion, Neoplastic lesion

## Introduction

A large body of evidence indicates that catecholamines are involved in the control of liver growth. Their effects are mediated by α- and ß-adrenergic receptors (α- and ß-ARs), whose expression and responsiveness undergo changes in various physiological and pathological states (Lefkowitz et al., 1984; Michalopoulos, 1990; Refsnes et al., 1992; Kajiyama and Ui, 1998).

Rodent hepatocarcinogenesis has been shown to be accompanied by either quantitative or qualitative changes in the α- and ß-AR subtype populations. Treatment of rats with certain chemical carcinogens has been reported to be associated with a transient marked increase in adrenergic responsiveness of the liver cell adenylate cyclase (Christoffersen et al., 1972; Christoffersen and Berg, 1975; Boyd and Martin, 1976; Refsnes et al., 1986). This increased adrenergic responsiveness, that has been attributed to an increase in the density of ß-ARs on the hepatocyte membrane (Refsnes et al., 1986), is no longer demonstrable after the appearance of the liver preneoplastic or neoplastic lesions both in rats given 2-acetylaminofluorene (2-AAF) (Christoffersen et al., 1972) and in rats given 3'-methyl-4-dimethylaminoazobenzene (Boyd and Martin, 1976), despite continuous administration of the carcinogen. Hepatocellular carcinomas from rats given 2-AAF (Christoffersen et al., 1972) and rat transplanted hepatoma cells show either a comparable or a lower responsiveness to epinephrine or to the ß-adrenergic agonist, isoproterenol, than normal liver cells (Emmelot and Bos, 1971; Lacombe et al., 1976; Okamura and Terayama, 1976; Matsunaga et al., 1984; Miyamoto et al., 1985, 1989; Garcia-Sainz et al., 1989; Sanae et al., 1989).

Recently, we have examined the distribution of ß$_1$-adrenergic receptors in the rodent liver by an immunohistochemical method, using an antibody specific for the ß$_1$-AR subtype (Cardani and Zavanella, 2001). In this study, which was carried out on archival livers from untreated Fischer 344 rats used as controls in a previous study of hepatocarcinogenesis (Zavanella et al., 1994), a clear positive reaction was found in the hepatocytes of both female and male rats from different age groups. Since spontaneous putative preneoplastic lesions, represented by basophilic foci or glycogen-retaining foci, are common in the livers of senescent F344 rats (Eustis et al., 1990), we had the chance to observe that the density of ß$_1$-ARs is much lower in the preneoplastic lesions than in the surrounding normal

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parenchyma (Cardani and Zavanella, 2001). Thus, it seemed of interest to examine the ß$_1$-AR expression in chemically induced liver tumors, using the animals employed in the previous study of hepatocarcinogenesis (Zavanella et al., 1994).

We report here our observations on the distribution of ß$_1$-ARs in both spontaneous and diethylnitrosamine (DEN)-induced preneoplastic or neoplastic liver lesions of F344 rat.

## Materials and methods

### *Experimental design*

Female and male F344 rats from 3 groups of animals used in a previous initiation-promotion experiment of hepatocarcinogenesis (Zavanella et al., 1994) were examined. Diethylnitrosamine (DEN) was used as a tumor initiating agent (Scherer and Emmelot, 1975) and phenobarbital (PB) as a promoter of hepatocarcinogenesis (Peraino et al., 1973; Pitot et al., 1978). Briefly, at six weeks of age two groups of rats, D2 and D4, were initiated with a single i.p. injection of 200 mg/kg diethylnitrosamine. The promoting regimen was started 17 days after DEN administration. Rats in group D4 were continuously treated with phenobarbital (PB), added to the basal diet at 0.05%, 6 days per week. Since the compounds tested in the study of hepatocarcinogenesis had been administered 6 days per week by gavage, the control group (D1) as well as D2 and D4 groups were given distilled water by gavage (10 ml/kg) 6 days per week. Four animals from each group were sacrificed after 2, 4 or 8 months of PB treatment and the surviving animals (3-8 per group) were sacrificed after 21 months. One week after the beginning of the promoting regimen, the animals to be killed after 2 months were given a single hepatotoxic dose of $CCl_4$ (2 ml/kg, by gavage), as a stimulus for liver cell proliferation (Columbano et al., 1981). Animals were fasted for 24 h before killing for visualization of liver glycogen-retaining foci. Animals were taken off PB and distilled water treatment for 1 week before they were killed. All necropsies were performed in the morning. Standard liver samples from the right anterior and the left lobes were fixed in 10% neutral buffered formalin and embedded in paraffin. Median lobes were also examined in a few rats killed after 21 months.

### *Immunohistochemistry*

Serial sections of 4 µm were incubated with a rabbit polyclonal antibody specific for the carboxy terminus of ß$_1$-ARs (Santa Cruz, Biotechnology, Inc.) following antigen retrieval in a microwave oven (Cattoretti et al., 1993; Shi et al., 1995). The peroxidase-conjugated EnVisionTM + Single Reagent (DAKO) was used as a visualization system, following the instructions of the manufacturer. The chromagen was 3,3'-diaminobenzidine tetrahydrochloride (Sigma Aldrich, Milan, Italy). Slides were then counterstained with hematoxylin. As negative controls, adjacent sections were incubated 1, without primary antibody, 2, with rabbit normal serum and 3, with primary antibody preadsorbed with ß$_1$-AR blocking peptide (Santa Cruz Biotechnology). Primary antibodies were also preincubated with ß$_2$- and ß$_3$-AR peptides used to obtain antibodies specific for the C-terminus of ß$_2$- or ß$_3$-ARs (Santa Cruz Biotechnology). Rat placenta was used as positive tissue control. Further details are given in Cardani and Zavanella (2001).

Altered cell foci and neoplastic lesions were examined in ß$_1$-AR, hematoxylin-eosin and hematoxylin-periodic acid-Schiff-stained adjacent sections. The nomenclature used for liver lesions is based on the classification reported by Eustis et al. (1990).

Putative preneoplastic lesions induced by DEN are represented by glycogen-retaining, basophilic and several types of enzyme-altered foci, whose potential to attain the malignant state is generally enhanced by PB (Scherer and Emmelot, 1975; Pitot et al., 1978; Schulte-Hermann et al., 1981; Pereira, 1982; Goldsworthy et al., 1984; Estadella et al., 1984; Barbason et al., 1985; Sato et al., 1984). The frequency and the time of onset of preneoplastic and neoplastic lesions depend on experimental conditions. F344 rats initiated with a single i.p. injection of 200 mg/kg of DEN and subjected to partial hepatectomy develop putative liver preneoplastic lesions after 4 weeks (Solt et al., 1977; Tatematsu et al., 1979; Ogawa et al., 1980), neoplastic nodules after 35 weeks, and hepatocellular carcinomas after 50 weeks (Tatematsu et al.,1988). In DEN-initiated and PB-promoted rats (PB 0.05% in the basal diet) neoplastic nodules are demonstrable after 20 weeks and the incidence of hepatocellular carcinomas is higher than in rats given DEN alone after a time interval of 50 weeks (Tatematsu et al., 1988).

## Results

The distribution of ß$_1$-ARs in the livers of control rats from group D1, given distilled water by gavage, was similar to that previously observed in the livers of untreated rats (Cardani and Zavanella, 2001). In the normal liver parenchyma there were no appreciable differences in ß$_1$-AR distribution between the livers of control rats and the livers of DEN or DEN+PB treated rats. Within the liver lobule a clear zonation is observed, with the ß$_1$-AR positivity most evident in pericentral zone hepatocytes and a gradual fading of the immunostaining from pericentral to periportal zone hepatocytes (Fig. 1A,B). No positive reaction is found in liver sections incubated without primary antibody, or with rabbit non-immune serum, or with anti-ß$_1$-AR preadsorbed with ß$_1$-AR blocking peptide. ß$_1$-AR immunoreactivity is still present in sections incubated with anti-ß$_1$-AR preadsorbed with ß$_2$- or ß$_3$-AR blocking peptide.

In non-initiated rats (group D1) small basophilic or

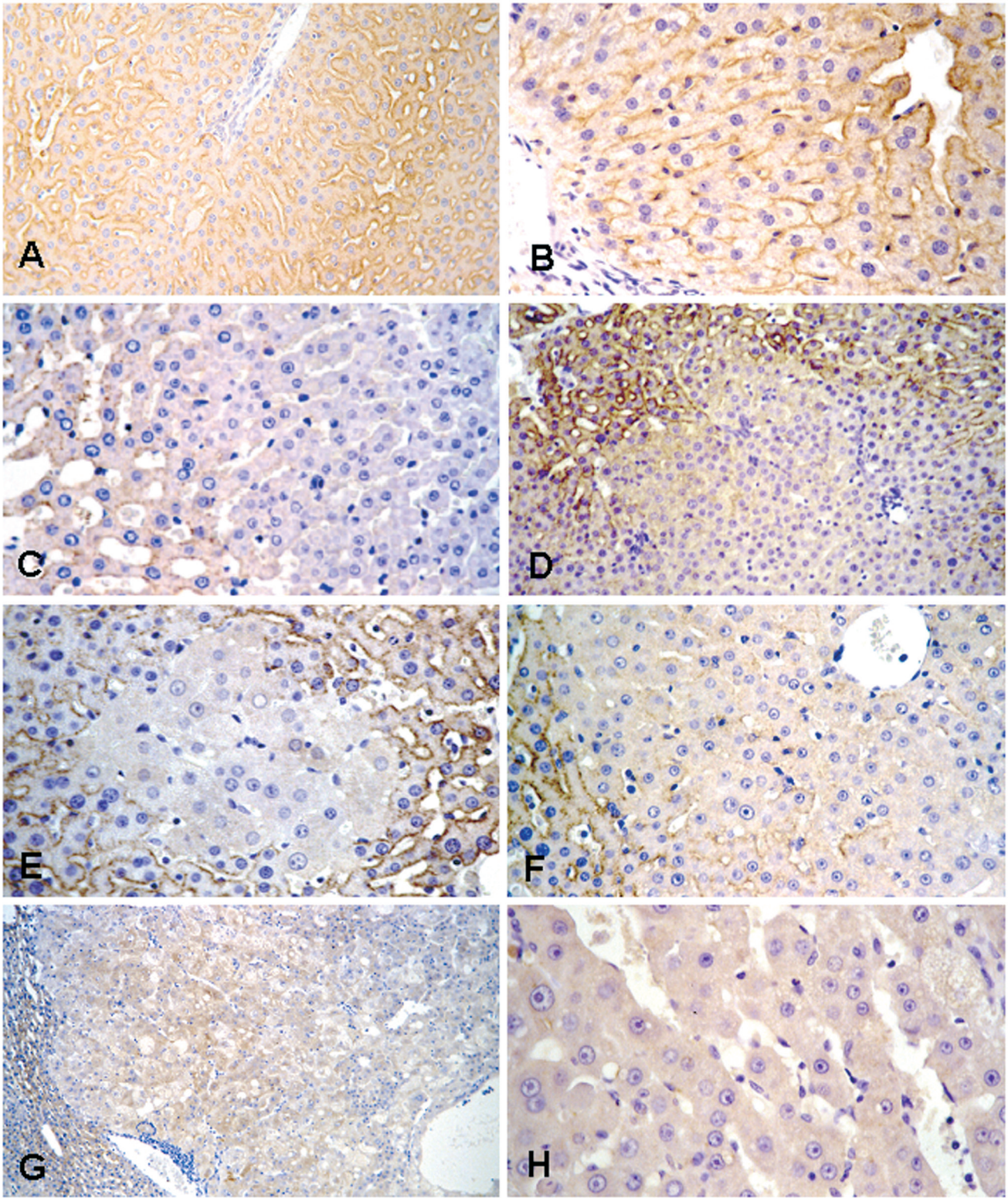
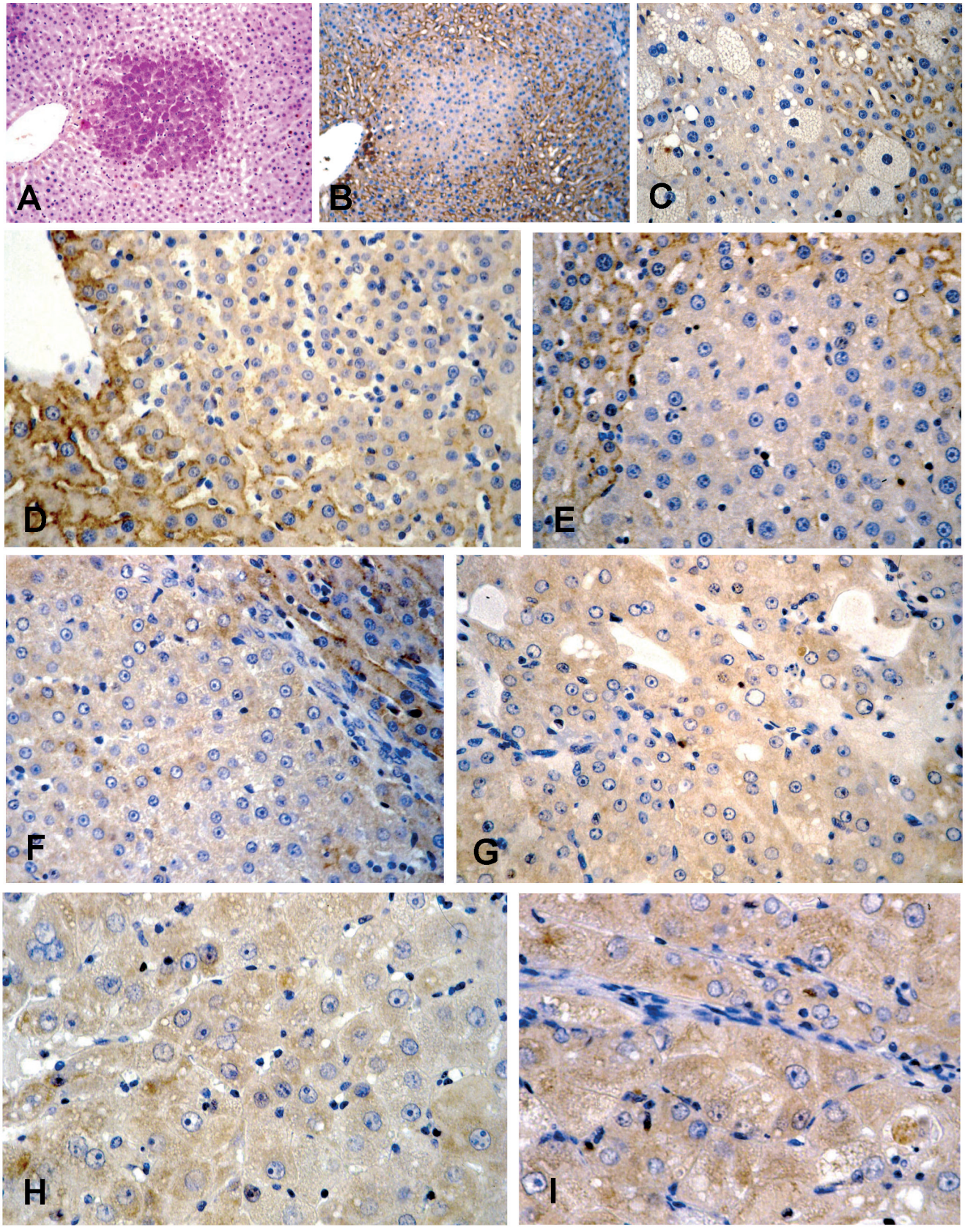



**Fig. 1.** Distribution of $\beta_1$-adrenoreceptors ($\beta_1$-ARs) in the normal liver parenchyma and in preneoplastic or neoplastic liver lesions of female F344 rats. **A.** Zonation of $\beta_1$-AR immunoreactivity in the normal parenchyma of the left liver lobe of a DEN+PB-treated rat (D4) killed after 4 months of experimentation (original magnification x 25). **B.** $\beta_1$-AR immunoreactivity in the normal parenchyma of the left liver lobe of a DEN-treated rat (D2) killed after 4 months of experimentation (original magnification x 400). **C.** Basophilic focus of the right liver lobe of a control rat (D1) killed after 21 months of experimentation: focal cells appear to be devoid of $\beta_1$-ARs (original magnification x 400). **D.** Basophilic focus of the right liver lobe from a D2 rat killed after 2 months of experimentation: note the lower density of $\beta_1$-ARs in focal cells than in the surrounding liver parenchyma (original magnification x 160). **E.** Glycogen-retaining focus of the right liver lobe from a D4 rat killed after 2 months of experimentation: focal cells appear to be devoid of $\beta_1$-ARs (original magnification x 400). **F.** Glycogen-retaining hepatocellular adenoma of the right liver lobe from a D2 rat killed after 21 months of experimentation: $\beta_1$-AR expression is lower in the focal lesion than in the surrounding normal liver parenchyma on the left side of the photomicrograph (original magnification x 400). **G.** Hepatocellular carcinoma of the median liver lobe from a D2 rat killed after 21 months of experimentation: note lower density of $\beta_1$-ARs in neoplastic cells as compared to normal liver parenchyma on the left side of the photomicrograph (original magnification x 25). **H.** Hepatocellular carcinoma of the right liver lobe from a D4 rat killed after 21 months of experimentation: no $\beta_1$-AR immunoreactivity is appreciable in neoplastic cells (original magnification x 400).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mixed cell foci, were occasionally observed at the time points of 4 or 8 months of promotion. At 21 months, all the animals had basophilic foci sometimes with a nodular appearance and larger than a liver lobule. At this time point, glycogen-retaining foci were also present in all male rats and in a few female rats. Hepatocellular adenomas were occasionally observed. In all DEN-initiated rats (group D2) and DEN+PB-treated rats (group D4) various types of altered cell foci with variable degrees of cellular atypia were present at all the time points considered. In DEN+PB-treated rats the majority of preneoplastic lesions were represented by glycogen-retaining foci and there were extensive areas of swelling and vacuolation of hepatocytes. Hepatocellular adenomas, showing variable degrees of cellular atypia, were present in a few rats sacrificed at the time point of 8 months and in all the rats from groups D2 or D4 sacrificed at 21 months. At 21 months, hepatocellular carcinomas were also present in rats of both sexes (Zavanella et al., 1994).

The number and types of spontaneous and chemically-induced liver lesions examined for ß$_1$-AR expression, as assessed by the immunohistochemical staining, are reported in Tables 1 and 2. ß$_1$-AR density was arbitrarily quantified on the basis of both the frequency of preneoplastic or neoplastic cells showing a positive immunostaining and the intensity of immunoreaction in the positive cells.

From our immunohistochemical study it appears that all types of preneoplastic lesions show a low density of

**Table 1.** ß$_1$-adrenoreceptors (ß$_1$-ARs) density in preneoplastic and neoplastic liver lesions of female F344 rats sacrificed after 2, 4, 8 or 21 months of experimentation.

| MONTHS | TYPES OF LESIONS | D1, DISTILLED WATER | | | | | D2, DEN | | | | | D4, DEN + PB | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. RATS | No. OF LESIONS EXAMINED | ß$_1$-AR[a] | | | No. RATS | No. OF LESIONS EXAMINED | ß$_1$-AR | | | No. RATS | No. OF LESIONS EXAMINED | ß$_1$-AR | | |
| | | | | - | ± | - | | | - | ± | + | | | - | ± | - |
| 2 months | | 4[b] | | | | | 4 | | | | | 4 | | | | |
| | GRC foci[c] | | 0[d] | | | | | 9 | 4 | 2 | 3 | | 7 | 6 | 1 | 0 |
| | MC foci[e] | | 0 | | | | | 1 | 1 | 0 | 0 | | 0 | | | |
| 4 months | | 3 | | | | | 3 | | | | | 3 | | | | |
| | BC foci[f] | | 0 | | | | | 1 | 0 | 0 | 1 | | 1 | 1 | 0 | 0 |
| | GRC foci | | 0 | | | | | 2 | 1 | 1 | 0 | | 7 | 3 | 2 | 2 |
| | MC foci | | 1 | 0 | 0 | 1 | | 2 | 0 | 0 | 2 | | 0 | | | |
| 8 months | | 4 | | | | | 4 | | | | | 4 | | | | |
| | BC foci | | 6 | 1 | 2 | 3 | | 2 | 1 | 1 | 0 | | 1 | 1 | 0 | 0 |
| | GRC foci | | 0 | | | | | 18 | 9 | 3 | 6 | | 34 | 26 | 6 | 2 |
| | MC foci | | 0 | | | | | 0 | | | | | 2 | 1 | 0 | 1 |
| 21 months | | 6 | | | | | 6 | | | | | 4 | | | | |
| | BC foci | | 43 | 29 | 7 | 7 | | 20 | 14 | 5 | 1 | | 5 | 5 | 0 | 0 |
| | GRC foci | | 2 | 2 | 0 | 0 | | 52 | 44 | 7 | 1 | | 38 | 35 | 2 | 1 |
| | MC foci | | 1 | 1 | 0 | 0 | | 6 | 3 | 3 | 0 | | 11 | 8 | 3 | 0 |
| | HA[g] | | 0 | | | | | 11 | 10 | 1 | 0 | | 14 | 11 | 2 | 1 |
| | HC[h] | | 0 | | | | | 2 | 2 | 0 | 0 | | 1 | 1 | 0 | 0 |

[a]: ß$_1$-AR immunoreactivity : -, absent or nearly absent; ±, weak; +, moderate. ß$_1$-AR immunoreactivity was considered nearly absent when a minimal fraction of altered cells showed a positive reaction or when a diffuse but hardly detectable immunostaining was present. ß$_1$-AR immunoreactivity was considered weak or moderate on the basis of both the frequency of ß1-AR positive cells and the intensity of immunostaining. [b]: Number of rats examined at each time interval. [c]: Glycogen-retaining cell foci. [d]: Number of liver lesions examined. [e]: Mixed cell foci. [f]: Basophilic cell foci. [g]: Hepatocellular adenomas. [h]: Hepatocellular carcinomas.

**Fig. 2.** ß$_1$-AR-immunoreactivity in the normal liver parenchyma and in preneoplastic and neoplastic liver lesions of male F344 rats. **A.** Glycogen-retaining focus demonstrated by the periodic acid-Schiff reaction in the left liver lobe of a DEN-treated (D2) rat killed after 8 months of experimentation (original magnification x 160). **B.** The same focus as Figure A: clear reduction of ß$_1$-AR-immunoreactivity as compared to the surrounding normal liver parenchyma (original magnification x 100). **C.** Glycogen-retaining focus of the left liver lobe of a DEN+PB-treated (D4) rat killed after 8 months of experimentation: note the lower density of ß$_1$-ARs in focal cells than in the adjacent normal cells on the right side of the photomicrograph (original magnification x 400). **D.** Basophilic focus in the left liver lobe of a D2 rat killed after 21 months of experimentation: note the lower density of ß$_1$-ARs in focal cells than in the normal liver parenchyma on the left side of the photomicrograph (original magnification x 400). **E.** Glycogen-retaining focus of the left liver lobe from a D2 rat killed after 21 months of experimentation: ß$_1$-AR expression is lower in the focal lesion than in the surrounding normal liver parenchyma. (original magnification x 400). **F.** Hepatocellular adenoma in the right liver lobe from a D2 rat killed after 21 months of experimentation: clear reduction in ß$_1$-AR immunoreactivity as compared to normal liver parenchyma on the right side of the photomicrograph (original magnification x 400). **G.** Hepatocellular carcinoma in the right liver lobe from a D4 rat killed after 21 months of experimentation: note the absence of ß$_1$-AR immunoreactivity (original magnification x400). **H, I.** Hepatocellular carcinoma in the right liver lobe from a D4 rat killed after 21 months of experimentation: no ß$_1$-AR immunoreactivity is appreciable in neoplastic cells (original magnification x 400).

the ß$_1$-ARs both in control rats D1, and in DEN-treated (D2) or DEN+PB treated rats (D4) (Figs. 1C-E, 2B-E). The majority of the focal lesions show a reduction in the intensity of immunostaining as compared to the adjacent normal liver parenchyma and no immunostaining is detectable in several altered cell foci. The density of ß$_1$-ARs is generally higher in the peripherally rather than in the centrally located focal cells (Fig. 1D) and appears to be somehow related to that of the surrounding normal parenchyma. However, focal lesions completely devoid of ß$_1$-ARs could be observed even in ß$_1$-AR rich areas of liver parenchyma (Fig. 1E). Hepatocellular adenomas and hepatocellular carcinomas also appear to be characterized by a very low density of ß$_1$-ARs (Figs. 1F-H, 2F-I). A variability in the ß$_1$-AR expression was often observed within the same neoplastic lesion, extensive areas completely devoid of ß$_1$-ARs being mingled with areas showing a barely detectable or a weak immunostaining.

No differences in liver lesion ß$_1$-AR immunoreactivity between female and male rats could be observed. In Figure 3, the findings from female and male rats are illustrated by pooling the data pertaining to all the preneoplastic lesions as well as all the neoplastic lesions.

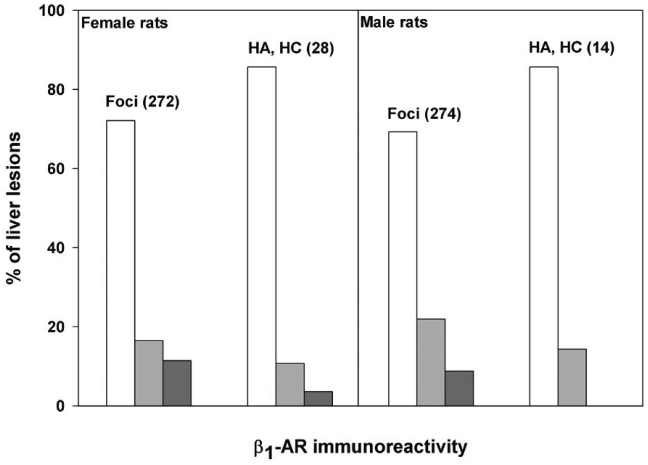


**Fig. 3.** Percent of altered cell foci and of neoplastic liver lesions (HA, HC) showing absent or nearly absent (white bars), weak (gray bars) or moderate (dark gray bars) ß$_1$-AR immunoreactivity in female and male rats. In parentheses total number of lesions examined.

## Discussion

In our immunohistochemical study, the density of ß$_1$-ARs in the preneoplastic or neoplastic lesions could be directly compared with that of adjacent and distant normal liver parenchyma. Our findings suggest that preneoplastic and neoplastic transformation is accompanied by a decrease in ß$_1$-AR expression. The reduction in ß$_1$-AR expression is most evident in hepatocellular adenomas and carcinomas, which often appear to be completely or almost completely devoid of ß$_1$-ARs.

**Table 2.** ß$_1$-adrenoreceptors (ß$_1$-ARs) density in preneoplastic and neoplastic liver lesions of male F344 rats sacrificed after 2, 4, 8 or 21 months of experimentation.

| MONTHS | TYPES OF LESIONS | D1, DISTILLED WATER No. RATS | No. OF LESIONS EXAMINED | ß$_1$-AR[a] - | ± | - | D2, DEN No. RATS | No. OF LESIONS EXAMINED | ß$_1$-AR - | ± | + | D4, DEN + PB No. RATS | No. OF LESIONS EXAMINED | ß$_1$-AR - | ± | - |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 months | | 4[b] | | | | | 4 | | | | | 4 | | | | |
| | GRC foci[c] | | 0[d] | | | | | 4 | 4 | 0 | 0 | | 4 | 3 | 0 | 1 |
| 4 months | | 3 | | | | | 3 | | | | | 3 | | | | |
| | BC focie | | 0 | | | | | 0 | | | | | 1 | 1 | 0 | 0 |
| | GRC foci | | 0 | | | | | 1 | 1 | 0 | 0 | | 8 | 6 | 2 | 0 |
| 8 months | | 4 | | | | | 4 | | | | | 4 | | | | |
| | BC foci | | 1 | 1 | 0 | 0 | | 2 | 1 | 0 | 1 | | 3 | 2 | 1 | 0 |
| | GRC foci | | 0 | | | | | 31 | 19 | 9 | 3 | | 67 | 42 | 17 | 8 |
| | MC foci[f] | | 0 | | | | | 0 | | | | | 1 | 1 | 0 | 0 |
| | HA[g] | | 0 | | | | | 0 | | | | | 1 | 1 | 0 | 0 |
| 21 months | | 6 | | | | | 3 | | | | | 3 | | | | |
| | BC foci | | 36 | 25 | 9 | 2 | | 10 | 7 | 2 | 1 | | 0 | | | |
| | GRC foci | | 40 | 27 | 10 | 3 | | 58 | 45 | 9 | 4 | | 1 | 1 | 0 | 0 |
| | MC foci | | 4 | 3 | 0 | 1 | | 1 | 0 | 1 | 0 | | 1 | 1 | 0 | 0 |
| | HA | | 1 | 0 | 1 | 0 | | 6 | 5 | 1 | 0 | | 3 | 3 | 0 | 0 |
| | HC[h] | | 0 | | | | | 1 | 1 | 0 | 0 | | 2 | 2 | 0 | 0 |

[a]: ß$_1$-AR immunoreactivity : -, absent or nearly absent; ±, weak; +, moderate. See note Table 1. [b]: Number of rats examined at each time interval. [c]: Glycogen-retaining cell foci. [d]: Number of liver lesions examined. [e]: Basophilic cell foci. [f]: Mixed cell foci. [g]: Hepatocellular adenomas. [h]: Hepatocellular carcinomas.

These observations might be in line with those of Emmelot and Bos (1971) on transplanted anaplastic rat hepatoma, originally induced by 4-dimethylaminoazobenzene administrations, and those of Christoffersen et al. (1972) on 2-AAF induced hepatocarcinomas. According to these Authors, the stimulatory effect of adrenalin on adenylate cyclase is lower in tumor cells than in normal liver cells from untreated rats (Emmelot and Bos, 1971) or from carcinogen-treated rats in which tumors had not yet developed (Christoffensen et al, 1972).

However, in these early studies there is no mention of the types of adrenergic receptors involved in adenylate cyclase stimulation. At present, the only literature data our morphological findings can be compared with are the pharmacological data on the density of binding sites for ß-AR ligands in normal and tumor liver cells. Unfortunatly, there is currently no information on the density of ß-adrenergic binding sites in focal cells isolated from any type of preneoplastic liver lesion.

A dramatic difference in the number of ß-adrenoreceptors between Reuber H35 hepatoma cell and normal BRL-1 liver cell lines has been observed (Leichtling et al., 1978). While normal hepatocytes possess 2000-5000 ß-ARs per cell, each H35 cell possesses fewer than 10 ß-ARs, as assessed by binding with the ß-antagonist [$^{125}$I]-iodohydroxybenzylpindolol. Cortinovis et al. (1985) also reported that no binding sites for a ß-adrenergic antagonist (CGP-12177) are demonstrable on Morris hepatoma MH3924 cells. Thus, these tumor cell lines appear to be lacking in either ß$_1$ or ß$_2$ adrenoreceptors. Opposite results have been obtained in studies on several serially transplanted ascites hepatomas originally induced by treatment with dimethylaminoazobenzene (Zajdela hepatoma, AH 130, AH 13, AH44, AH66, AH109A, AH7974). In these studies, receptor binding assays were carried out on plasma membrane preparations of ascites tumor cells from tumor-bearing rats and of normal liver cells from control rats or from primary normal liver cultures. The number of binding sites for the ß-antagonists $^{3}$H-dihydroalprenolol (Matsunaga et al., 1984) or [$^{125}$I]-iodocyanopindolol (Sanae et al., 1989, 1992), in ascites hepatoma cells have been demonstrated to be equal or higher than in normal rat hepatocytes. The ß-ARs of all these ascites hepatoma cells show the properties of ß$_1$-subtype (Lacombe et al., 1976; Garcia-Sainz et al., 1989; Miyamoto et al., 1985, 1989; Sanae et al., 1989, 1992).

At present, we have no explanation for these discrepancies apart from the different experimental models employed. Certainly, the process of hepatocarcinogenesis is accompanied by changes in ß-AR expressions. Our findings seem to indicate that a reduction in ß$_1$-AR expression is a recurrent feature of both spontaneous and DEN-induced preneoplastic and neoplastic liver lesions of F344 rat. Whether a relationship does exist between reduction in ß$_1$-ARs density and liver tumor growth remains an open question.

It is noteworthy that in the normal liver acinus of untreated rats, a heterogeneous distribution pattern has been found for the expression of ß$_1$-ARs, whose density is higher in the pericentral (zone 3) than in the periportal (zone 1) hepatocytes (Cardani and Zavanella, 2001). A gradual fading of ß$_1$-AR immunoreactivity from zone 3 to zone 1 has been found also in the normal liver acinus of DEN-initiated or DEN+PB-treated rats (present study). In the liver of control rats, a lobular zonation for cell turnover has also been demonstrated, cell proliferation rate being higher in the periportal and intermediate zones than in the pericentral zone (Zajicek et al., 1985; Geisler et al., 1994; Bralet et al., 1994). Thus, it is tempting to speculate that liver cell proliferation is inversely related to ß$_1$-AR expression. Further work is currently underway to test this hypothetical relationship in normal liver parenchyma and in spontaneous or chemically induced preneoplastic and neoplastic liver lesions.

*Acknowledgements.* This work was supported in part by FIRST and in part by Lega Italiana per la Lotta contro i Tumori.

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Accepted March 30, 2005