CHANGES IN PLASMA CATECHOLAMINE AND CORTICOSTERONE LEVELS AND GENE EXPRESSION OF KEY ENZYMES OF CATECHOLAMINE BIOSYNTHESIS IN PARTIALLY HEPATECTOMIZED RATS

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Objective. To reexamine the possible role of catecholamines and corticosterone in the early period of liver regeneration after partial hepatectomy (PH) in conscious cannulated rats under carefully controlled conditions which would allow to obtain reliable information about sympathetic-adrenomedullary function after PH in the rat in vivo.

Methods. Plasma levels of catecholamines (epinephrine - EPI, norepinephrine – NE) were estimated by radioenzymatic assay and these of corticosterone by competitive protein binding assay. The total RNA was isolated from the adrenals and tyrosine hydroxylase (TH) mRNA expression was estimated by hybridisation with cDNA after Northern blot. The level of immunoreactive protein was measured by using a monoclonal antibody to rat TH, visualized by Western light chemiluminiscent detection system and analyzed by densitometry. The level of TH in adrenals was estimated with the aid of ^{3}H-tyrosine and TH cofactor DL-6-methyl-5,6,7,8-tetrahydropterine and the formed radioactive water was measured by scintillation spectrometry.

Results. The plasma level of norepinephrine (NE), epinephrine (EPI) and corticosterone rapidly increased 20 min after PH or sham operation (laparotomy). Although the increase of plasma NE was about the same after both PH and laparotomy, that of EPI and corticosterone in PH rats was significantly higher as compared to the laparotomy. One hour after the surgery plasma NE levels in both groups decreased to the basal value and remained still unchanged 4 and 24 h later. At the interval of 4 h the plasma level of EPI and corticosterone in PH was higher than in laparotomized controls, but after 24 h the EPI levels returned to basal values. Adrenal tyrosine hydroxylase (TH) mRNA level was significantly elevated in both PH and laparotomized rats, however 24 h after the surgery they returned to the baseline. Adrenal TH immunoprotein levels and TH activity were significantly elevated in both groups 4 h after the surgery, while 24 h later they returned to the baseline in laparotomized rats but remained elevated in PH rats. Adrenal phenylethanolamine N-methyl-transferase (PNMT) mRNA levels were increased 4 h after both the PH and laparotomy and declined within 24 h.

Conclusions. The first peak of catecholamine and corticosterone levels might result from unspecific stressor associated with the surgery. These levels could be accompanied with the mechanism of the rat liver regeneration. Prolonged elevations of EPI found after PH seems to be specific for liver regeneration indicating that the rise in the adrenal TH mRNA appears to be translated into immunoreactive protein which further leads to the elevation of TH activity. These results contrast markedly with previous studies indicating that the regeneration is modulated predominantly by norepinephrine.

Key words: Partial hepatectomy – Catecholamines – Corticosterone – Tyrosine hydroxylase (TH) mRNA – TH immunoreactive protein – TH level

The signals controlling rat liver regeneration consist of the substances circulating in the blood, such as hormones and growth factors. These are involved in the triggering, progression and termination of

hepatocyte replication. Considering this, about twelve hormones have been proposed as regulators of liver regeneration (Bucher 1991). Changes in plasma levels of several hormones were observed after rat liver resection and they were thought to regulate energy metabolism in the pre-replicative phase (Bucher 1991). We recently observed that also the hormones of the anterior pituitary displayed significant changes in blood level after partial hepatectomy in rats (Knopp et al. 1991).

The involvement of adrenergic system in liver regeneration was proposed on the basis of data obtained after the surgical interruption of hepatic innervation (Maros 1970; Vaptzarova et al. 1973; Ungvary et al. 1974). The suggestion that the incorporation of ^{3}H-thymidine into the liver DNA was stimulated by catecholamines resulted from the experiments using chemical sympathectomy by the long-term treatment with 6-hydroxydopamine (Royse and Morley 1984) or guanethidine (Ashrif et al. 1974).

In the early phase of rat liver regeneration an increase in the concentration of ornithine decarboxylase activity (ODC) (Thrower and Ord 1974) and decrease of 2',5'-oligoadenylate synthase activity (Smekens et al. 1983) was observed. The sensitivity of regenerating liver to alpha blockers (phenoxybenzamine, phentolamine) and glucocorticoid blocker 17-alpha-hydroxyprogesterone suggested that norepinephrine and glucocorticoids might be implicated in the liver regeneration (Throwed and Ord 1974). After the addition of norepinephrine to the culture of hepatocytes in the presence of TGFß and EGF, the DNA synthesis was more rapid than that in the cells without this neurotransmitter (Cruise et al. 1985). Thus, those *in vivo* and *in vitro* studies suggest the participation of sympathetic nervous system in liver regeneration. The mechanism of catecholamine action on rat liver regeneration seems to be indirect and arising from the factors appearing after PH and modulating the catecholamine binding to alpha$_1$-adrenoreceptor, to the breakdown of PIP_2 (Houck and Michalopoulos 1989) and decreasing the mito-inhibitory effect of TGF beta.

Circulating catecholamines could be involved in the promotion of the rat liver regeneration. In spite of several suggestions on the importance of sympathetic-adrenomedullary system in this phenomenon there is no direct evidence for the changed secretion of catecholamines in well controlled studies. Previous data showing that plasma catecholamine levels increased at various time after PH are unequivocal (Cruise et al. 1987). In the mentioned study plasma catecholamine levels were measured in the blood collected from abdominal aorta and vena cava under ether anesthesia. However, ether anesthesia itself is followed by a substantial rise of plasma epinephrine and norepinephrine even without any concomitant surgery (Chiuch and Kopin 1978; Michalikova et al. 1991). Moreover, the concentration of catecholamines was determined in pooled plasma from three animals.

In the present study, we reexamined the changes in plasma catecholamine and corticosterone levels in conscious cannulated rats under carefully controlled conditions. This approach allows to obtain reliable information about sympathetic-adrenomedullary function after PH in the rat. In order to explore the background of changes in circulating catecholamines after partial hepatectomy, we examined the gene expression, immunoprotein level and activity of the key enzymes of catecholamine synthesis such as tyrosine hydroxylase (TH) and phenylethanolamine-N-methyltransferase (PEMT) in the adrenal gland was investigated.

Materials and Methods

Animals. Male Wistar rats (200-220 g) were obtained from Charles River Europe (Sulzfeld, Germany). The experiments started at least 7 days after their transfer to the animal room. The animals were housed 4-5 per cage under light-controlled conditions (lights on from 6.00 to 18.00 h) and a room temperature of 22 °C. Food and water were available ad libitum.

Cannulation procedure was performed according to Chiuch and Kopin (1978). The animals were anesthetized using sodium pentobarbital (40 mg/kg i.p.). A polyethylene tubing (PE 50; 0.75 m long, 0.58 mm in internal diameter) filled with saline and containing 300 IU of sodium heparin/ml was inserted into the ventral caudal tail artery. The catheter was tunneled under the skin and excited at the nape. A spring wire protected the catheter. After surgery, each rat was housed in an individual plastic cage, with the protected catheter extending out of the cage.

After 20-24 h, a control blood sample was taken from each of rat. Then partial hepatectomy or sham

operation were performed as described by Higgins and Anderson (1933) under light ether anesthesia. Further blood samples were collected at 20, 60, 240 min and 24 h after surgery.

Blood sampling. Blood samples (0.5-0.8 ml) were collected via the catheter at indicated times, and the same volume of heparinized saline (100 IU/ml) was administered intraarterially after each blood sample was obtained. Repeated blood sampling using this method does not affect plasma levels of catecholamines or corticosterone (Kvetnansky et al. 1978; Dobrakovova et al. 1989; Graesler et al. 1989). The blood was centrifuged at 3,000 g and 4 °C for 15 min and the plasma was stored at -70°C until assayed.

Plasma catecholamines were measured in 50 ěl aliquots of plasma by the radioenzymatic assay described previously (Peuler and Johnson 1977). Catecholamines present in plasma aliquots were converted into their labeled O-methylated derivatives by using S-[^{3}H]-adenosylmethionine (Amersham, UK) and a lyophilized catechol-O-methyltransferase isolated from rat liver. The O-methylated derivatives of the amines were then extracted along with unlabeled carrier compounds, separated by thin-layer chromatography, eluted and reacted with periodate. The detection limit was 5 pg NE or EPI per tube. Total plasma corticosterone was measured by the competitive protein binding technique (Murphy 1967).

Isolation of RNA and Northern Analysis. After decapitation the adrenals were removed, cleaned of fat tissue, transferred into sterile Eppendorf tubes, and immediately frozen in liquid nitrogen. Total RNA was isolated according to Chomczynski and Sacchi (1987) using RNAzol (Tel-Test, Inc.) and analyzed by Northern blots. Aliquots of total RNA samples (one fourth) were fractionated on 1.3 % agarose gels containing 2.2 M formaldehyde, 1 x MOPS buffer (20 mol.l^{-1} MOPS, pH 7.0, 5 mmol.l^{-1} sodium acetate, 1 mmol.l^{-1} EDTA) as described previously by Kilbourne and Sabban (1990), transferred to nitrocellulose and baked for 2 hours at 80 °C in a vacuum oven. Northern blot filters were prehybridized in 0.08 ml/cm^2 of 50 % formamide, 5 x Denhardt's, 5 x SSPE, 0.4 % SDS, and 100 ěg/ml salmon sperm DNA at 42 °C for 4 h. Hybridizations were performed using the following probes: 5' 1.1-kilobase EcoRI fragment from rat TH cDNA. The autoradiograms were scanned with a LKB Pharmacia Uppsala scanning densitometer using exposures that were within the linear range.

Measurement of immunoreactive TH protein levels. Adrenals were homogenized in 0.05 mol.l^{-1} potassium phosphate, pH 6.65/0.2 % Triton X-100 and centrifuged at 10,000 x g for 10 min at 4 °C. Supernatant proteins were fractionated by 10 % SDS-PAGE gel electrophoresis and electrotransferred to nitrocellulose membranes. The levels of immunoreactive protein were measured by using a monoclonal antibody to rat TH (Boehringer, Mannheim), visualized by using the Western light chemiluminiscent detection system (Amersham, UK) and analyzed by densitometry.

Measurement of TH activity. Adrenals were weighed and TH was yed by a modification of the method described by Nagatsu et al. (1964). Adrenals were homogenized in 0.05 mol.l^{-1} potassium phosphate, pH 6.65/0.2 % Triton X-100 and centrifuged at 10,000 x g for 10 min at 4 °C. Aliquots of supernatant were incubated at 37 °C for 15 min with a mixture containing L-[3,5^{3}H]-tyrosine and TH cofactor DL-6-methyl-5,6,7,8-tetrahydropterine (Calbiochem). Addition of acid after incubation terminated the conversion of labeled L-[3,5 ^{3}H] tyrosine to DOPA and the formed radioactive water was measured by scintillation spectrometry.

Statistical analysis. All data are presented as means±S.E. One-way analysis of variance was used to analyze data (Anova test).

Results

Plasma epinephrine and norepinephrine levels. Plasma EPI levels (Fig. 1) increased rapidly and significantly after both PH and laparotomy. After the peak values at 20 min a significant difference was found in further intervals between laparotomized and PH rats. One hour after laparotomy the EPI levels declined to control levels seen before surgery and remained unchanged throughout the following 24 h period. In contrast, plasma EPIlevel in rats with PH remained significantly elevated as compared to rats with laparotomy. Twenty four hours after PH the plasma EPI levels were only slightly higher compared to laparotomized rats.

Plasma NE levels (Fig. 2) increased rapidly 20 min after both The PH and laparotomy, but after 60 min they returned to control values. Smaller

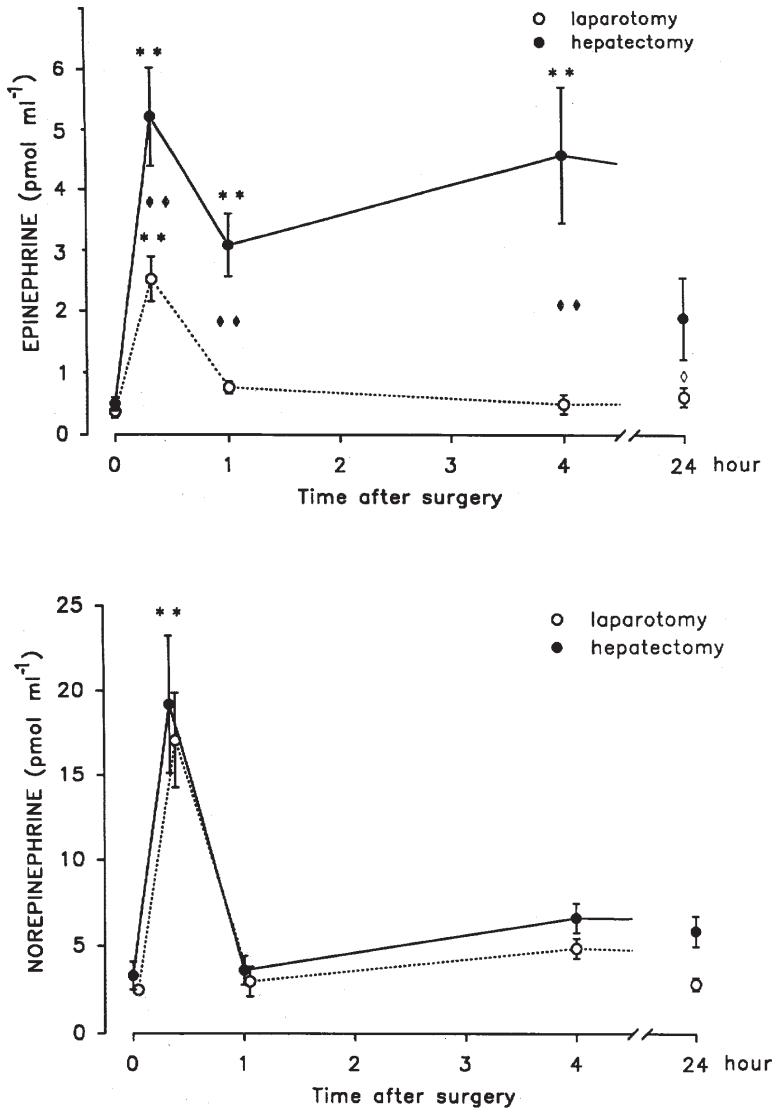

Fig. 1 and 2 Plasma levels of EPI and NE in partially hepatectomized or laparotomized rats. Each point represents the mean±S.E. for eight rats per group. ** – P<0.01 compared to controls (time zero before surgery), ♦♦ – P<0.01 (compared to laparotomy).

increments in plasma NE levels seen after PH compared with laparotomy were not statistically significant.

Effect of ether anesthesia on plasma EPI and NE levels. In an attempt to obtain information on the effect of ether anesthesia on plasma catecholamine levels a separate experiment was performed in intact rats. As shown in Fig. 3, the exposure of rats to ether for 2 minutes produced a significant ($P<0.01$) elevation in plasma NE and EPI levels, while 20 min later such levels returned to baseline.

Plasma levels of corticosterone. Plasma levels of corticosterone (Fig. 4) increased rapidly after both the PH and laparotomy. However, in laparotomized rats the increased levels lasted for 4 h after the surgery and returned to control value at 24 h. After PH a similar course of plasma corticosterone was recorded with a significant increments at 1, 4 h in comparison with laparotomized rats. Plasma corticosterone levels 24 h after PH remained significantly elevated as compared to controls and laparotomized rats.

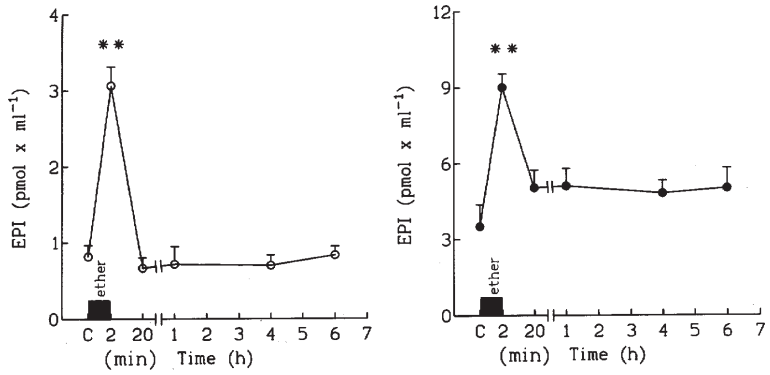

Fig. 3 Plasma levels of NE and EPI in rats after exposure to ether anesthesia (1 min). Each point shows the mean±S.E. for eight rats per group. ** – P<0.01 compared to control (time zero before anesthesia).

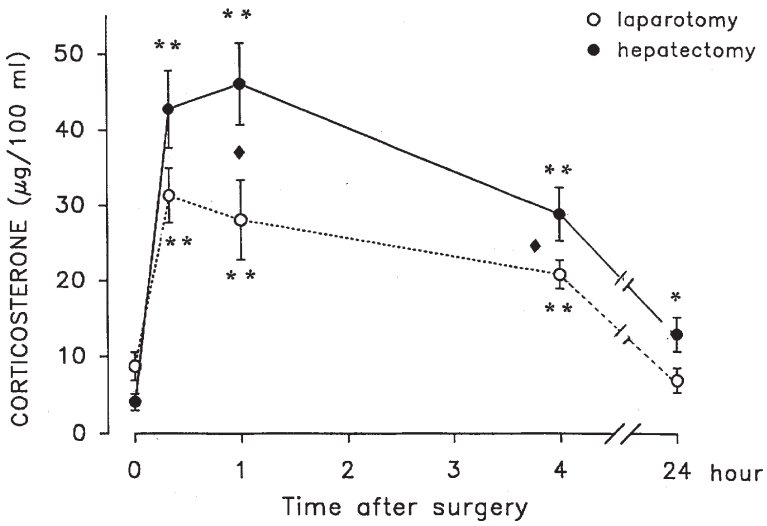

Fig. 4 Plasma corticosterone levels in laparotomized and partially hepatectomized rats. Each point shows mean ± S.E. for eight rats per group. ** – P<0.01 compared to control (time zero), ♦ – P<0.05 laparotomy versus PH.

Effect of PH on the regulation of catecholamine synthesizing enzymes in adrenal glands. *TH mRNA levels:* Both laparotomy and PH increased TH mRNA levels in the rat adrenals 4 h after the surgery. After this interval TH mRNA levels declined sharply and in both groups at 24 and 120 h remained similar to these in zero time interval. (Fig. 5). *TH immunoprotein levels:* Adrenal TH immunoprotein levels (Fig. 6) after both PH and laparotomy increased markedly and 24 h after laparotomy immunoprotein levels were similar to baseline level, while in PH rats they remained still significantly elevated. *TH activity:* As shown in Fig. 7, the activity of tyrosine hydroxylase in the rat adrenal gland increased significantly 4 h after both the PH and laparotomy, while 24 h after the laparotomy its activity returned to control levels, but after the partial hepatectomy adrenal TH activity remained still elevated. *PNMT mRNA levels:* Adrenal PNMT mRNA levels (Fig. 8) increased significantly 4 hours after both laparotomy and PH (P<0.01) and at 24 h interval remained elevated in compared to control values.

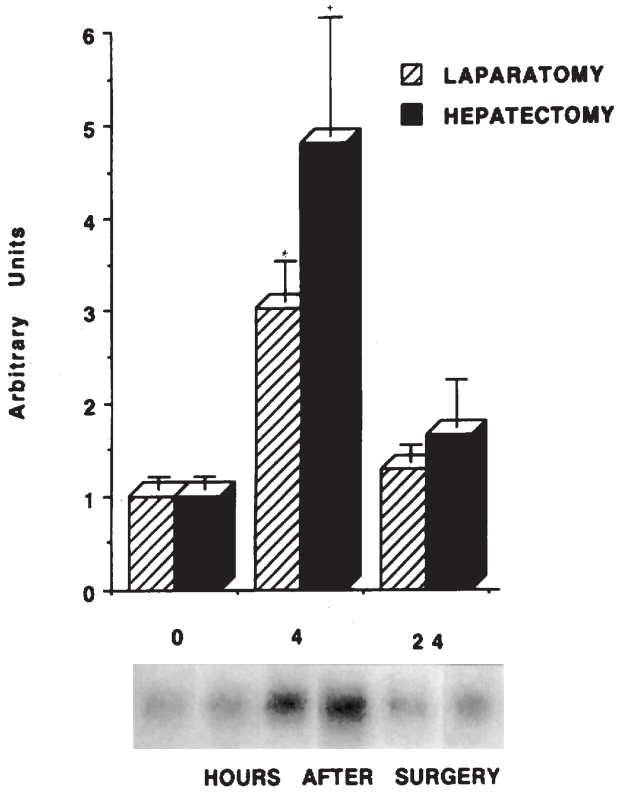

Fig. 5 Effect of partial hepatectomy and laparotomy on adrenal TH mRNA levels at the indicated intervals after surgery. Values are expressed as mean±S.E. for six rats per group. * – P<0.01 versus time zero.

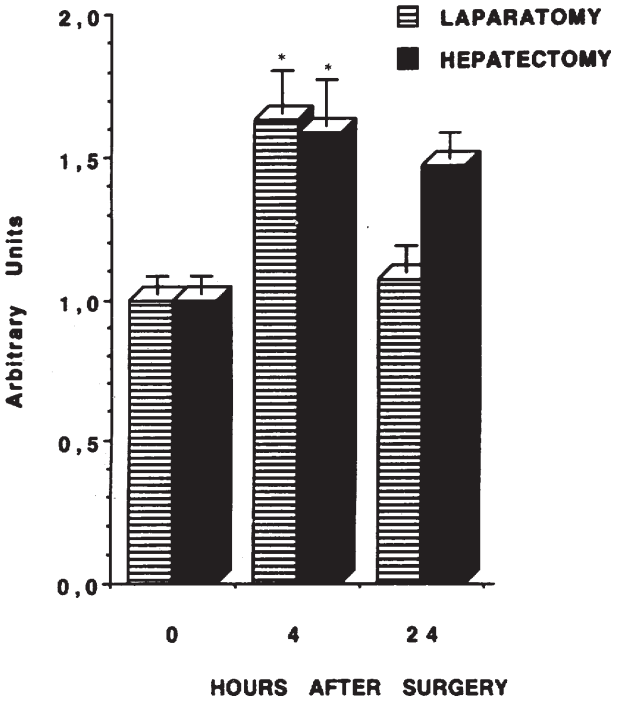

Fig. 6 Effect of partial hepatectomy and laparotomy on TH adrenal enzyme activity. Values are expressed as mean±S.E. for six rats per group. * – P<0.05; ** – P<0.01 versus time zero, + – P<0.01 versus appropriate laparotomized group.

Discussion

The present results demonstrate an increase in the plasma levels of catecholamines and corticosterone in conscious cannulated rats after laparotomy and partial hepatectomy. The major part of the elevated plasma levels of catecholamines at short intervals after PH and laparotomy might be caused by unspecific stressors such as handling, transfer, surgery and ether anesthesia. In the later time intervals the elevated levels of epinephrine and corticosterone seem to be specific for partial hepatectomy. As stated before, for measurement of plasma levels of catecholamines some authors used the blood collected under ether anesthesia (Cruise et al. 1987). However, these results were not statistically evaluated. With respect to their findings the results presented here show considerably different time course of plasma EPI and NE levels in rats after PH.

Previous studies indicated (Cruise et al. 1987; Houck and Michalopoulos 1989) the role of norepinephrine in the process of the rat liver regeneration. The results presented in this study raise the question whether the small, but not significant increments of plasma NE as compared to these after laparotomy in later time intervals might play a role in the rat liver regeneration. Our results show that partial hepatectomy does not show any specific effect on the sympathetic activity (on the base of unchanged levels of plasma NE) and therefore we do not support the idea that NE might play a dominant role in the mechanism of the rat liver regeneration. It was suggested that the effect of norepinephrine in regenerative process results in the potentiation of the mitogenic effect of epidermal growth factor (EGF) (Cruise et al. 1986) considered as complete hepatocyte mitogen. Moreover, norepinephrine in hepatocyte cell cultures was found to alter phosphoinositol cascade (Cruise

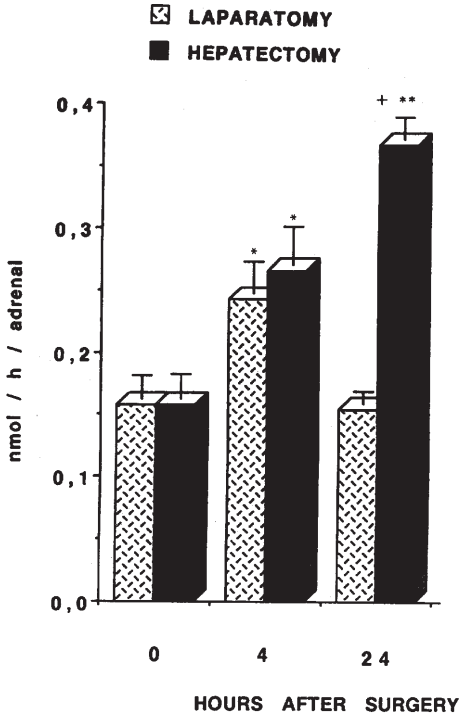

Fig. 7 Effect of partial hepatectomy and laparotomy on TH immunoprotein levels in adrenals. Values are mean±S.E. for six rats per group. * – P< 0.05 versus time zero.

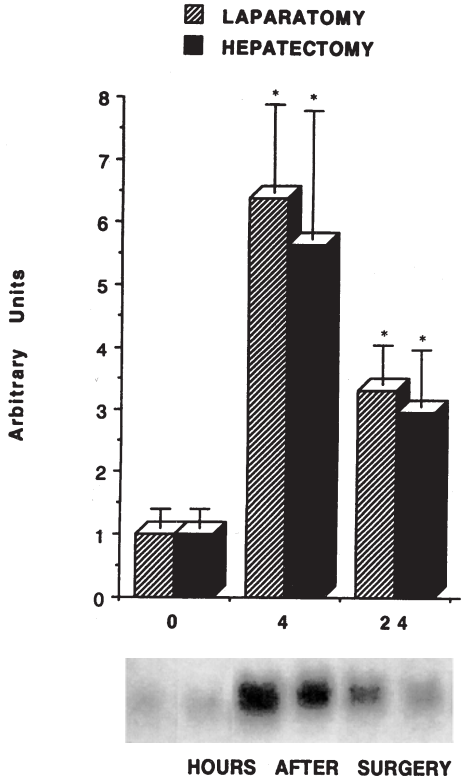

Fig. 8 Effect of partial hepatectomy and laparotomy on adrenal phenylethanolamine N-methyltransferase mRNA levels. Values are expressed as mean±S.E. for six rats per group. * – P<0.01 versus time zero.

et al. 1989). Results of plasma levels of norepinephrine after PH demonstrated here do not strength such a possibility of its actions. Of course, we do not have any evidence about NE levels in the liver or about possible changes of adrenergic receptors which might affect the mitogenesis. However, plasma NE levels reflect to great extent the activity of the sympathoneural system.

One of the stimulatory pathways involved in the control of rat liver proliferation after PH is the cyclic AMP (cAMP) system. Early in the regeneration process there is an increase in the concentration of cAMP in rat liver which is maximal about 4 h after the surgery with the second peak at 12 h (Thrower and Ord 1974). With regard to a role of catecholamines in the proliferation of liver cells after PH only indirect effect of agents which interfere with the catecholamine receptors and cAMP system was demonstrated (Thrower and Ord 1974). Plasma epinephrine levels found in our experiments support the idea that the peaks of cAMP concentration at 4 h and 12 h after PH might be due to increased circulating epinephrine. However, it was found that the first peak in cyclic AMP concentration is not essential for DNA synthesis after PH while the role of second peak is uncertain. Nevertheless, a persistent elevation of plasma level of epinephrine starting from 20 min and lasting for several hours after PH may participate on the mitotic stimulus.

The present results support a two step hypothesis for the induction of hepatocyte proliferation by stress hormone EPI which may activate macrophages to produce interleukin 6 (Koga and Ogasavara 1991). The recorded changes in plasma corticosterone after PH might be important to alter adrenergic receptor sensitivity because a possible participation of glucocorticoid in elevation of cAMP levels through the inhibition of cAMP-phosphodiesterase was also ob-

served (Tanigawa et al. 1978). Further studies suggested that in rats both norepinephrine and glucocorticoids are implicated in the induction of ornithine decarboxylase activity (Thrower and Ord 1974). The sharp rise and persistent elevation of plasma levels of corticosterone observed after PH in our experiments confirm that corticosterone may also contribute to hepatocyte mitogenesis.

Expression of various genes in the residual liver of rats was monitored during several hours after PH. Our findings suggest that the immediate increase of catecholamines in the peripheral blood was presumably due to its release from tissues and was not result of removal of 2/3 of the bulk hepatic monoamine oxidase, that degrades plasma catecholamines. Further we show that at 4 h time interval the increased plasma levels of catecholamines are at least the result of adrenal TH activity in both laparotomized and PH rats.

Several types of stressors were found to produce a very rapid increase in TH activity without any changes in the number of enzyme molecules (Weiner et al. 1978; Fluharty et al. 1985). However, we found that there was an increase in immunoreactive TH protein after both laparotomy and PH. The differences in TH mRNA, TH activity and TH immunoreactivity after PH or laparotomy are in agreement with the findings showing that the activation and induction of TH are two different processes (Kvetnansky and Sabban 1993). PNMT mRNA levels in the adrenal of rats 4 h after laparotomy and PH displayed an elevation with the magnitude comparable to that found in rats which underwent the immobilization stress. PNMT is predominantly regulated by glucocorticoids and only in a small degree by the nervous activity (Axelrod and Reisine 1984). Thus, we suppose that the relative high PNMT gene expression found in our experiments was presumably caused mainly with the increased plasma levels of corticosterone.

In summary, the present study showed that partial hepatectomy in rats evokes the increase in plasma levels of epinephrine and corticosterone. While plasma levels of norepinephrine were transient and caused mainly by surgery, plasma levels of epinephrine and corticosterone persisted for several hours. These changes are accompanied with the expression and activation of catecholamine synthesizing enzymes. The pattern of responses of plasma epinephrine found after partial hepatectomy supports the idea that this hormone might play a role in the prereplicative and corticosterone in both the prereplicative and replicative phase of the rat liver regeneration. These results contrast markedly with previous studies indicating that the regeneration is modulated predominantly by norepinephrine.

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BOOK REVIEW

THE IGF SYSTEM, MOLECULAR BIOLOGY, PHYSIOLOGY, AND CLINICAL APPLICATIONS

Edited by Ron G. Rosenfeld and Charles T. Roberts (Oregon Health Sciences University, Portland), 787 pages, Humana Press

More than 40 yr elapsed from the finding of "sulfation factor" by Salmon and Daughaday which has been later "transformed" into insulin-like growth factors, the purification and sequencing of which has been accomplished about 20 yr later. The book presented by Ron G. Rosenfedl and Charles T. Roberts and edited by Humana Press may be considered a true encyclopaedia on the currect status of art in this rapidly developing field.

The book is divided into four main sections: I. Molecular biology of the IGF system, II. Biological actions of the IGFs, III. IGF physiology, IV. Clinical aspects of the IGFs, each sections containing several chapters written by a total of 74 well known experts in this field. Expert editorial work apparently resulted in handy organisation of the text of each chapter which follows the unified hierarchy of titles and subtitles. In addition, each chapter contains the Introduction and Summary and even the appropriate cross-references to the other chapters. The average number of about 150 references included into each of 31 chapters makes a total of about 5000 references which go up to 1997.

Severeal instructive and carefully selected figures and tables bring convincing evidence on the main facts and findings described in the text.

Undoubtedly, this valuable book will be useful not only for those dealing with various aspects of basic reasearch in this and related fields, but also for those teaching several premedical disciplines. However, several clinicians including endocrinologists, pediatricians, gynecologists will find unique and valid information to enlarge the field of their theoretical basis as well as their diagnostic and therapeutic skills.

Pavel Langer