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Inducibility of rat liver cytochrome P-450IA1 (P-450c) mRNA during the partial inhibition of protein synthesis

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A labile protein, responsible for repressing the transcription of the cytochrome P-450IA1 gene, has been postulated on the basis of observations describing "superinduction" of gene expression when Hepa 1c1c7 cells are exposed to an inhibitor of protein synthesis, cycloheximide (CHX*) [1–3]. An attempt to observe this phenomenon *in vivo* was not successful, possibly as a result of cycloheximide-induced depression of labile cytochrome P-450IA1 gene *activator* proteins [4]. The protocol reported here attempts to bypass this obstacle by allowing for the interaction of these potential "activator" proteins with 3-methylcholanthrene (3-MC) prior to the partial inhibition of protein synthesis *in vivo*.

Materials and methods

Materials. Cycloheximide was purchased from the Sigma Chemical Co., St. Louis, MO; L-[4,5-^{3}H]leucine (122.5 Ci/mmol) and Nuclear Chicago Solvent (NCS) were from the Amersham Corp., Arlington Heights, IL; Nytran (0.45 μm) membranes were from Schleicher & Schuell, Inc., Keene, NH.

Some of the plasmids used in these studies were obtained from the following: pRSA57, a cDNA clone for rat serum albumin [5], was provided by Drs. James Bonner and Thomas Sargent; clone 46, a cDNA clone for mouse cytochrome P-450IA1 [6], was a gift from Drs. Daniel Nebert and Masahiko Negishi. All other materials have been described previously [7, 8].

Methods. Male 4-week-old Sprague–Dawley rats that were obtained from Sasco Inc., Omaha, NE, were provided Lab Chow (Ralston Purino Co.) and water *ad lib*. The rats were injected i.p. as follows: CHX (3.0 mg/kg body weight) was administered 2 and 6 hr after 3-MC (25 mg/kg body weight); labeled leucine was injected 8 hr after treatment with 3-MC and 1 hr before the animals were killed. This group was referred to as 3-MC/CHX. The 3-MC/NaCl group received 0.9% NaCl in place of CHX, while the CO/NaCl group received corn oil (the vehicle of 3-MC) and 0.9% NaCl in place of 3-MC and CHX respectively. The CO/CHX group was treated with corn oil and CHX.

Hepatic protein was isolated as described by Cook *et al.* [9]. The trichloroacetic acid-insoluble precipitate was redissolved in 6 vol. (w/v) of NCS at 50%. The incorporated radioactivity was determined directly using a Beckman LS-150 liquid scintillation counting system.

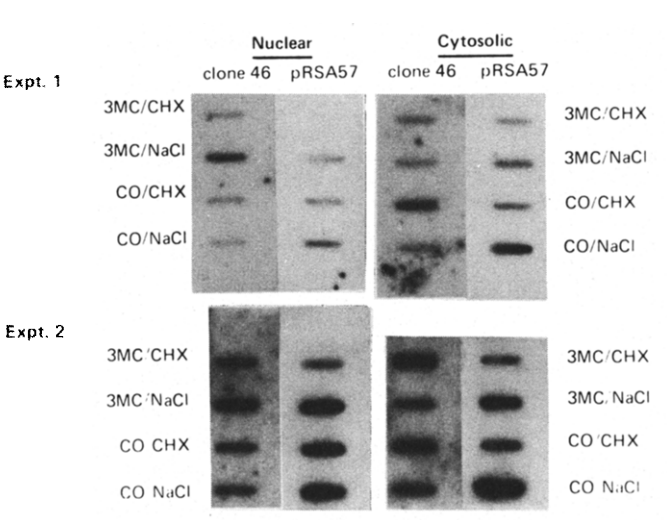
Total hepatic nuclear and cytosolic RNA were isolated essentially as described by Lamers *et al.* [10]. The application of RNA onto a Nytran membrane, prehybridization, hybridization, washing, and conditions for the removal of the probe were as specified by the membrane manufacturer. Radiolabeled DNA for use as hybridization probes was prepared by nick-translation [11]. The intensity of the hybridization signal was determined using an LKB model 2202 UltroScan laser densitometer and Gel Scan software. The intensity of hybridization, which is expressed in arbitrary units, refers to the integrated area of peak absorbance at 633 nm.

Results and discussion

Cycloheximide administration has been demonstrated to result in an inhibition of the incorporation of labeled precursor amino acids into hepatic protein *in vivo* [12]. In our studies, the inhibition of [^{3}H]leucine incorporation into hepatic protein is indicated in Table 1. A 50–70% inhibition of protein synthesis was maintained in the liver over the duration of the experiments (CO/NaCl vs CO/CHX and 3-MC/NaCl vs 3-MC/CHX). 3-Methylcholanthrene administration alone caused a 50% stimulation in the incorporation of [^{3}H]leucine into protein of the liver (3-MC/NaCl vs CO/NaCl).

The effects of 3-MC and CHX treatments upon the steady-state levels of cytochrome P-450IA1 mRNA are indicated in Fig. 1. The hybridization to pRSA57, a probe for the albumin gene, was used to control for differences in retention of RNA on the membrane, and for experimental artifacts. The administration of CHX to control rats, i.e. CO/NaCl vs CO/CHX, resulted in an average increase in steady-state cytochrome P-450IA1 mRNA of 2.1- and 6.2-fold in nuclear and cytosolic compartments respectively. Cycloheximide has been reported to stimulate hepatic nuclear RNA synthesis by 12 hr after treatment [13]. However, as demonstrated in Fig. 1, no marked effect on rat serum albumin mRNA levels was observed. The "general" stimulation of nuclear RNA synthesis, therefore, may be attributable to "specific" nuclear RNA subpopulations. A similar stimulation of cytochrome P-450IA1 mRNA levels

* Abbreviations: CHX, cycloheximide; 3-MC, 3-methylcholanthrene; NCS, Nuclear Chicago Solvent; and CO, corn oil.



	Intensity of Hybridization Relative to CO/NaCl sample	
	Nuclear	Cytosolic
3MC/CHX	34.3	4.5
3MC/NaCl	21.6	2.4
CO/CHX	2.6	8.7
CO/NaCl	1.0	1.0
3MC/CHX	4.9	9.5
3MC/NaCl	2.0	1.5
CO/CHX	1.6	3.7
CO/NaCl	1.0	1.0

Fig. 1. Hybridization of nuclear and cytosolic RNA to nick-translated clone 46 and pRSA57. Total hepatic RNA was isolated from nuclear or cytosolic fractions. Nuclear RNA, 1 μg, and cytosolic RNA, 2 μg, immobilized on a Nytran membrane, were hybridized to radiolabeled clone 46 (probe for the P-450IA1 gene) or pRSA57 (probe for the albumin gene) as described. In experiment 1, the Nytran membrane that had been hybridized to radiolabeled clone 46 was cleared of the latter and rehybridized to pRSA57. In experiment 2, duplicate membranes were prepared and each was hybridized to either clone 46 or pRSA57. The intensity of the positive signal on the autoradiogram was determined by laser densitometry as described in the text. The intensity of the positive signal to pRSA57 was used to normalize for possible variations in the amount of RNA fixed to the membrane, and for other nonspecific effects. In separate experiments, we found no change in albumin mRNA activity as a result of the 3-MC or CHX treatment.

in cultured mouse hepatoma 1c1c7 cells by cycloheximide has been observed [1]. In addition, both nuclear and polysomal β- and γ-actin mRNA levels were observed to be induced by cycloheximide treatment of quiescent AKR-2B mouse embryo cells [14]. It is possible that this "apparent" induction of specific RNA levels by cycloheximide merely reflects a decreased synthesis of rapidly turned-over proteins that are required for the degradation of some but not all RNA species [15].

The administration of 3-MC to rats resulted in a 2.0–21.6- and 1.5–2.4-fold relative increase in hybridization to nuclear and cytosolic cytochrome P-450IA1 respectively. The addition of CHX to the 3-MC protocol effected a further 1.6–2.5- and 1.9–6.3-fold increase respectively (Fig. 1). This "superinduction" occurred despite the 70% reduction in the specific activity of liver protein (Table 1). This phenomenon was not observed previously when inhibition of protein synthesis was achieved prior to 3-MC treatment [4]. This discrepancy may also rely on other differences between the two studies such as: our normalization to the rat serum albumin mRNA level in order to correct for nonspecific variations in cytochrome P-450IA1 mRNA levels; and our use of cytochrome P-450IA1-specific probes [8] versus probes that recognize both cytochrome P-450IA1 and P-450IA2 [4]. The "superinduction" phenomenon has been observed previously for cytochrome P-450IA1 mRNA in cultured mouse Hepa 1c1c7 cells treated with both 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and cycloheximide as compared to those cells treated with only TCDD, or only cycloheximide [1]. In this system, "superinduction" was attributed to an increase in cytochrome P-450IA1 gene transcription [2, 3], involved specific DNA sequences [3] and, therefore, could not be attributed solely to a decrease in cytochrome P-450IA1 mRNA degradation.

In summary, these results are in concert with the existence of a specific labile repressor of cytochrome P-450IA1 gene expression *in vivo*. The details of this trans-regulatory mechanism remain to be elucidated.

Table 1. [^{3}H]Leucine incorporation into hepatic protein*

Group	Specific activity (cpm/g protein)
CO/NaCl	11,678 (1.0)†
CO/CHX	5,354 (0.5)
3-MC/NaCl	17,756 (1.5)
3-MC/CHX	4,000 (0.3)

* Liver protein was isolated at 1 hr after i.p. administration of 20 μCi of L-[4,5-^{3}H]leucine (sp. act. = 1225 Ci/mmol), and the specific activity of the labeled protein was determined. The counting efficiency for tritium was 30–35%. These data are representative of two experiments.

† Relative to CO/NaCl group.

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Na^+ and pH dependent transport of foscarnet via the phosphate carrier system across intestinal brush-border membrane

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Foscarnet (trisodium phosphonoformic acid, PFA*, Fig. 1) is a potent inhibitor of herpesvirus replication [1, 2] and is also effective against the AIDS retrovirus (LAV/HTLV-III) [3]. PFA has been found to be absorbed completely in rabbits following oral administration [4], while approximately 30% absorption occurs in mice and rats (unpublished data from Astra Läkemedel AB.) and about 12–22% in humans†. These fairly high values of oral availability, despite the high hydrophilicity, and the considerable species difference in the availability suggest a participation of some specific mechanism for PFA absorption. The

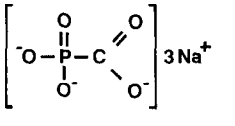

Fig. 1. Chemical structure of PFA.

* Abbreviations: PFA, foscarnet (trisodium phosphonoformic acid); Hepes, *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid; Mes, 2-(*N*-morpholino)-ethanesulfonic adic; and FCCP, carbonylcyanide-4-trifluoromethoxyphenylhydrazone.

† Sjövall J, Karlsson A, Ogenstad S, Sandström E and Saarimäki M, 27th Interscience Conference of Antimicrobial Agents and Chemotherapy, New York, Abstr. No. 1083, p. 286, 1987.