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Activity Change of Ornithine Decarboxylase (ODC) after Hepatectomy

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Abstract

Ornithine decarboxylase (ODC) catalyzes the first and key step in the polyamine biosynthetic pathway. Ornithine decarboxylase is known to the enzyme that increase substantially its activity in regenerating liver. We found that activity and mRNA level for ODC increase significantly after partial hepatectomy in the rat. After laparotomy, there was significant decrease in activity; however, mRNA content was unaltered in contrast to previous reports of no change in ornithine decarboxylase and thymidine kinase after sham hepatectomy. This may be mediated by the decrease in food intake after hepatectomy. Therefore it is necessary to examine the effect of food intake after hepatectomy on the ODC activity and mRNA level in the future.

Key words : ornithine decarboxylase, Northern blot, hepatectomy

INTRODUCTION

Ornithine decarboxylase (ODC; EC 4.1.1.17) is the rate limiting enzyme in polyamine pathway and converts ornithine to putrescine. Ornithine decarboxylase catalyzes the first and key step in the polyamine biosynthetic pathway. All animal cells so far examined contain ODC activity except for Chinese hamster ovary cell ODC-mutants generated by mutagenesis. The enzymes from a variety of mammlian sources are specific for the L isomer of ornithine and have Km values of about 0.1 mM. Yeast ODC has a Km of 0.09mM which is similar to the mammalian enzymes (1-3).

In the case of mouse kidney ODC induction caused by androgens, the amount of ODC protein increased 400~500 fold with a 4~10 fold increase in the half-life of the enzyme indicating that the rate of synthesis must be increased 50~100 fold. However, ODC mRNA was shown to increase only about 10 fold. This suggested that translational stimulation was involved. In rat liver, marked induction of ODC activity by both thioacetamide treatment and by feeding preceded by only a several fold increase in the activity of ODC mRNA suggesting involvement of post-transcriptional processes (4,5).

ODC has an extremely rapid turnover rate, which in conjunction with its synthesis being regulated, allows for a means of bringing about rapid change in enzyme activity. The turnover of ODC also changes under various conditions and seems to be subject to regulation.

Polyamines stimulated an increase in ODC turnover which was slow initially but soon accelerated to a more rapid decay. Immunochemical studies have confirmed that polyamines accelerate the decay of prelabeled ODC protein in a time-dependent manner.

The polyamine-induced acceleration of decay was inhibited by cycloheximide but not by actinomycin D indicating that protein, but not RNA, synthesis was required for enzyme induction (6).

Thymidine kinase (TK; EC 2.7.2.21) is a slavage pathway enzyme involved in DNA synthesis. Partial (70%) hepatectomy in the rat causes large increases in the activites of ornithine decarboxylase and thymidine kinase as the remnant liver regenerates (7). Sham hepatectomy (laparotomy) has been reported to have no effect on either enzyme (8,9). During studies of *in vitro* translation products of regenerating liver resol-

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ved by two-dimensional gel electrophoresis, they noted that, in addition to being increased after partial hepatectomy, the activites of ODC was low after sham hepatectomy. Since food consumption is decreased after surgery, the possibility that reduced caloric intake could effect ODC activity (10,11).

The objectives of this study are to provide informations on the changes of ODC enzyme activity and mRNA level after hepatectomy.

MATERIALS AND METHODS

Materials

Reagents for RNA extraction, gels and hybridization studies and enzyme assays were from BRL laboratory (Gaithersbury, MD), and Sigma-Aldrich (St. Louis, MO). α-[^{32}P]dATP and [1-^{14}C] ornithine were perchased from New England Nuclear (Boston, MA) and [^{3}H] tymidine was perchased from Amhersham (Arlington Height, IL). All other reagents were of analytical grade.

ODC assay

ODC activity was determined by measuring the release of $^{14}CO_2$ from L-[1-^{14}C]ornithine at 37° C. The assay mixture contained 0.4mM L-ornithine, 0.125 μCi of L-[1-^{14}C] ornithine (58.5mCi/mmol), 0.05mM pyridoxal 5′-phosphate, 2.5mM DTT, 25mM Tris-HCl, pH 7.4, and enzyme in a total volumn of 125μl. The reaction was stopped by addition of 0.8ml of 40 % TCA. $^{14}CO_2$ was trapped for 30min with 120μl of methylbenzethonium hydroxide in center wells and subsequently countered by liquid scintillation. ODC units are pmol CO_2 released in 30min per mg protein of cytosol supernatant. The decarboxylation of L-[1-^{14}C] ornithine was shown to be ODC specific by including 1mM α-difluro-methylornithine (DFMO) in control assays (4,12,13).

Treatments

Male Sprague-Dawley (SD) rats (190~210g, Bio Science Labs. North Dakota State University Experimental Station) were maintained on a cycle of twelve hours light (8 am to 8 pm) and twelve hour darkness (8 pm to 8 am). Water and chow were available *ad libitum*. Partial hepatectomy was performed by resecting the left and median lobes of the liver under either anesthesia between 8 and 10 am. Sham hepatectomy was carried out by resecting a small piece of omental fat during leparotomy. Rats were killed for investigation and assay 48 hours later (8).

Starving and pair-feeding

Food intake was recorded before and after sham hepatectomy. On the day of sham hepatectomy (8 am to 8 pm) the rats involved for treatments began to eat around a 5 pm. The amount of food consumed by the sham-operated rats between 5 pm and 8 am was measured in the next morning (7).

A group of rats was matched to the same schedule: food was not provided between 8 am and 5 pm, and the next moring between 8 am and 5 pm, the amount of food consumed by the rats undergoing sham hepatectomy was measured.

RNA isolation and blots analysis

Total RNA was isolated by the guanidine thiocyanate method. Poly (A) RNA was isolated using oligo d (T) cellulose.

For Northern blot analysis, 5mg of poly(A) RNA was fractionated on 1.2% agarose gel contaning 2.2 M formaldehyde and blotted onto nitrocellulose or nylon membranes. The blots were prehybridized for 6hrs at 42° C in solution containing 50% formamide, 3× SSC, 5× Denhart's solution (1× Denhardt's contains 0.1% each of bovine serum albumin, polyvinyl pyrrollidone and Ficoll), 0.1% SDS, 10% dextran sulfate and 100mg/ml denatured herring sperm DNA. Hybridization used the same solution with the Riboprobe for 12days at 42° C. The blots were washed twice at room temperature in 2× SSC, 0.1% SDS for 5 min each followed by washing twice at 50° C in 0.1 % SSC, 0.1× SDS for 20min each. Blots were exposed to Kodak XAR films with intensifying screens at −70° C (12,14).

Riboprobe preparation

Plasmid pHOD48, which was constructed as shown in Fig. 1, contains a *Hind*III fragment of mouse cDNA

clone pOD48. This plasmid DNA was used as a template for ^{32}P-labeled Riboprobe synthesis for 1 hours at 37°C with T7 RNA polymerase. This plasmid DNA template as linearized prior to preparation of run-off transcripts. Riboprobe transcripts made by this method have a specific activity of approximately $3\sim8\times10^8$ cpm/mg RNA (12,14).

Quantitation and statistics

Blots were quantitated by densitometry. Comparisons between experimental groups were made by analysis of variance. Significant differences were set at $p<0.05$. All data were expressed as mean±SE (standard error).

RESULTS AND DISCUSSION

After hepatectomy (Table 1), ODC activity increased significantly ($p<0.05$) from 11.7±0.4 in normal liver (control) to 90.5±3.2 at 24 hours and 73.7±6.6 at 48 hours. After sham hepatectomy, ODC activity decreased significantly to 5.5±0.2 at 24 hours and 9.1±0.6 at 48 hours.

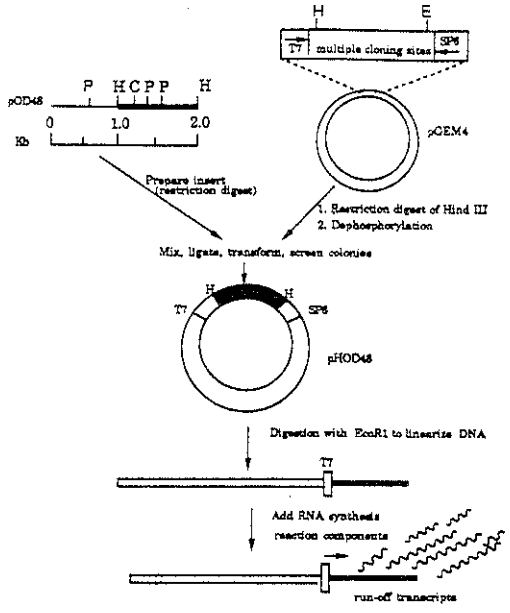

Fig. 1. Schematic diagram of Riboprobe preparation. Plasmid pOD48 is a mouse ODC cDNA clone. A 900bp *Hind*III fragment from pOD48 was subcloned into plasmid pGEM4 DNA. The orientation of the subclone insert was determined by restriction mapping and sequence analysis of run off transcripts. Radiolabeled riboprobe transcripts was prepared with T7 RNA polymerase. SP6, SP6 RNA polymerase promoter; T7, T7 RNA polymerase promoter; P, *Pst*I; H, *Hind*III; C, *Hin*III.

The possibility that reduced food consumption was the cause of the declines in ODC activities after sham hepatectomy was investigated in starved and pair-fed rats.

During three days before sham hepatectomy nine rats consumed 25±1g lab chow per day. After sham hepatectomy, the first rat began to eat at 5 pm the day of surgery and the nine rats consumed 15±1g food between 5 pm and 8 am the next morning (significantly different, $p<0.05$, from daily intake before surgery). Food intake after partial hepatectomy was quite similar. Twelve rats were pair-fed on this schedule : between 8 am and 5 pm no food was available and between 5 pm and 8 am next morning each rat was provided 15g food. In all cases, the entire 15g was consumed by these rats. Another group of 12 rats was starved 24 hours (8 am to 8 am).

ODC activity in the pair-fed rats (Table 1) was 5.0±1.1, significantly less ($p<0.05$) than normal. ODC activity was further redued to 3.2±0.3 in rats starved for 24 hours. This was significantly less ($p<0.05$) than normal and sham-operated rats.

Liver ODC mRNA content was determined in Northern blot of poly (A) RNA from normal, 24 hour partial and sham hepatectomy, and 24 hour pair-fed and starved rats (n=8~11 per group). As determined in Fig. 2, ODC mRNA content were increased at the 24 hour interval after partial hepatectomy. In contrast, there was no consistent effect of sham hepatectomy, pair

Table 1. Response of ornithine decarboxylase activity

Treatment	ODC
Normal(n=20)	11.7±0.4
Partial (24h, n=12)	90.5±3.2[a]
Partial (48h, n=12)	73.7±6.6[a]
Sham (24h, n=12)	5.5±0.2[a]
Sham (48h, n=12)	9.1±0.6[a]
Pair-fed (24h, n=12)	5.0±1.1[a]
Pair-fed (24h, n=12)	3.2±0.3[a,b]

[a] Significantly different than normal : [b]significantly less different than 24h Sham. Partial : 70% partial hepatectomy ; Sham hepatectomy
Data are mean±SE (standard error)

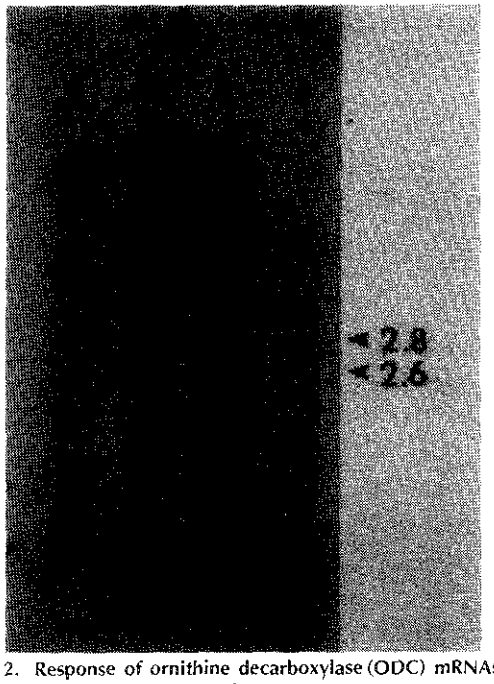

Fig. 2. **Response of ornithine decarboxylase (ODC) mRNAs from rat liver to partial hepatectomy.**
Lanes 1, 2, 3 and 4 rats were treated as described in MATERIALS AND METHODS. mRNA was isolated from total RNA, fractionated on 1.2% agarose gel, transferred onto nitrocellulose membranes, and blotted with Riboprobe. Blots were exposed to Kodak XAR films with intensifying screens at −70°C.

feeding or starving on the hepatic mRNA contents of ODC on Northern blot, and these groups were not significantly different from normal.

Partial hepatectomy is known to increase the activities of ODC as the liver regenerates, a fact confirmed in the present studies (7). Our results show that these elevations are accompanied by increases in ODC mRNA contents at the 24 hour interval. A previous study has demonstrated similiarly increased ODC mRNA levels during the first 6 hours after parital hepatectomy. Other studies showed that the increase in TK activity are accompanied by increased TK mRNA levels (8,9). This result also indicated that the rat has two TK mRNAs, both of which are increased 24 hours after partial hepatectomy. This has been observed in rat muscle mRNA blots using a cloned fragment of the human TK cDNA.

The present study shows that ODC activities are significantly reduced after sham hepatectomy. Previous reports have indicated no effect of sham hepatectomy on ODC or TK enzyme activity (8). One reason for these differences may be the larger number of rats used in the present studies. The effect of sham hepatectomy is small compared with that of partial hepatectomy and may be missed; however, it is consistent and is clearly statistically significant.

The fact that pair-feeding and starving lower ODC activities suggests that decreased food intake after surgery is the cause of the decline. This in turn raises the possibility that a dietary factor or an endogenous factor controlled by nutrient consumption influence these enzymes and may be involved in their physiological control in the liver. Consistent with this hypothesis is the observation that starved rats had the lowest enzyme levels, where pair-fed rats had levels that were close to the sham-operated rats. Previous many studies have also found that liver ODC activity is reduced by starvation (9,10,12).

Such factors are not required, however, since partial hepatectomy induces substantial increases, when food consumption is similiar to sham operated rats. This changes in the amount of ODC protein could be brought about by changes in both the rate of synthesis and the rate of degradation of the protein (4).

The decrease in ODC enzyme activity in sham hepatectomy, starvation and pair-feeding were not accompanied by any consistent changes in hepatic mRNA content increase after partial hepatectomy, possibilities for control in regenerating liver exist at both pretranslational and posttranslational levels. Many accumulated evidence has been indicated that ODC is regulated by multiple mechanisms including transcription, translation, post-translational activation, and enzyme degradation (6,12).

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절제수술후에 일어나는 생쥐 오르니틴 탈탄산효소(ODC)의 변화

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요 약

오르니틴 탈탄산효소(ODC)는 putrescine, spermine, 그리고 spermidine과 같은 polyamine을 생산하는 생합성기작의 첫단계이고 또한 율속단계에 있는 효소이다. 그리고 이 효소는 간이 재생될 때 갑작스럽게 효소의 역가가 증가한다고 알려져 있다. 본 연구에서는 생쥐의 간을 부분 절제수술 후에는 오르니틴 탈탄산효소와 mRNA의 변화가 급격하게 증가하였으나, 간을 절제하지 않은 수술에서는 mRNA의 양적인 변화가 없었으나 효소역가는 훨씬 감소된 값을 보였다. 그러나 mRNA와 효소역가의 무변화를 보였다는 여러 연구와는 상반된 결과를 보여주고 있다. 그러므로 보다 세밀한 분석은 수술 후 음식물의 투여가 본 효소의 역가변화 그리고 mRNA의 양적 변화에 미치는 영향에 대한 연구가 필요하다 하겠다.