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EFFECT OF ADRENERGIC AND Ca^{2+} ANTAGONISTS ON INCREASED ORNITHINE DECARBOXYLASE EXPRESSION IN REGENERATING RAT LIVER*

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Abstract—Partial hepatectomy (PH) (70% resection) causes within 4 hr an accumulation of ornithine decarboxylase (EC 4.1.1.17, ODC) mRNAs concomitant with an increase in ODC activity, maximum values being observed at 8 and 16 hr, respectively. In the early hours of hepatic regeneration, enhancement of transcriptional-rate of ODC gene, demonstrated by nuclear run-on analysis, can account for the accumulation of ODC mRNAs. The involvement of catecholamines in these processes is demonstrated by using prazosin and propranolol, specific antagonists of α_1 and β adrenoceptors, respectively. Prazosin reduces almost completely the rise of ODC activity at 4 hr, without affecting mRNA levels. At 16 hr, enzyme activity and mRNAs increase, however, over the values observed in regenerating liver of prazosin-untreated animals. These findings suggest that α_1-receptor activation triggers positive control signals for ODC gene expression at the early time of liver regeneration and, on the contrary, negative signals at later times by mainly post-transcriptional and transcriptional mechanisms, respectively. Propranolol reduces similarly the initial 4 hr-rise of ODC activity. These results indicate that activation of both α_1- and β-adrenoceptors causes the large increase in ODC activity. Pharmacological manipulation of intracellular Ca^{2+} levels by verapamil, a Ca^{2+}-channel blocker, or neomycin, an inhibitor of Ca^{2+} release from endogenous stores, diminishes ODC activity at 4 and 16 hr after PH. ODC mRNA levels, which are not modified at 4 hr, increase over the values of partially hepatectomized rat liver at 16 hr. Trifluoperazine inhibits both ODC activity and mRNA accumulation at the times studied. As a working hypothesis it is proposed that Ca^{2+}-mediated processes induced by catecholamines are involved in ODC gene expression during the prereplicative phase of liver regeneration.

The entry of quiescent animal hepatocytes into the cell cycle after partial hepatectomy (PH)¶ (70% resection) is triggered by hormones and growth factors circulating in the blood [1–8]. The progression of liver cells towards DNA synthesis is controlled essentially by autocrine and paracrine factors secreted by the hepatic cells which stimulate appropriate receptors [9]. Sequential expression of the proto-oncogenes c-*fos*, c-*myc*, p-53 and c-*ras*, which occurs during the prereplicative stage of liver regeneration upon stimulation by humoral factors, is involved in growth and division of hepatocytes after PH [10, 11]. Thus, different and possibly interdependent mechanisms regulate liver compensatory growth, either at the initial hypertrophic phase which lasts for 12–16 hr or later at the replicative stage when the hepatocytes undergo DNA replication (24 hr) and mitosis (30 hr).

To gain further insight into the humoral control of liver regeneration, used as model of normal cell growth, we studied some aspects of the hormone-dependent gene expression of L-ornithine decarboxylase (ODC), a key enzyme in polyamine biosynthesis [12]. Increase in ODC activity, which is one of the earliest biochemical events of the prereplicative phase occurring in the remnant liver after PH, has been implicated in the regulation of this growth process [13–16]. In fact, irreversible inhibition of ODC activity by DL-α-difluoromethylornithine (F_2-MeOrn) impairs this process [17–20].

There is evidence that catecholamines interact with α_1-adrenoceptors to stimulate hepatocyte DNA synthesis [6] and to regulate DNA content in regenerating liver [21], through the action of Ca^{2+} as intracellular messenger [22]. Ca^{2+} movements and/or phosphatidylinositol turnover seem to be especially important for liver cell proliferation [23, 24]. Changes in cyclic AMP concentration may have an additional controlling effect in the early phases of hepatic regeneration [25]. Regulation of ODC expression by these factors is likely to be a complex process.

This study attempts to clarify the level of ODC gene control in regenerating rat liver and the different mitogenic signalling pathways involved. ODC mRNA accumulation, nuclear transcription-rate at various times after PH and their relationship with ODC activity have been determined. The pattern of

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¶ Abbreviations: PH, partial hepatectomy; ODC, L-ornithine decarboxylase; F_2-MeOrn, DL-α-difluoromethylornithine; IP_3, D-*myo*-inositol 1,4,5,-triphosphate; Tris–HCl, 2 - amino - 2 - hydroxymethylpropane -1,3- diol hydrochloride; SDS, sodium dodecyl sulfate.

ODC activity and mRNA levels were then investigated in the liver of hepatectomized rats given α_1- or β-adrenergic blocking agents as well as inhibitors of Ca^{2+} channels, of intracellular non-mitochondrial Ca^{2+} release or of calmodulin, a Ca^{2+}-binding modulator protein.

The results demonstrate that PH causes a rapid and long-lasting accumulation of ODC mRNAs, associated with an increase in ODC activity. Catecholamines seem to exert a positive control on ODC expression at the early time of hepatic regeneration, and a negative control at a later stage through the activation of α_1-adrenoceptors and modulation of intracellular Ca^{2+} levels. Moreover, β-adrenoceptor activation is also implicated in the increase of ODC activity observed at early times. The mechanisms involved are most likely to be post-transcriptional and transcriptional during the two phases respectively.

MATERIALS AND METHODS

Materials. DL-[1-^{14}C]ornithine (58 mCi/mmol), [^{32}P]deoxycytidine triphosphate (dCTP) (3000 Ci/mmol) and [^{32}P]uridine triphosphate (UTP) (3000 Ci/mmol) were purchased from Amersham International Ltd. (Amersham, Bucks, U.K.). Prazosin was from Pfizer (Pomezia, Rome, Italy). Propranolol hydrochloride, verapamil hydrochloride, trifluoperazine dihydrochloride and neomycin were from the Sigma Chemical Co. (St Louis, MO, U.S.A.). F_2-MeOrn (MDL 71782) was generously given by Merrell Dow Research Institute (Strasbourg, France). All other chemicals were of the highest grade available.

Animals and treatments. Male Wistar rats (150–170 g) were purchased from Charles River (Calco, Como, Italy) and maintained under a 12 hr light/12 hr dark cycle in a temperature-controlled environment with food and water available *ad lib*. Rats were partially hepatectomized by the method of Higgins and Anderson [26], under oxygen–ether anesthesia.

The inhibitors were administered to rats according to these schedules: prazosin (1 mg/kg body wt) [27, 28], propranolol (10 mg/kg body wt) [27, 28], verapamil (25 mg/kg body wt) [29] or trifluoperazine (30 mg/kg body wt) [29] dissolved in saline was injected i.p. at the time of the surgical operation; neomycin (100 mg/kg body wt) dissolved in saline was injected s.c. daily for 6 days before the operation, and the rats were killed approximately 24 hr after the last injection [30]. At the effective dose used, not all the drugs caused apparent toxic side effects. For sham-operation, rats were anesthetized, an abdominal incision was made and the liver was manipulated but not removed. Some of these rats were treated with prazosin or propranolol at the dose above reported.

The time of surgery varied, but all the animals were killed between 10.00 and 12.00 a.m. to minimize variations in ODC activity resulting from circadian rhythm. The livers were quickly frozen in liquid nitrogen after the removal for RNA analyses and ODC assay or immediately used for nuclei preparation.

RNA extraction and analysis. Liver samples were homogenized in 4 M guanidine isothiocyanate, and total RNA was collected by centrifugation in 5.7 M CsCl containing 4 M sodium acetate (pH 5) at 35,000 rpm in a Beckman SW40 Ti rotor for 16 hr. The pellet was resuspended in 10 mM 2-amino-2-hydroxymethylpropane-1,3-diol hydrochloride (Tris–HCl) buffer (pH 8.0), 5 mM EDTA, 1% sodium dodecyl sulfate (SDS), and the RNA was purified by two extractions in chloroform/isobutanol (4/1, v/v) and was precipitated in ethanol.

For Northern blot analysis, the RNA (20 μg) was heated at 65° for 5 min in a denaturing buffer = 50% formamide/2.2 M formaldehyde/1 × Mops (1 × Mops = 18 mM 3-(*N*-morpholino)propane-sulfonic acid/0.4 M sodium acetate/50 mM EDTA). Loading buffer (50% glycerol/0.05% xylene cyanide/0.05% blue bromophenol) was added to the samples after cooling at room temperature and prior to gel electrophoresis in 1% agarose containing 2.2 M formaldehyde. Running buffer was 1 × Mops. The gel was then washed and transferred onto a nylon filter (Hybond-N, Amersham) equilibrated in 20 × SSC (1 × SSC = 0.15 M sodium chloride/15 mM trisodium citrate). The nylon filters were hybridized with [^{32}P]-labelled probe (2 × 10^6 cpm/mL) in 10% (w/v) dextran sulfate, 50% formamide, 1 × Denhardt's solution and competing DNA and RNA [31]. The probe utilized was the 750 base pairs Pst I fragment obtained from the pOD48 plasmid containing the ODC cDNA [32], kindly provided by Dr P. Coffino (University of California, San Francisco). The probe was labelled by primer extension using a multiprimer Kit (Amersham, No. RPN. 1601 Z). Hybridization was performed at 42° for 18 hr. Hybridized filters were washed twice by 500 mL of a 300 mM NaCl solution containing 30 mM sodium citrate and 0.1% SDS at room temperature, and twice by 500 ml of a 30 mM NaCl solution containing 3 mM sodium citrate and 0.1% SDS at 52°. Hybridized filters were exposed to Kodak XAR-S film with intensifying screens at −70°.

RNA slot blots were performed with a Schleicher and Schuell manifold. RNA samples of 20 μg were added to the denaturing buffer and processed as above reported. Samples were vacuum-filtered through the manifold onto a nylon membrane equilibrated with 20 × SSC. The filter was baked *in vacuo* at 80° for 2 hr, and hybridized as described above.

RNA molecular weights were estimated by using a RNA ladder (BRL) as standard. Densitometric analyses were carried out by using a LKB ultrascan.

In vitro *nuclear transcription assay.* Nuclei from regenerating livers, at 4 and 8 hr after PH, and from normal liver were prepared as previously described [33]. *In vitro* transcription was carried out according to Groudine *et al.* [34] using radiolabelled UTP. Nuclear RNA was isolated as described by Gariglio *et al.* [35] by extraction with phenol/chloroform and chloroform, followed by isopropyl alcohol precipitation. The pellets were resuspended in 10 mM Tris–HCl (pH 8.0) containing 1 mM EDTA. Free ^{32}P was eliminated on a Sephadex G-50 spin column (Boehringer-Mannheim Biochemicals, Penzberg, F.R.G.). The same number of cpm of ^{32}P-labelled nuclear RNA were hybridized for each sample. The hybridization was performed on nylon filters, as

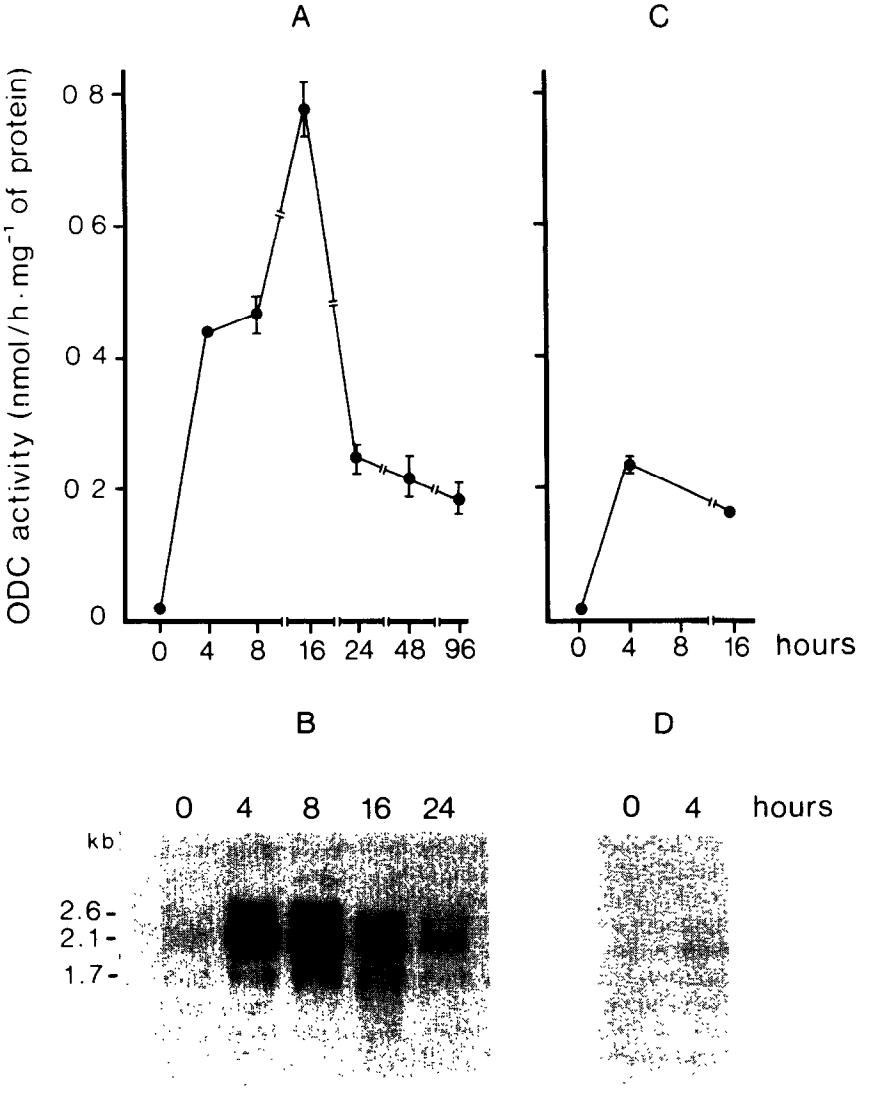

Fig. 1. Time-course of hepatic ODC activity and mRNA levels after PH and sham-operation. ODC activity (A, C) and mRNA levels (B, D) in regenerating liver of partially hepatectomized rats (A, B) and in the liver of sham-operated rats (C, D) were measured. As reported in Materials and Methods, ODC activity was assayed as $^{14}CO_2$ release from labelled ornithine, and total ODC RNA was analysed by Northern blot. Approximate size of the mRNA species was estimated from RNA standards run in parallel. For ODC activity, each point is the mean ± SE of five animals. SE is not shown when enclosed within the symbol.

described for DNA hybridization, except that in this case 10 μg of linearized plasmid had been immobilized on the nylon filters using a slot blot apparatus.

ODC activity assay. ODC activity was determined by measuring the release of $^{14}CO_2$ from [1-^{14}C]ornithine by the method of Jänne and Williams-Ashman [36]. In brief, livers were homogenized in 9 vol. of a buffer containing 0.25 M sucrose and 20 mM Tris–HCl (pH 7.1). The homogenate was centrifuged at 18,000 *g* for 30 min at 2° and the cytosol immediately used for the enzyme assay. The reaction mixture, in a final volume of 0.5 mL, contained the cytosolic supernatant (about 3 mg of protein), 50 mM Tris–HCl (pH 7.1), 0.1 mM pyridoxal phosphate, 5 mM dithiothreitol, 1 mM L-ornithine and 0.5 μCi of [1-^{14}C]ornithine, so that its final specific activity was 1 mCi/mmol. All samples were preincubated for 20 min. Blanks were obtained by preincubation in the presence of 4 mM F_2-MeOrn, a specific irreversible inhibitor of ODC [37]. The reaction was started by addition of the labelled plus cold ornithine solution. The incubation was carried out for 1 hr at 37° in test tubes closed with a stopper crossed by a needle, which supported a 6 mm disc of paper (Schleicher and Schuell), wetted with Protosol (New England Nuclear) for trapping the released $^{14}CO_2$.

Table 1. Effects of adrenoceptor antagonists on ODC activity and mRNA levels in the liver of hepatectomized rats*

	PH		PH + prazosin		PH + propranolol	
	ODC		ODC		mRNA	
Hours	Activity	mRNA	Activity	mRNA	Activity	mRNA
0	20 ± 2	0.2	—	—	—	—
4	439 ± 28	0.7	74 ± 24‡	0.6	155 ± 56‡	0.5
8	463 ± 45	1.4	525 ± 130	1.0	612 ± 165	1.1
16	777 ± 79	1.2	1406 ± 229†	2.4	894 ± 141	1.2
24	243 ± 35	1.0	576 ± 149†	1.2	398 ± 24†	0.9

* Prazosin (1 mg/kg body wt) or propranolol (10 mg/kg body wt) was administered intraperitoneally to rats at the time of PH. ODC activities and mRNA levels were determined at the indicated times as described in Material and Methods. ODC activities are expressed as pmol/hr/mg protein (± SE, N = 5), and ODC mRNA as absorbance of a typical slot blot experiment (see Fig. 3).

† $P < 0.05$ (Dunnett's test).

‡ $P < 0.01$ (Dunnett's test).

Protein content of supernatants was determined by the procedure of Lowry *et al.* [38].

RESULTS

Kinetic of ODC activity and mRNA accumulation in regenerating rat liver

The availability of an ODC cDNA probe made it possible to verify the hypothesis that a specific RNA modulation is involved in the well-known increase in ODC activity after PH [13–15]. As shown in Fig. 1A, ODC activity increased within 4 hr (20-fold) after PH. Thereafter, it remained constant until 8 hr and peaked (40-fold) at 16 hr. By 24 hr after PH the enzyme activity declined significantly, but was still higher than control value at 96 hr.

The kinetic analysis of ODC mRNA levels in regenerating rat liver was performed on total RNA isolated at different times of hepatic regeneration by Northern blot. A typical autoradiogram from these blots is reported in Fig. 1B. The ODC-labelled probe hybridized with three distinct RNA species: two major species of ~2.6 and 2.1 kb, and a minor species of ~1.7 kb. These RNA species were markedly induced at 4 hr of regeneration (~4-fold) to reach maximum levels at 8 hr (~7-fold) as determined by scanning densitometry (Fig. 1B, Table 1), and declined slightly, thereafter. The three hybridizing-ODC RNA bands seemed to be co-ordinately regulated, and the 2.1 kb mRNA was the main hybridizable message at the times studied. A mouse instead of a rat probe can be utilized because mouse-rat cDNA homology is known to be about 94% [39] in the coding region.

In the liver of sham-operated rats a marked increase in ODC activity was also observed at 4 hr (about 10-fold) (Fig. 1C). This increase was transient and smaller than that of regenerating liver at 4 hr, and was not associated with significant accumulation of mRNA (Fig. 1D).

In vitro *nuclear transcription assay*

The relative rates of ODC gene transcription were next examined by using the nuclear run-on transcription assay in isolated nuclei. The *in vitro* labelled RNA transcripts of nuclei isolated from normal and

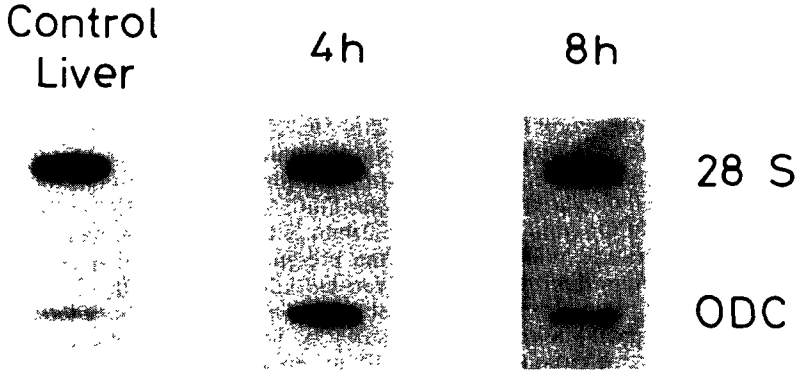

Fig. 2. PH-induced transcription of ODC gene. Nuclei from normal or regenerating rat liver at 4 and 8 hr after PH were isolated. The ^{32}P-labelled run-on transcripts from each nuclear reaction sample were hybridized to excess denatured ODC cDNA or 28S RNA probe as reported in Materials and Methods.

regenerating liver, at 4 and 8 hr after PH, were hybridized to excess denatured ODC cDNA (Fig. 2). Measurement of the transcription rate of ribosomal 28S RNA was used as control since this rate did not vary during liver regeneration. ODC gene transcription rate was markedly increased at 4 hr after PH diminishing to values close to control, thereafter (8 hr).

Effect of adrenergic receptor antagonists on ODC activity and mRNA levels

Table 1 shows the effects of prazosin an α_1-receptor antagonist, and of propanolol a β-receptor antagonist on ODC activity and mRNA levels from liver of hepatectomized rats. Prazosin and propranolol inhibit the inositol phospholipid turnover and the adenyl cyclase/cyclic AMP system activated by catecholamines, respectively. Therefore, they prevent the cell metabolic events mediated by these two transducing mechanisms [5, 6, 40]. The treatment of rats with prazosin and propranolol at the time of PH prevented the increase of ODC activity occurring at 4 hr by 85 and 65%, respectively. The effects of the adrenergic receptor antagonists were, however, transient since the enzyme activity was similar to control value at 8 hr. Thereafter, the α_1-blocker superinduced ODC activity by 1.8-fold at 16 hr and 2.4-fold at 24 hr. A similar induction by the β-blocker occurred only at 24 hr (1.7-fold).

The two adrenergic blocking agents administered separately did not change ODC activity at 4 hr after sham-operation (data not shown). It is known that inhibition of catecholamine biosynthesis or depletion of nervous terminals from catecholamines prevents the enhancement of ODC activity due to laparatomy. In fact, presence of catecholamines in blood stream is sufficient to facilitate the stimulatory action of glucocorticoid on ODC activity in sham-operated rat liver [41].

Administration of prazosin and propranolol reduced the increase in ODC mRNA levels in regenerating liver at 4 hr by 20 and 40%, respectively, and at 8 hr by 33 and 25%, respectively (Table 1, Fig. 3). Thereafter, prazosin but not propranolol treatment induced a 2-fold increase in ODC mRNA relative to untreated hepatectomized rats.

Effects of antagonists of Ca^{2+} movements and trifluoperazine on ODC activity and mRNA levels

The role of Ca^{2+}-mediated process(es) in the regulation of ODC activity and gene expression during liver regeneration was evaluated. The consequences of blockade of exogenous Ca^{2+} uptake was investigated using verapamil, which is an inhibitor of the plasmalemma slow Ca^{2+} channels [29, 42]. Adrenergic stimulation of α_1-adrenoceptors also triggers release of Ca^{2+} from endoplasmic reticulum. This release is coupled to the generation of D-*myo*-inositol 1,4,5-triphosphate (IP_3) by the enzymatic hydrolysis of plasmalemma phosphatidylinositol-4,5-diphosphate. Therefore, we studied the effect of a subacute treatment by neomycin, an inhibitor of IP_3-mediated Ca^{2+} release at the level of intracellular non-mitochondrial pools [30, 43, 44].

As reported in Table 2, verapamil decreased ODC activity by 55% at 4 hr after PH. Thereafter, enzyme activity increased in parallel to control. Neomycin depressed ODC activity by about 40% at 4 and 16 hr. The slight differential effects of verapamil and neomycin could result from either dissimilar pharmacokinetics of the drugs or different involvement of the two Ca^{2+} signalling systems in ODC induction. Trifluoperazine, an inhibitor of the Ca^{2+}-binding protein calmodulin [29, 45], was more effective than the other drugs at suppressing ODC activity (71 and 63% inhibition at 4 and 16 hr after PH, respectively).

As seen in Table 2 and Fig. 4, Ca^{2+}-movement antagonists did not affect ODC mRNA levels of 4 hr-regenerating liver, while they caused a striking ODC mRNA accumulation at 16 hr. In contrast, trifluoperazine reduced ODC mRNA levels by about 25% at 4 and 16 hr. Following all three drug treatments, the mRNA levels were relatively higher than those of ODC activity.

In no case were modifications of ODC activity and mRNA levels observed in livers of sham-operated rats (data not shown).

DISCUSSION

The importance of polyamines in tissue growth is reflected by the tight regulation at multiple levels of ODC, one of the key enzymes of the polyamine biosynthetic pathway [12, 46–49]. The data, presented in this paper, confirm the previously described biphasic increase in ODC activity in regenerating rat liver after PH [13–16], and demonstrate that it is associated with an accumulation in ODC mRNAs levels, maximum accumulation occurring at 8 hr. Induction of ODC activity by various growth stimuli is also accompanied by an increase in ODC mRNA levels in kidneys from androgen-treated mice [50, 51], rat pheochromocitoma cells treated with nerve growth factor [52] and fibroblasts stimulated by serum and growth factors [53].

An apparent relation between ODC activity and mRNA levels can be established at 4 and 16 hr of hepatic regeneration. However, the rise in ODC activity after PH (about 20-fold at 4–8 hr and 40-fold at 16 hr) is larger than that of ODC mRNA (4-, 7- and 6-fold at 4, 8 and 16 hr, respectively) (Fig. 1; Tables 1 and 2). This suggests that in addition to control on transcription, regulatory mechanisms may affect the efficiency of ODC RNA translation and/or ODC activity as well as the enzyme protein half-life in regenerating liver as it has been shown for other cell systems [48, 49]. The stress due to sham-operation increases transiently ODC activity [41], and causes a slight accumulation of ODC mRNA. It is likely, however, that the marked and long-lasting modifications in ODC expression observed after PH are more extensively related to the regenerative stimulus.

Two major species of 2.6 and 2.1 kb and a minor species of 1.7 kb of hybridizable ODC mRNA are detected in rat liver and are induced by PH. The presence of three mRNA species of similar size has been found in rat tissues, such as hepatoma cells stimulated by 12-*O*-tetradecanoylphorbol-13-acetate [54] and recently in regenerating liver [55], but not in other rodent and human tissues. These observations suggest that in rat liver either some different splicing

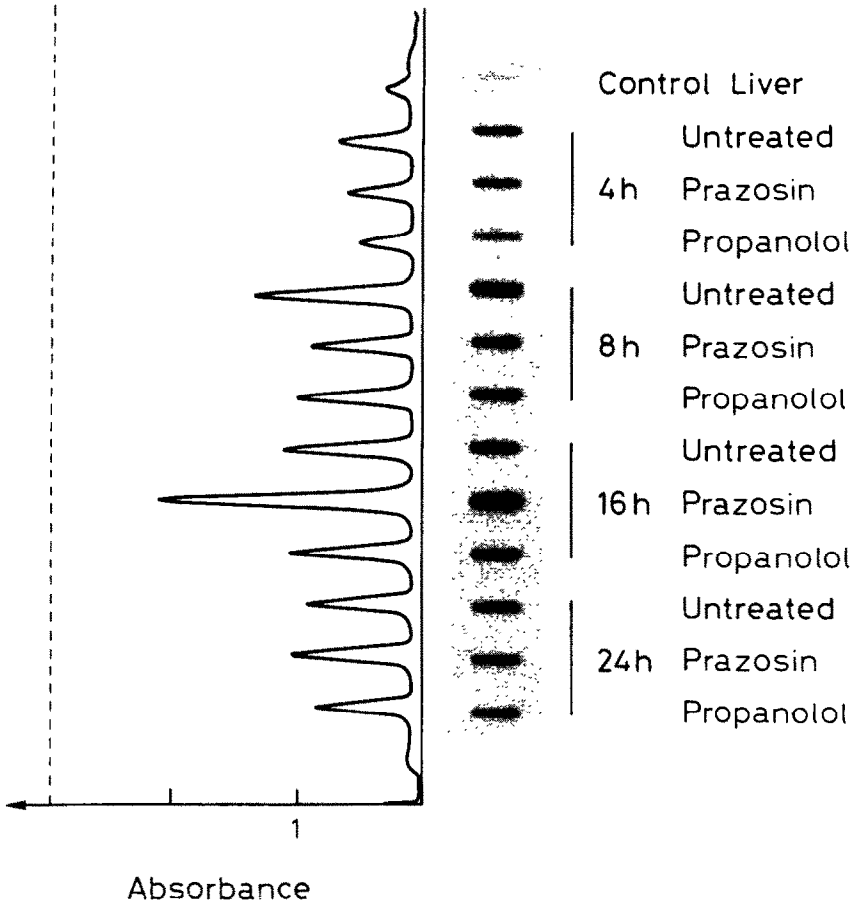

Fig. 3. Effects of adrenergic receptor antagonists prazosin and propranolol on ODC mRNA levels. Partially hepatectomized rats were treated with prazosin or propranolol as reported in Materials and Methods. Total ODC RNA was analysed by slot blot technique and RNA levels were determined by densitometric scanning.

Table 2. Effects of Ca^{2+} movement antagonists on ODC activity and mRNA levels in the liver of hepatectomized rats*

	PH ODC		PH + verapamil ODC		PH + neomycin ODC		PH + trifluoperazine ODC	
Hours	Activity	mRNA	Activity	mRNA	Activity	mRNA	Activity	mRNA
4	531 ± 58	0.9	241 ± 22‡	1.0	333 ± 43‡	0.9	152 ± 14‡	0.7
16	754 ±66	1.3	469 ± 58†	2.1	409 ± 46‡	2.0	285 ± 47‡	1.0

* Verapamil, neomycine and trifluoperazine were administered to partially hepatectomized rats, and ODC activities and mRNA levels were determined at the indicated times as described in Materials and Methods. ODC activities are expressed as pmol/hr/mg protein (± SE, N = 5), and mRNA as absorbance of a typical slot blot experiment (see Fig. 4).

† $P < 0.05$ (Mann-Whitney's test).

‡ $P < 0.01$ (Mann-Whitney's test).

processes or structural gene and pseudogene transcription can exist. In fact, ODC belongs to a multigene family [49, 56, 57]. It cannot be considered, however, established that the 1.7 kb species does not derive from an artefactual process during isolation.

The measurement of ODC transcripts in nuclei isolated from normal and regenerating rat liver by nuclear run-on assay gives evidence that PH increases ODC gene transcriptional rate. In fact *in vitro* nuclear incorporation of labelled ribo-

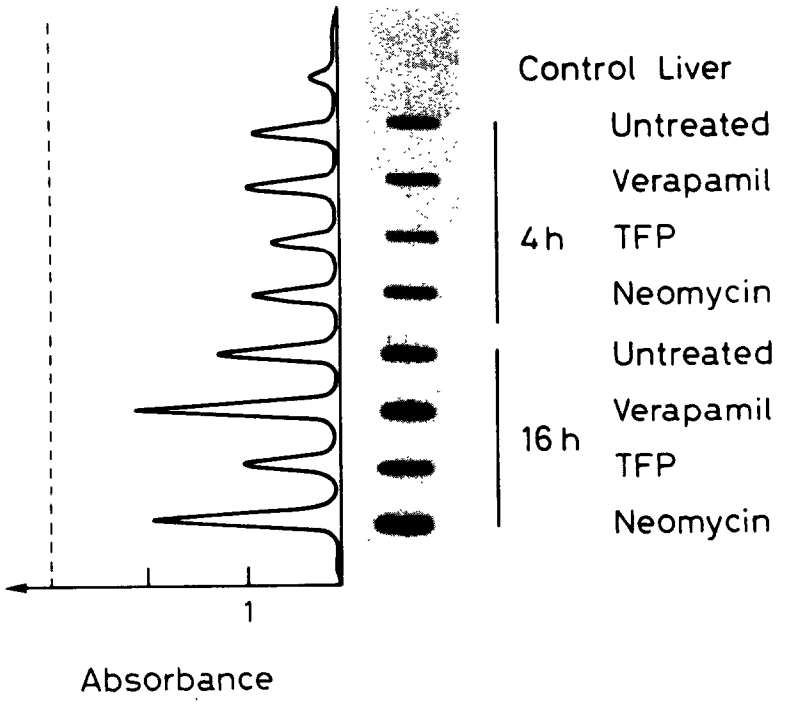

Fig. 4. Effects of Ca^{2+} antagonists and trifluoperazine on ODC mRNA levels. Partially hepatectomized rats were treated with the Ca^{2+} antagonists verapamil, neomycin, or with trifluoperazine as reported in Materials and Methods. Total ODC RNA was analysed by slot blot technique and RNA levels were determined by densitometric scanning.

nucleotide triphosphate into mRNAs is markedly elevated at 4 hr, but decreases at 8 hr. A more detailed kinetic study should be necessary to clarify the fact that ODC gene transcriptional rate decreases at 8 hr, whereas ODC mRNA levels are higher at 8 hr than at 4 hr. However, it cannot be excluded that the elevated transcriptional rate observed at 4 hr lasts possibly until 6–7 hr.This may account for the higher level of ODC mRNA at 8 hr than at 4 hr, since the half-life of ODC mRNA has been found to be greater than 4 hr in other systems [51, 58, 59].

It is well known that catecholamines play direct (via effects on the liver) and indirect (through modulation of epidermal growth factor and insulin/glucagon levels) roles in the process of liver regeneration in the rat [5]. Our results using prazosin and propranolol, α_1- and β-adrenergic receptor antagonists, respectively, indicate that the ODC gene expression after PH is mediated via adrenoceptor stimulation. Prazosin inhibits ODC activity at 4 hr after PH, without affecting ODC mRNA levels. In contrast, at 16 hr it provokes an accumulation of mRNAs over the levels of hepatectomized-untreated rats and a superinduction of ODC activity. Propranolol exerts a similar inhibitory effect on ODC activity at 4 hr. The early effects result most likely from a direct action of the pharmacological agents, while the biochemical changes observed at later time periods occur probably as secondary responses to the chain of events initiated by compound administrations.

These findings are further evidence in favour of a dual phase regulation of ODC in regenerating liver [60, 61]. Moreover, they suggest a role of catecholamines through α_1- and β-adrenoceptor activations in this process, causing a stimulation on ODC activity within the first hours of regeneration independent on ODC RNA transcription. This primary effect is followed by a negative control on both ODC mRNA accumulation and activity via initial α_1-adrenoceptor activation.

In this study we also used drugs which act at different cellular levels to modify intracellular Ca^{2+} concentrations. Verapamil inhibits slow Ca^{2+} channels localized in the plasmalemma, preventing the uptake of exogenous Ca^{2+} [29, 42, 62]. Neomycin inhibits the IP_3-induced Ca^{2+} release from endogenous stores by chelating IP_3, as reported in rat hepatocytes [44]. This can be the mechanism of the inhibitor action in regenerating liver where IP_3 formation is stimulated by α_1-adrenoceptor activation [6]. However, a block of MgATP-dependent Ca^{2+} uptake cannot be excluded [44]. Verapamil and neomycin inhibit ODC activity enhancements and cause accumulation of ODC mRNAs at 16 hr like α_1-blocking. In fact, it is known that verapamil may affect also the activity of α_1-adrenoceptors [29, 62]. In contrast with Ca^{2+} inhibitors, trifluoperazine, an inhibitor of calmodulin [29, 45], diminishes both ODC mRNA levels and activity. Therefore, the negative effect on ODC gene expression, mediated via Ca^{2+}, seems to be calmodulin-independent. Since trifluoperazine is known to inhibit also protein kinase C [63], a firm conclusion cannot, however, be drawn.

At present we can suggest that intracellular

increase of Ca^{2+}, due to PH activation of α_1-adrenoceptors, may be involved in the negative control of ODC expression in the late prereplicative phase of liver regeneration, but further investigations would be needed to verify this hypothesis.

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