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THE SUBSTITUTED PYRIDINES METYRAPONE AND NICOTINAMIDE ARE INDUCERS OF 5-AMINOLEVULINATE SYNTHASE AND CYTOCHROME P-450 IN HEPATOCYTE CULTURE

URS GIGER* and URS A. MEYER†
Division of Clinical Pharmacology, Department of Medicine,
University Hospital Zürich, CH-8091 Zürich, Switzerland

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Abstract—The effects of metyrapone and nicotinamide, two substituted pyridines, were studied in cultured chick embryo hepatocytes, a system characterized by preserved inducibility of cytochrome P-450 hemoproteins. Both metyrapone and nicotinamide caused a dose-dependent increase in cytochrome P-450 concentration. Their inducing potencies differed by one to two orders of magnitude and correlated with the known difference in the binding affinity of these two pyridines to cytochrome P-450. The increase of cytochrome P-450 concentration after metyrapone and nicotinamide was additive to the induction of cytochrome P-450 by phenobarbital and β-naphthoflavone and was abolished by cycloheximide. Treatment of hepatocyte cultures with metyrapone resulted in an increase in a microsomal protein with an apparent mol. wt of 52,000. In addition, induction of cytochrome P-450 by the substituted pyridines was associated with enhanced 5-aminolevulinate synthase, the rate-limiting enzyme of heme biosynthesis. These data suggest that in cultured chick embryo hepatocytes the substituted pyridines metyrapone and nicotinamide induce cytochrome P-450.

Initial evidence for the multiplicity of cytochrome P-450 hemoproteins and their overlapping substrate specificity was largely derived from the differential effects of various inducers, inhibitors and activators of these enzymes [1, 2]. Metyrapone (2-methyl-1,2-bis-(3-pyridiyl)-1-propanone) was discovered as an inhibitor of steroid hydroxylation in adrenal microsomes and mitochondria [3, 4], but it also inhibits or activates the oxidation of numerous drugs and other lipophilic compounds in liver microsomes [5–8]. Inhibition of drug metabolism by metyrapone was also reported in animals *in vivo* [9]. Nicotinamide (pyridine-3-carboxylic acid amide), another substituted pyridine, is a vitamin and also is formed in the liver. Its effects on microsomal drug oxidation are similar to those of metyrapone [10]. Both metyrapone [11] and nicotinamide [10, 12] are ligands of cytochrome P-450 and their interactions with the hemoprotein results in a type II binding spectrum. Moreover, metyrapone serves as a substrate for cytochrome P-450 dependent monooxygenase reactions [13], although its major metabolic transformation in the liver is reduction to metyrapol [14]. Nicotinamide also is metabolized by a cytochrome P-450 dependent *N*-oxidation reaction in the liver [15, 16]. As both substituted pyridines are used in the diagnosis and therapy of human diseases it is surprising that their effects on hepatic drug metabolism *in vivo* are largely unknown.

In the present report, the effects of metyrapone and nicotinamide on cytochrome P-450 concentration and function were investigated in cultured chick embryo hepatocytes. This culture system is characterized by preserved inducibility of cytochrome P-450 hemoproteins under chemically defined conditions, even with phenobarbital-type inducers [17, 18]. This is in contrast to rat hepatocytes in primary culture, where drug-mediated induction of cytochrome P-450 cannot be directly demonstrated in the initial phase of culture [19]. In cultured chick embryo hepatocytes the substituted pyridines metyrapone and nicotinamide induced cytochrome P-450. A preliminary account of this study has been presented at the National Meeting of the Swiss Society of Experimental Biology, Lausanne, Switzerland, 1981, and has been published in abstract form [20].

MATERIALS AND METHODS

Materials. Chick embryos (Shaver hybrids) were obtained from Wolff Poultry Farm (Volketswil, Switzerland). Williams'E medium containing 2.5 mM glucose and calcium-free Hanks balanced salt solution were prepared by the Pharmacy of the University Hospital, Zurich, Switzerland, which also supplied sodium phenobarbital. Collagenase type I (160 IU/mg), 3,3',5-triiodothyronine, benzo(a)pyrene and cycloheximide were purchased from Sigma Chemical Co. (St. Louis, MO). Metyrapone (2-methyl-1,2-bis-(3-pyridyl)-1-propanone) were obtained from Fluka Chemicals (Buchs, Switzerland) and nicotinamide (pyridine-3-carboxylic acid amide) from Hoffman-La Roche (Basle, Switzerland); α- and β-naphthoflavone was purchased from Aldridge (Milwaukee, WI) and 7-ethoxycoumarin from Boeh-

* Present address: Department of Pharmacology, College of Medicine, University of Florida, Box J-267 JHMHC, Gainesville, FL 32610, U.S.A.
† To whom correspondence should be addressed.

ringer Co. (Mannheim, West Germany). SKF 525A (2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride) was supplied by Smith, Kline & French Laboratories (Philadelphia, PA). 1,4[^{14}C]Succinate, sp. act. 116 mCi/mmole was from the Radiochemical Centre (Amersham, U.K.). Lithium dodecyl sulfate was obtained from BDH Chemicals Co. (Poole, U.K.). All other chemicals or biochemicals were obtained from either Merck (Darmstadt, West Germany) or Fluka Chemicals.

Preparation and culture of chick embryo hepatocytes. Hepatocytes were isolated from 15 day old chick embryos under sterile conditions by *in situ* perfusion and *in vitro* incubation with collagenase (0.05% collagenase–Hanks balanced salt solution) as described [17, 21]. This procedure yielded approximately 10^8 cells per gram liver (two chick embryo livers). Cells (1.5×10^7) in 10 ml Williams'E medium containing 2.5 mM glucose and 1.5 μM triiodothyronine were plated in a 10 cm Falcon culture dish and incubated at 37° in a humidified incubator in a 5% CO_2: 95% air atmosphere for 44 hr. The medium was changed after 24 hr. Metyrapone, nicotinamide, cycloheximide and phenobarbital were dissolved in water and filtered (Millipore, 0.22 μM). β-Naphthoflavone was dissolved in dimethylsulfoxide; the final concentration in culture of dimethylsulfoxide did not exceed 0.1% (v/v).

Preparation of homogenates and microsomes and analytical assays. Tissue fractionation was performed at 4° as follows: monolayers of two dishes from the same experimental group were washed twice with cold 0.25 M sucrose, scraped off with a rubber policeman, suspended in a final volume of 10 ml 0.25 M sucrose, homogenized with a glass–Teflon Potter–Elvehjem homogenizer (clearance between 0.08 and 0.14 mm; 1200 rpm, 11 complete strokes) and sonicated for 5 sec (Kontes cell disruptor, type 881440, Kontes, Vineland NY, 130 watts/cm^2). The homogenate was centrifuged at 10,000 *g* for 12 min. The supernatant fraction was then spun at 105,000 *g* for another 90 min to sediment the microsomal fraction. The microsomes were suspended in 0.1 M sodium phosphate buffer, pH 7.4.

Microsomal cytochrome P-450 concentration was measured in microcuvettes as described previously by Althaus *et al.* [17]. Since during fractionation metyrapone and nicotinamide were washed out and carbon monoxide displaces the metabolic intermediates [2] of the remaining substituted pyridines from cytochrome P-450 [11], there probably was little interference of these compounds with the measurement of cytochrome P-450 by spectral analysis. This is important because according to Jonen *et al.* [22], displacement is not complete when high amounts of metyrapone (2.5 mM) are present in microsomal preparations before saturation with carbon monoxide resulting in a reduced absorbance at 450 nm. 7-Ethoxycoumarin deethylase and aryl hydrocarbon hydroxylase activities were determined by the methods of Greenlee and Poland [23] and Gelboin and Nebert [24], respectively. Microsomal proteins from cultured hepatocytes were analyzed by one-dimensional slab gel electrophoresis. Microsomal suspensions (1 mg protein per ml) were treated with 2% lithium dodecyl sulfate [25] and 50 mM dithiothreitol for 30 min. 15 μg microsomal protein were applied per well. Electrophoresis was performed at 4° with a discontinuous buffer system [26] on a 1 mm lithium dodecyl sulfate gel containing a gradient of 7.5 to 12.5 % acrylamide. Proteins were stained with Coomassie blue. Bovine albumine, pyruvate kinase, fumarase and aldolase were used as marker proteins.

From one culture dish per experimental group a cell homogenate was prepared in 0.5 ml 0.9% NaCl containing 10 mM Tris, 0.1 mM pyridoxalphosphate and 0.5 mM EDTA, pH 7.4. 5-Aminolevulinate synthase activity was determined in this homogenate by the method of Strand *et al.* [27] as modified by Yoda *et al.* [28]. Protein concentration was measured by the method of Lowry *et al.* [29] with bovine serum albumin as standard.

Cysteine and cystine were included in our culture medium at the concentrations (0.2 mM) present in commercial Williams'E medium. In contrast to primary culture of adult rat hepatocytes [30] omission of these two amino acids during 24 or 44 hr of culture caused neither enhanced basal levels nor changes in the inducibility of cytochrome P-450 by phenobarbital or β-naphthoflavone in cultured chick embryo hepatocytes (data not shown).

RESULTS

The effect of various doses of metyrapone on cytochrome P-450 concentrations in cultured chick embryo hepatocytes is shown in Fig. 1. The presence

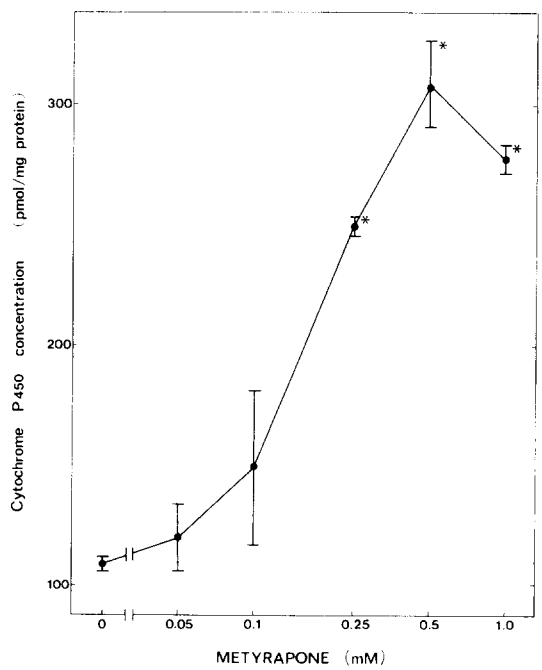

Fig. 1. Effect of metyrapone on microsomal cytochrome P-450 in cultured chick embryo hepatocytes. Hepatocytes from 15 day old chick embryos were cultured for 44 hr in Williams'E medium containing 1.5 μM triiodothyronine. Monolayers were exposed to various concentrations of metyrapone during the entire culture period. Each point represents the mean ± S.E. of 3–9 experiments; *$P \leq 0.001$, compared to the untreated control (Student's *t*-test).

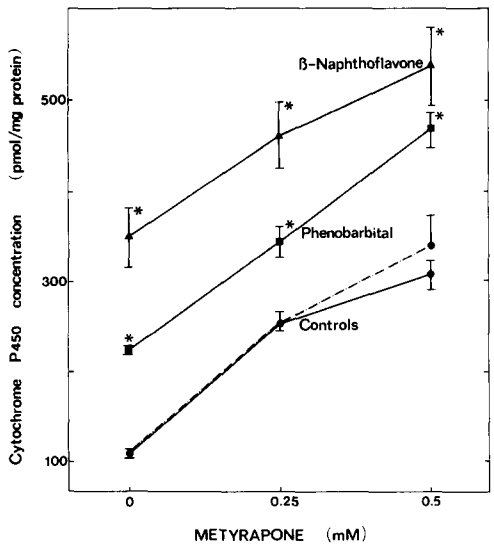

Fig. 2. Effect of metyrapone on induction of microsomal cytochrome P-450 by phenobarbital and β-naphthoflavone in cultured chick embryo hepatocytes. Monolayers, cultured from the beginning (●——●) or just for the last 20 hr of culture (●—·—●). Phenobarbital (0.4 mM; (■——■) and β-naphthoflavone (11 μM; ▲——▲) were added for a final 16 hr induction period to cultures treated with metyrapone during the entire culture period. Each point represents the mean ± S.E. of 3–9 experiments; *P ≤ 0.01, compared to the corresponding controls.

of metyrapone in the medium from the beginning of culture throughout the entire culture period (44 hr) resulted in a dose-dependent increase in cytochrome P-450 levels. A metyrapone concentration of 0.5 mM led to a maximal increase in the cytochrome P-450 concentration. The final concentration of cytochrome P-450 in metyrapone treated cultures was higher than control levels of cytochrome P-450 observed in chick embryo liver *in ovo* in this [31] and other laboratories [32]. Concentrations of metyrapone above 1 mM were cytotoxic, as judged by phase contrast micrography of the hepatocyte monolayers. The peak of the carbon monoxide binding difference spectrum from microsomes of metyrapone-treated cultures was at 450 nm, as is seen after induction with phenobarbital [17]. There was a similar augmentation of cytochrome P-450 independently if monolayers were exposed to metyrapone from the beginning or just for the last 20 hr of culture (Fig. 2.). Addition of 0.4 mM phenobarbital or 11 μM β-naphthoflavone during the final 16 hr of culture induced cytochrome P-450 about 2-fold and 3-fold, respectively (Fig. 2). Metyrapone present at two different concentrations concomitant with these classical inducer compounds had an additive effect on cytochrome P-450 induction (Fig. 2).

Since metyrapone is a widely used and potent inhibitor and activator of monooxygenase reactions *in vitro* [5–8], 7-ethoxycoumarin deethylase and aryl hydrocarbon hydroxylase activities were also studied in microsomal fractions from metyrapone-treated cultures (Table 1). The metyrapone-mediated increase in cytochrome P-450 concentration was

Table 1. Effect of metyrapone on two monooxygenase activities in cultured chick embryo hepatocytes

Metyrapone (mM)	Time of presence (hr)	Inducing compound (28–44 hr)	7-Ethoxycoumarin deethylase activity		Aryl hydrocarbon hydroxylase activity	
			pmoles OH-coumarin	pmoles OH-coumarin	pmoles OH-benzpyrene	pmoles OH-benzpyrene
			mg protein · min	pmole P-450 · min	mg protein · min	pmole P-450 · min
—	—	—	332 ± 31(6)	3.05	163 ± 31(5)	1.45
0.25	24–44	—	447 ± 27(3)†	1.78	130 ± 17(3)	0.52
0.5	24–44	—	558 ± 58(4)*	1.69	150 ± 25(3)	0.45
0.25	0–44	—	551 ± 72(4)†	2.21	188 ± 38(3)	0.76
0.5	0–44	—	594 ± 74(4)*	1.93	202 ± 43(3)	0.66
		Phenobarbital (0.4 mM)	709 ± 47(4)*	3.19	213 ± 55(4)	0.96
0.25	0–44	(0.4 mM)	654 ± 87(3)*	1.92	194 ± 58(3)	0.57
0.5	0–44	(0.4 mM)	809 ± 30(3)*	1.73	194 ± 30(3)	0.42
		β-Naphthoflavone (11 μM)	1304 ± 118(4)*	3.75	962 ± 149(4)*	2.76
0.25	0–44	(11 μM)	1220 ± 108(3)*	2.65	700 ± 98(3)*	1.52
0.5	0–44	(11 μM)	1285 ± 147(3)*	2.39	917 ± 106(3)*	1.70

Monolayers were treated with metyrapone, phenobarbital and β-naphthoflavone as described in Fig. 2. 7-Ethoxycoumarin deethylase and aryl hydrocarbon hydroxylase activities were determined in microsomes. Cytochrome P-450 concentrations were from Fig. 2. Results are expressed as the mean ± S.E. with the numbers of experiments in parentheses. *P ≤ 0.01 and †P ≤ 0.05, compared to the untreated control (Student's *t*-test). P-450, cytochrome P-450.

Table 2. Effect of cycloheximide on cytochrome P-450 induction by metyrapone

Additions (24–44 hr present in the medium)	Cytochrome P-450 concentration (pmoles/mg protein)	
	Control	Cycloheximide (5 μM)
None	109 ± 3(9)	51 ± 7(4)*
Metyrapone (0.5 mM)	332 ± 41(4)	73 ± 4(3)*

Monolayers, cultured for 44 hr, were exposed to cycloheximide and/or metyrapone for the last 20 hr and cytochrome P-450 concentration was determined in microsomes. Results are expressed as the mean ± S.E. with the numbers of experiments in parentheses. *$P \leq 0.01$, compared to the untreated or metyrapone-treated controls (Student's *t*-test).

accompanied by a slightly enhanced 7-ethoxycoumarin deethylase activity when expressed per mg microsomal protein, whereas aryl hydrocarbon hydroxylase activity was not significantly changed. Metyrapone did not change 7-ethoxycoumarin deethylase nor aryl hydrocarbon hydroxylase activity when these activities were induced with phenobarbital or β-naphthoflavone. However in regard to product formation expressed per pmole cytochrome P-450, metyrapone reduced both control and induced activities (Table 2). Addition of SKF 525A (2-diethylaminoethyl-2,2-diphenylvalerate, 100 μM) and α-naphthoflavone (100 μM) diminished both monooxygenase activities in microsomes from metyrapone treated cultures by approximately 50%. The *in vitro* effect of metyrapone (100 μM) on monooxygenase activities in microsomes from untreated, phenobarbital and β-naphthoflavone treated cultures was comparable to the inhibitory effects of SKF 525A (data not shown).

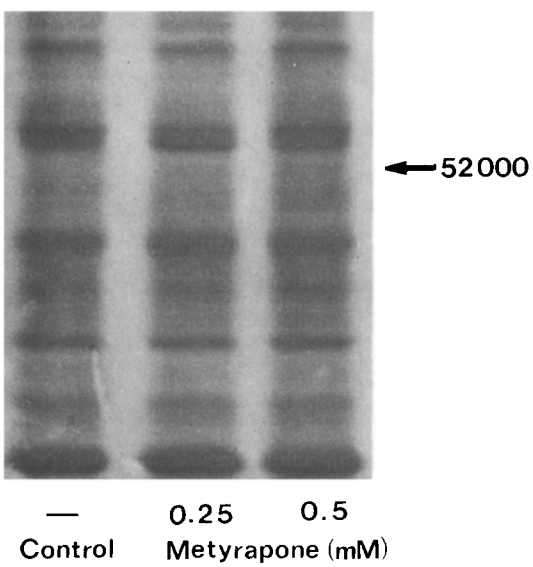

Fig. 3. The electrophoretic pattern of microsomal proteins from cultured chick embryo hepatocytes treated with metyrapone. Monolayers were exposed to metyrapone throughout the entire culture period. Microsomes were prepared and a lithium dodecyl sulfate polyacrylamide gel electrophoresis was performed as described in Materials and Methods.

Addition of metyrapone to monolayers for 44 hr caused an increase of a single microsomal electrophoretic band of apparent mol. wt of 52,000 as compared to the untreated control (Fig. 3). The increased appearance of this electrophoretic band after metyrapone treatment was dose-dependent.

When chick embryo hepatocyte monolayers were exposed to 5 μM cycloheximide for the last 20 hr of culture cytochrome P-450 concentration was reduced to 50% of controls. Moreover, cycloheximide completely blocked the metyrapone-mediated increase in cytochrome P-450 concentration (Table 2).

Metyrapone, either present from the beginning or for the last 20 hr of culture, significantly augmented 5-aminolevulinate synthase activity. In fact, the metyrapone-mediated effect was additive to the well known increase of 5-aminolevulinate synthase activity produced by phenobarbital (Fig. 4).

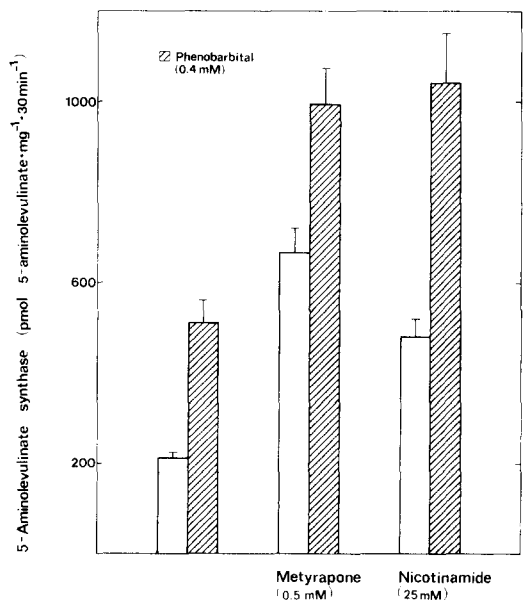

Fig. 4. Effect of metyrapone and nicotinamide on 5-aminolevulinate synthase activity in cultured chick embryo hepatocytes. Monolayers were exposed to metyrapone (0.5 mM) or nicotinamide (25 mM) from the beginning of culture. Phenobarbital (0.4 mM) was added for the final 16 hr of culture (hatched bars). 5-Aminolevulinate synthase activity was determined in homogenates. Each bar represents the mean ± S.D. of 2–4 experiments.

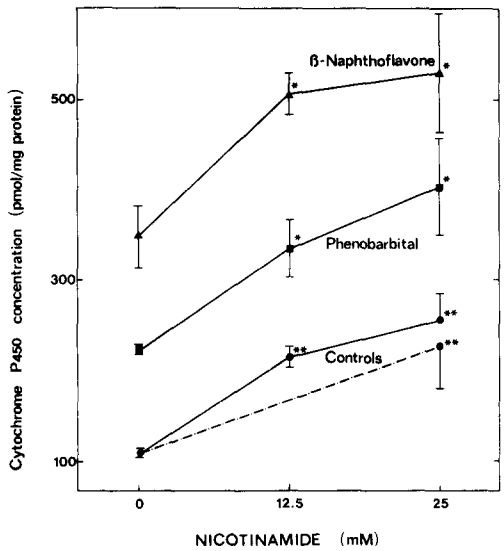

Fig. 5. Effect of nicotinamide on microsomal cytochrome P-450 in cultured chick embryo hepatocytes. Monolayers were exposed to nicotinamide from the beginning of culture (●——●) or just for the last 20 hr of culture (●—·—●). Phenobarbital (0.4 mM; ■——■) and β-naphthoflavone (11 μM; ▲——▲) were added for a final 16 hr induction period to cultures treated with metyrapone during the entire culture period. Each point represents the mean ± S.E. of 3–9 experiments; *$P \leq 0.01$, compared to the corresponding controls; **$P \leq 0.01$ compared to the untreated control (Student's *t*-test).

The effects of nicotinamide were similar to those of metyrapone, but the potency of nicotinamide was less. Treatment of cultured chick embryo hepatocytes with 12.5 and 25 mM nicotinamide resulted in significant increases in cytochrome P-450 concentration (Fig. 5), the peak of the carbon monoxide binding spectrum occuring at about 450 nm. Lower doses of nicotinamide were ineffective and concentrations above 25 nM were highly cytotoxic, as judged by increasing appearance of rounded and floating cells. Analogous to the effects of metyrapone, the nicotinamide-mediated increase in cytochrome P-450 levels was of the same magnitude if nicotinamide was present in the medium from the beginning of culture or only for the last 20 hr and was additive to the inductive response by phenobarbital and β-naphthoflavone (Fig. 5). Moreover, the effect of nicotinamide on cytochrome P-450 was prevented by cycloheximide (data not shown) and 5-aminolevulinate synthase activity was increased by nicotinamide. This latter effect was additive to the increase observed with phenobarbital (Fig. 4). It is worth noting that the basal concentration of nicotinamide in Williams'E medium is 8.2 μM and therefore negligible with regard to the concentrations used in these experiments.

DISCUSSION

The present study suggests that in cultured chick embryo hepatocytes the two substituted pyridines metyrapone and nicotinamide induce cytochrome P-450. The effect of metyrapone and nicotinamide was dose-dependent and was of the same magnitude if the two substituted pyridines were added from the beginning or only for the last 20 hr of a 44 hr culture. However, nicotinamide appeared to be 50 to 100 times less potent than metyrapone. The difference in potency correlates well with the different binding affinities of these two pyridines to cytochrome P-450 as shown in rat liver microsomes [12]. The maximal response after metyrapone led to concentrations of cytochrome P-450 above those observed in chick embryo liver *in ovo* [31, 32]. These latter data make it unlikely that metyrapone and nicotinamide increase cytochrome P-450 by solely stabilizing preexisting hemoproteins, as appears to be the case in adult rat hepatocyte culture, where nicotinamide [12, 33–36] and metyrapone [12, 37] partially prevent the rapid initial loss in cytochrome P-450 which is typical for this culture system [33–38]. To produce this 'protective' effect these compounds have to be present from the beginning of culture [33, 37].

The substituted pyridine-mediated increase in cytochrome P-450 in cultured chick embryo hepatocytes was dependent on protein synthesis, as cycloheximide, at a concentration previously shown to inhibit general protein synthesis by 93% [21, 35], lowered basal concentration of cytochrome P-450 to 50% within 20 hr and completely prevented the increase in cytochrome P-450 caused by metyrapone or nicotinamide. If one assumes a virtually complete inhibition of synthesis of apocytochrome P-450 by cycloheximide, the observed rate of decrease in cultured chick embryo hepatocytes would be consistent with an average half-life of less than 20 hr and is in accordance with previous studies in rats *in vivo* [39–41] and in cultured chick embryo hepatocytes [42]. In primary cultures of adult rat hepatocytes cycloheximide has different and controversial effects on cytochrome P-450. Guzelian and Barwick [43] found partial prevention of the loss of cytochrome P-450 by cycloheximide, while Villa *et al.* [35] could not demonstrate such an effect. Moreover, in these rat hepatocyte cultures, cycloheximide did not influence the effect of metyrapone on cytochrome P-450 [12, 35, 37].

Treatment of hepatocytes with metyrapone resulted in an increased appearance of a microsomal protein with an apparent mol. wt of 52,000. This increase was dose-dependent and corresponded to the spectral increase of cytochrome P-450. It is likely that this increase in a microsomal protein of apparent mol. wt of 52,000 on lithium dodecyl sulfate polyacrylamide gel electrophoresis reflects induction of a molecular form of cytochrome P-450, because a protein with similar electrophoretic properties was previously induced by phenobarbital and tentatively identified as cytochrome P-450 [17].

The induction of cytochrome P-450 by metyrapone and nicotinamide was accompanied by an increased activity of 5-aminolevulinate synthase (EC 2.3.1.37), the first and rate-limiting enzyme of heme biosynthesis [44]. Increased heme formation during induction of cytochrome P-450 is required to supply prosthetic heme for newly synthesized apocytochrome P-450 [31]. In agreement with the observation of increased 5-aminolevulinate synthase activity De

Matteis [46] described increased porphyrin accumulation after metyrapone in cultured chick embryo hepatocytes. Moreover, 5-aminolevulinate synthase is induced by metyrapone in chick embryo liver *in ovo* [32]. These findings further suggest that metyrapone is an inducer of apocytochrome P-450 and heme synthesis.

The molecular form (or forms) of cytochrome P-450 induced by metyrapone and nicotinamide has certain similarities to a phenobarbital-induced hemoprotein. Thus, the peak of the carbon monoxide binding spectrum after induction by these substituted pyridines was precisely at 450 nm. The apparent mol. wt of 52,000 of microsomal protein induced by these compounds corresponds to the mol. wt of a phenobarbital induced cytochrome P-450 in cultured chick embryo hepatocytes [16]. Finally, the differential increase in microsomal monooxygenase activities caused by previous exposure of the monolayers to metyrapone revealed an increase in 7-ethoxycoumarin deethylation, whereas aryl hydrocarbon hydroxylase activity remained unaffected. This resembles the induction pattern of monooxygenase activities produced in cultured chick embryo hepatocytes by phenobarbital [17]. More precise identification and characterization of the cytochrome(s) P-450 induced by substituted pyridines will require ligand affinity studies [22], purification and the use of monospecific antibodies.

When product formation of these two monooxygenase activities was expressed per pmole cytochrome P-450 it became evident that pretreatment of cultures with metyrapone reduced these activities. This could mean either that metyrapone and nicotinamide induce forms of cytochrome P-450 with low substrate affinities for 7-ethoxycoumarin and benzo(a)pyrene, or that metyrapone present in the culture and/or bound to cytochrome P-450 was not completely removed during microsomes preparation and affected thereby the *in vitro* monooxygenase activities. The inhibitory effect of metyrapone on certain monooxygenase activities *in vitro* is of course well known [5–8].

As discussed above, the effects of metyrapone and nicotinamide appear to differ in several respects from those observed in primary cultures of adult rat hepatocytes [12]. The latter system is characterized by a massive initial decrease in cytochrome P-450 concentration which can partially be prevented by metyrapone and nicotinamide. Moreover, neither 5-aminolevulinate synthase nor cytochrome P-450 are inducible by drugs and chemicals in the initial phase of adult rat hepatocyte culture. It therefore was suggested that ligand formation of metyrapone and nicotinamide with cytochrome P-450 may prevent its rapid destruction in adult rat hepatocyte culture, because the effect of these substituted pyridines was correlated to the ability of metyrapone and nicotinamide to bind to cytochrome P-450 [12], and because there are numerous examples where ligands protect proteins from degradation [47]. In addition, Paine *et al.* [34] demonstrated in adult rat hepatocyte culture that the ability of nicotinamide to maintain cytochrome P-450 is apparently not related to increased nicotinamide–adenine dinucleotide content. More recent studies by the same authors do not exclude an additional effect of substituted pyridines on the synthesis of heme or the assembly of heme and apocytochrome P-450 [37].

Although metyrapone has been extensively investigated as an inhibitor and activator of hepatic cytochrome P-450 hemoprotein monooxygenase functions [1, 11], its potential as an inducer of cytochrome P-450, as demonstrated in this paper, has only recently been recognized. An increase in arylhydrocarbon hydroxylase activity by metyrapone was observed in three liver derived cell cultures by Owens and Nebert [48]. Bergmann *et al.* [49] showed that in rats repeated treatment with metyrapone caused a decrease in the hexobarbital sleeping time and an increase in ethylmorphine demethylation. Similarly, Feuer *et al.* [50] observed increased microsomal oxidation of hexobarbital and coumarin after repeated treatment of rats with metyrapone. More recently, nicotinamide was shown to cause an increase in cytochrome P-450 concentration after a single administration to rats [51]. A metyrapone-mediated increase in cytochrome P-450 concentration was observed in chick embryo liver *in ovo* by Rifkind *et al.* [32] and recently in our laboratory. Interestingly, SKF 525A, another well-known potent inhibitor and substrate of hepatic cytochrome P-450 catalyzed reactions, on prolonged administration also caused an increase in cytochrome P-450 concentration [52] and increased 5-aminolevulinate synthase activity in rats [45] and in chick embryos *in ovo* [32]. On the other hand, in rats nicotinamide reversed the induction of hepatic 5-aminolevulinate synthase by allylisopropylacetamide [53]. The potent effects of allyl-group containing compounds, such as allylisopropylacetamide, on 5-aminolevulinate synthase is related in part to rapid degradation of the heme moiety of cytochrome P-450 by adduct formation and consequent impaired feedback repression by heme of 5-aminolevulinate synthase [40, 54]. Similarly, SKF 525A also inhibited allylisopropylacetamide-mediated induction of 5-aminolevulinate synthase [51], whereas induction of 5-aminolevulinate synthase by propylisopropylacetamide, an analogue of allylisopropylacetamide, which does not contain an allyl-group and therefore does not form an adduct with prosthetic heme, was not prevented by SKF 525A. We suggest that nicotinamide P-450 may prevent the interaction of allylisopropylacetamide with the prosthetic heme from cytochrome P-450 and thereby prevent its destruction.

The fact that metyrapone and nicotinamide inhibit and activate microsomal hepatic monooxygenase activity may convincingly be explained by their strong binding affinity for specific forms of cytochrome P-450. If their properties here described as inducers of cytochrome P-450 are related to the same physico-chemical characteristics, they may be interesting model compounds to approach the still unknown mechanism of induction of cytochrome P-450 hemoproteins.

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