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The Role of Sympathetic Activity in the Early Phase of Liver Regeneration after Partial Hepatectomy

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ABSTRACT

This study was undertaken to confirm whether or not the sympathetic nervous system takes part in the liver regeneration after partial hepatectomy.

The male Sprague-Dawley rats were pretreated with I.P. injection of guanethidine 25 mg/kg: single dose (G-1); multiple doses once a day for 3 days (G-3), for 5 days (G-5), or for 6 days (G-6). The rats were subjected to partial hepatectomy (70.4 ± 1.99%) under light anesthesia of diethyl ether.

1) The systolic blood pressure of control rat was 98.0 ± 3.9 mmHg and was not affected by G-1. But after the pretreatment with G-3, G-5 or G-6, the pressure was markedly decreased by over 25%.
2) Both of plasma norepinephrine and epinephrine levels showed the marked increases 3 hrs after the hepatectomy. However, the increases are entirely inhibited by G-1 or G-6.
3) All the liver contents of putrescine, spermidine and spermine showed the significant increases 6 hrs after the hepatectomy and were not affected by G-1 or G-6 with the exception of the inhibition of putrescine increase by only G-6.

The present results suggest that the sympathetic activation appeared after partial hepatectomy seems not to play an important role in rat liver regeneration.

Key Words: Regenerating liver, Guanethidine, Polyamine, Plasma catecholamine

Abbreviations: FNBT; 4-fluoro-3-nitrobenzotrifluoride, NTP-polyamine; N-2′-nitro-4′-trifluoromethylphenyl polyamine, ODC; ornithine decarboxylase

INTRODUCTION

It is postulated that the diamine putrescine and the polyamine spermidine and spermine widely distributed in all of the mammalian organs, play several important roles in the control of cell growth, proliferation, and differentiation (Williams-Ashman and Canellakis, 1979; Pegg, 1986; Seiler, 1987).

Leffert *et al.* (1979) suggested that the liver regeneration occurred after partial hepatectomy is primarily controlled by five peptide hormones such as insulin, glucagon, epidermal growth factor, parathyroid hormone and calcitonin, and by two nonpeptide hormones such as thyroid hormones and glucocorticoids.

There is a general agreement that in mammalian systems the activity of ODC, the rate-limiting key enzyme in the polyamine biosynthetic pathway, is not governed by allosteric or covalent mechanisms but rather by a cyclic adenosine-3′, 5′-monophosphate (cyclic AMP)-mediated transcriptional cascade events: the activation of type I cyclic AMP-dependent protein kinase and the phosphorylation of nonhistone chromosomal proteins related to the synthesis of new messenger ribonucleic acid (mRNA) specific for ODC (Russell *et al.*, 1976; Russell and Haddox, 1979).

But Lau and Slotkin (1979) described that the activation of cardiac ODC in response to isoproterenol might result from a shift of the enzyme from its low

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affinity states for ornithine to a high affinity form. However, the increase of ODC by beta-adrenoceptor agonists seems to be caused by Ca^{2+}-influx and Ca^{2+}-calmodulin complex (Canellakis *et al.*, 1981; Veldhuis and Hammond, 1981).

Many studies (Copeland *et al.*, 1982; Guarnieri *et al.*, 1983; Womble and Russell, 1983) are supporting that $beta_2$-adrenoceptors are selectively coupled to the regulation of cardiac ODC following catecholamine stimulation. And hepatic hypertrophy and/or hyperplasia could be produced via stimulation of beta-adrenoceptor (Bronstad and Christoffersen, 1980).

On the other hand, Thrower and Ord (1974) reported that early in the liver regeneration, propranolol was ineffective in preventing the initial activation of ODC, but phenoxybenzamine and phentolamine delayed the rise of ODC activity.

However, polyamines may be, in a contrast view, involved in the activation of Ca^{2+}-fluxes through the plasma membrane (Koenig *et al.*, 1983).

Therefore, the present study was undertaken to evaluate the role of sympathetic system in the changes of polyamine contents of the remnant liver after partial hepatectomy in male rats pretreated with guanethidine.

MATERIALS AND METHODS

Materials

Guanethidine sulfate, and the hydrochlorides of putrescine, spermidine, spermine, and histidine were purchased from Sigma.

1,8-Diaminooctane, 4-fluoro-3-nitrobenzotrifluoride (FNBT), dimethylsulfoxide, and 2-methylbutane were from Aldrich. Acetonitrile was a chromatographic grade preparation of Merck. Other chemicals were analytical grade. Male Sprague-Dawley rats (weighing 150 to 180g) were obtained from Korea Experimental Animal Lab. Co.

Treatments of animals

Male Sprague-Dawley rats were kept 2 to a cage, and allowed to acclimate to a 12hr-light (7 AM to 7 PM) and 12hr-dark cycle for one week before being studied.

Guanethidine sulfate 25 mg/kg was intraperitoneally injected at noon once a day for 1, 3, 5 or 6 days. At 22 hours after the last injection, the rats were subjected to partial hepatectomy (Higgins and Anderson, 1931) of $70.4 \pm 1.99\%$ under light anesthesia of diethyl ether between 9 and 12 AM, and the remnant liver weight was estimated $29.7 \pm 1.45\%$ of prehepatectomy liver weight. Sham-operated rats underwent a similar laparotomy procedure.

Indirect measurement of rat blood pressure

The systolic blood pressure of rat tail was, after prewarming for 15 min, on a Narco temperature control unit for rats, measured with a Narco MK-IV physiography system equipped with a electrosphygmograph coupler (Narco Part No. 704-0070), using a pneumatic pulse transducer (Narco Part No. 705-0022) and a metal tubular cuff of 7/16 in i.d..

HPLC analysis of plasma catecholamine

The rat blood was, immediately after decapitation, collected from the trunk into a cold bottle containing 20 μl solution of heparin 100 units, disodium EDTA 0.4 μmole, and sodium sulfite 0.1 μmole, and then centrifugated at about $1000 \times g$ for 5 min at 4°C. The catecholamines of plasma 0.2 ml were extracted with acidified alumina (30 mg) as previously described (Anton and Sayre, 1962). Following careful rinsing of the alumina, the catecholamines were desorbed into 200 μl of 0.2 M perchloric acid, and then the percholate solution of 100 μl was diluted with the HPLC elution buffer of 100 μl. The mixture was clarified by centrifugation at $15{,}000 \times g$ for 5 min. at 4°C. The supernatant of 100 μl was applied to the HPLC system consisted of a Gilson 302/5 pump, a Gilson 141 EC detector, a Rheodyne 7125 injection valve, an Erma REC-ODS-1262 column (3 μm, 6×100 mm), and a Linear chart recorder of double channel.

The elution of catecholamines on the HPLC system was isocratically effected by 0.05 M sodium phosphate buffer, pH 3.7 containing disodium EDTA 0.02 mmole and 0.5 mmole sodium octanesulfonic acid as an ion-pairing agent, monitoring with the EC detector of a glassy carbon electrode set at + 0.75 V on the chart-recorder.

The capacity factors of norepinephrine and epinephrine were 4.67 and 8.75, respectively, and the recovery rates of them were as follows; norepinephrine: $76.8 \pm 6.4\%$ and epinephrine: $72.8 \pm 6.2\%$.

Polyamine measurement

Extraction and derivatization: The polyamine extraction was done below 6°C as previously describ-

ed (Choi *et al.*, 1989). Excised rat livers obtained after decapitation were homogenized in 5 vol. of cold 0.4 M perchloric acid and 2 mM disodium EDTA solution containing diaminooctane (100 μg) as an internal standard. The supernatant obtained from one ml of the homogenate was evaporated to dryness with streams of nitrogen gas at room temperature. According to the FNBT derivatization method originally reported by Spragg and Hutchings (1983), the dry residue obtained was redissolved in 100 μl of 1 M sodium carbonate, and then was treated with 300 μl of FNBT reagent (10 μg FNBT per ml of dimethyl sulfoxide). After cooling the reaction mixture, the N-2'-nitro-4'-trifluoromethylphenyl-polyamine (NTP-polyamine) derivatives were extracted twice with 2 ml of 2-methylbutane. The organic extract was evaporated to dryness with streams of nitrogen gas, and then was reconstituted with 1 ml of HPLC-grade methanol.

HPLC separation: The methanol solution of 20 μl was, according to the reverse-phase HPLC condition formulated by Spragg and Hutchings (1983), analysed by a HPLC system consisted of a Gilson 302/5 pump, a Knauer UV/VIS spectromonitor, an Erma ERC-ODS-1161 column (3 μm, 6×100 mm), a Rheodyne 7125 injection valve, and a Linear chart recorder of double channel.

The analysis of NTP-polyamines was effected by an isocratic elution of 80% aqueous acetonitrile mobile phase at the rate of 1.2 ml/min, monitoring at 242 nm.

The HPLC parameters of NTP-polyamines were as shown in our previous paper (Choi *et al.*, 1989).

RESULTS

Effect of guanethidine on blood pressure

The systolic pressure of the rat was not changed 22 hours after one dose of guanethidine 25 mg/kg. But the pressures of the rats pretreated with guanethidine 25 mg/kg once a day for 3, 5 or 6 days were significantly decreased down to 65-75% of the control values (Fig. 1).

Effect of guanethidine on the hepatectomy-induced increases of plasma catecholamines

The plasma norepinephrine level was not significantly changed by guanethidine 25 mg/kg once a day for 6 days. However, the plasma epinephrine level (14.87±0.81 pmole/ml) measured 22 hours after single injection of guanethidine 25 mg/kg, was

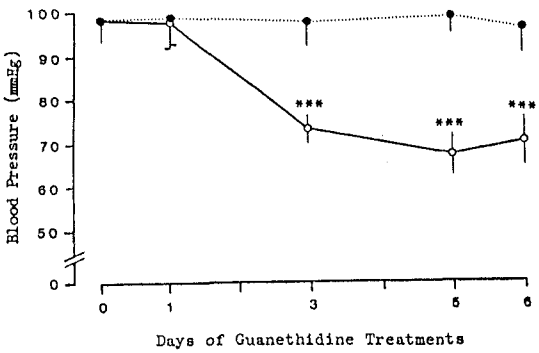

Fig. 1. Influence of guanethidine treatment on the blood pressure.
Data indicates the mean ± standard error of 4 samples.
*** indicates $p<0.005$.
●·····●: isotonic saline treatment
○——○: guanethidine treatment

significantly lower than that (23.45±1.51 pmole/ml) of the control rat, but the treatment with guanethidine 25 mg/kg once a day for 6 days did not affect the plasma epinephrine level (Fig. 2).

The plasma norepinephrine and epinephrine levels (20.38±2.23 pmole/ml and 56.06±4.63 pmole/ml, respectively) measured 3 hours after partial hepatectomy showed the marked increases over 370.0 or 239.1% of the control values (Fig. 2).

But the hepatectomy-induced increases of plasma catecholamines were significantly suppressed by the pretreatment with guanethidine 25 mg/kg single dose or once a day for 6 days (Fig. 2).

Effect of guanethidine on the short-term changes of polyamine contents in regenerating liver after hepatectomy

After partial (70.4±2.99%) hepatectomy, the remnant rat liver (29.7±2.45%) of the control group showed the rapid increase up to 34.7±2.52% of prehepatectomy liver weight by 6 hr. The weight recovery rate was not significantly affected by the treatment with guanethidine 25 mg/kg single dose or once a day for 6 days.

After partial hepatectomy, the putrescine content (118.7±14.1 nanomole/g wet weight) of prehepatectomy rat liver markedly increased to 660.2±24.6 nanomole/g wet weight by 6 hr, the spermidine content (873.8±27.7 nanomole/g wet weight) of the saline-treated control group rapidly increased to 1316.1±78.9 nanomole/g wet weight by 6 hr, and

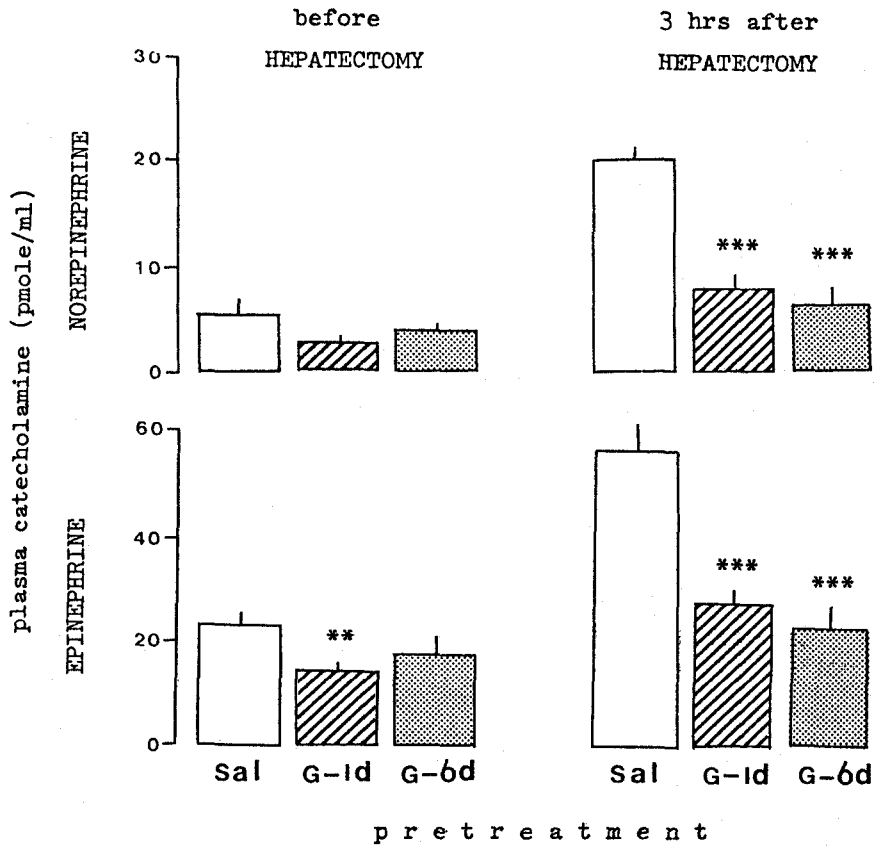

Fig. 2. Influences of partial hepatectomy and guanethidine treatment on the changes of plasma catecholamine levels. Data indicate the mean ± standard error of 4 samples.
** and *** indicate $p<0.01$ and $p<0.005$, respectively.
□: isotonic saline treatment
▨: guanethidine treatment for 1 day
⊡: guanethidine treatment for 6 days

the spermine content (875.6 ± 42.1 nanomole/g wet weight) moderately increased to 1155.1 ± 57.3 nanomole/g wet weight by 6 hr.

All of the polyamine increases were not affected by the guanethidine pretreatments with the exception of the inhibition of the putrescine increase (Fig. 3).

The increase of putrescine content (660.2 ± 24.6 nanomole/g wet weight) showed 6 hrs after partial hepatectomy, was not affected by single dose of guanethidine 25 mg/kg but significantly inhibited by the pretreatment with guanethidine once a day for 6 days (Fig. 3).

DISCUSSIONS

It has been well known that polyamines play a pivotal role in the compensatory hypertrophy (Bronstad and Christofferson, 1980) or regeneration (Thrower and Ord, 1974; Lindguist *et al.*, 1985) of several organs as well as in the control of cell growth, proliferation, and differentiation (Williams-Ashman and Canellakis, 1979; Tabor and Tabor, 1984).

The biosynthesis rates profoundly rise in the tissues stimulated by trophic stimuli such as hormones and growth factors (Leffert *et al.*, 1979; Russell and Haddox, 1979).

After hypophysectomy, there was a marked suppression in the increase of liver spermidine content after partial hepatectomy (Russell and Snyder, 1968). The increases of hepatic ODC activity and DNA synthesis (Thrower and Ord, 1974) and hepatic RNA synthesis (Thrower *et al.*, 1973) appeared after par-

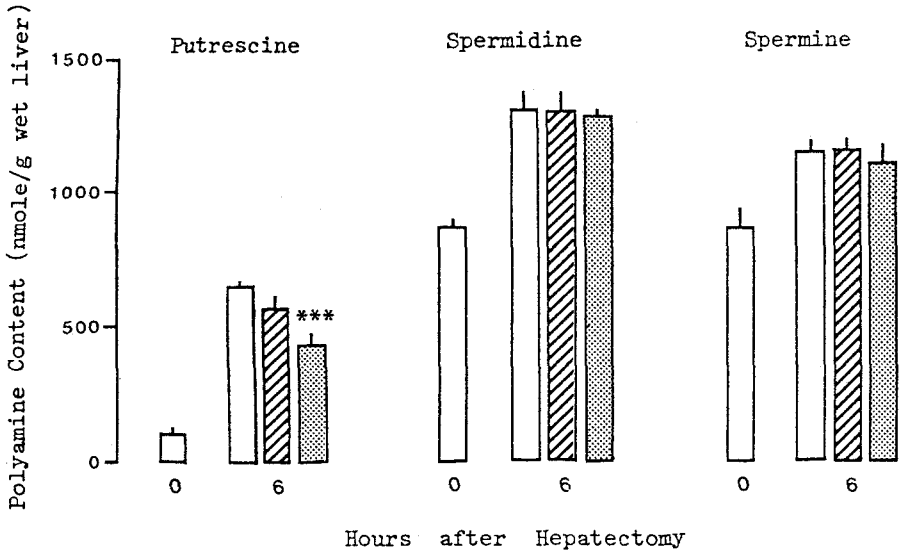

Fig. 3. Influence of guanethidine on the changes of polyamine contents in the regenerating liver. Data indicate the mean ± standard error of 4 samples.
*** indicates $p<0.005$.
□: isotonic saline
▨: guanethidine treatment for 1 day
▩: guanethidine treatment for 6 days

tial hepatectomy were delayed by the pretreatment with α-adrenoceptor blockades. But Bronstad and Christofferson (1980) proposed that hepatic hypertrophy and/or hyperplasia could be induced via stimulation of β-adrenoceptor, and many reports (Copeland *et al.*, 1982; Guarnieri *et al.*, 1983; Womble and Russell, 1983) are supporting that β_2-adrenoceptors are selectively coupled to the regulation of cardiac ODC activation by catecholamines.

And Goldstone *et al.* (1983) suggested that the influx and intracellular mobilization of Ca^{2+} could be increased by β-adrenoceptor stimulation (Goldstone *et al.*, 1983).

Recently, Choi *et al.* (1989) demonstrated that the increase of putrescine content in regenerating rat liver was not affected by yohimbine and atenolol but significantly inhibited by prazosin, propranolol, and nifedipine.

Therefore, the present study was undertaken to confirm whether or not sympathetic nervous system takes part in the regulation of the polyamine metabolism in regenerating rat liver.

As the result, the systolic blood pressure of rats was not changed 22 hrs after single injection of guanethidine 25 mg/kg but markedly decreased by multiple pretreaments with guanethidine once a day for 3, 5 or 6 days. The marked increases of plasma catecholamine levels appeared 3 hrs after partial hepatectomy were significantly suppressed by the pretreatments with single dose of guanethidine 25 mg/kg or multi-doses of guanethidine once a day for 6 days.

But the increase of putrescine content in regenerating rat liver after partial hepatectomy, was little affected by single dose of guanethidine but significantly inhibited by the pretreatment with multiple doses of guanethidine once a day for 6 days.

These results are somewhat different from those previously described (Thrower *et al.*, 1973; Thrower and Ord, 1974; Choi *et al.*, 1989), suggesting that the endogenous catecholamine liberated by partial hepatectomy followed by regeneration process may not play an important role in the activation of polyamine synthesis for the liver regeneration.

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= 국문초록 =

肝-部分切除 후 나타나는 再生過程에서 交感神經系의 役割에 關한 硏究

고려대학교 의과대학 약리학교실, +건국대학교 의과대학 신경정신과학교실

최상현 · 이중근 · 박청산+ · 전보권 · 천연숙

간-부분절제후 재생과정에서 간 polyamine 대사에 교감신경계가 촉진적으로 간여하는지를 확인하기 위하여 본 실험을 시행하였다. Guanethidine 25mg/kg를 1회(G-1), 또는 매일 1회씩 3(G-3), 5(G-5) 및 6일간(G-6) 각각 복강내-주사하고, diethyl ether 마취하에서 웅성 백서의 간을 부분(70.4±1.99%)-절제하여, 절제 전후의 혈압, 혈장 catecholamine치 및 간 polyamine 함량의 변동을 검토하여 다음의 성적을 얻었다.

1. 백서 꼬리에서 측정한 정상 수축기 혈압은 98.0±3.9mmHg이며, 이는 G-1에 별 영향을 받지 않았으나, G-3, G-5 및 G-6에 의하여는 25% 이상 현저히 저하되었다.

2. 혈장 norepinephrine 및 epinephrine치는 간-부분절제하고 3시간 후에 각각 20.38±2.23pmole/ml 및 56.06±4.63pmole/ml로써 현저한 증가를 보였으며 그 증가율은 G-1 및 G-6에 의하여 80.46% 이상 현저히 억제되었다.

3. 간-부분절제 전의 putrescine, spermidine 및 spermine 함량은 각각 118.6±14.1, 873.8±27.7 및 875.6±42.1nmole/g wet liver로서 절제후 6시간에 각각 5.5배, 1.5배 및 1.3배 이상 증가되었으며, putrescine의 증가만이 G-6에 의하여 유의하게 억제되었을 뿐 guanethidine-전처치에 별 영향을 받지 않았다.

이상의 성적으로 미루어 볼 때, 간-부분절제후 재생과정에서 나타나는 polyamine대사의 촉진현상에 교감신경성 catecholamine들이 직접적으로 중요한 역할을 수행한다고 보기는 어렵다.