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FORMATION OF AN INACTIVE CYTOCHROME P-450Fe(II)–METABOLITE COMPLEX AFTER ADMINISTRATION OF AMIODARONE IN RATS, MICE AND HAMSTERS

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Abstract—Administration of amiodarone hydrochloride (50–150 mg/kg i.p daily) to rats, mice or hamsters resulted in the *in vivo* formation of a cytochrome P-450Fe(II)–amiodarone metabolite complex absorbing at 453 nm, unable to bind CO and biologically inactive In rats, the amount of complex present in hepatic microsomes was small 24 hr after administration of a single dose of amiodarone (100 mg/kg i p) but was increased 2 5-times by pretreatment with phenobarbital and 8-times by pretreatment with dexamethasone phosphate In addition, the complex increased linearly with time as the doses of amiodarone were repeated daily When both enhancing factors were combined (treatment for 3 days with both dexamethasone and amiodarone), the amount of complex present in liver microsomes reached 0 78 nmol/mg protein or 40% of total cytochrome P-450 in rats In these rats, *in vitro* disruption of the complex with potassium ferricyanide suppressed its Soret peak at 453 nm, increased by 70% the CO-binding spectrum of dithionite-reduced microsomes, and restored several monooxygenase activities The 453 nm-absorbing complex was also formed *in vitro* upon incubation of amiodarone or *N*-desethylamiodarone with NADPH, EDTA and microsomes from dexamethasone-treated rats The formation of the complex was smaller with microsomes from phenobarbital-treated rats and was not detected with microsomes from control rats We conclude that amiodarone forms an inactive cytochrome P-450Fe(II)–metabolite complex in rats, mice and hamsters

Amiodarone has been widely used in Europe for the last two decades [1]. Initially employed in the treatment of angina pectoris [1], the drug was soon found to have potent antiarrhythmic properties, being effective against a variety of supraventricular or ventricular cardiac arrhythmias [2, 3]. Adverse reactions include various types of thyroid dysfunction [4], a bluish discoloration of the skin [5], corneal deposits [6], peripheral neuropathy [7], pulmonary fibrosis [8] and liver lesions [9].

Recently, several drug interactions have been reported in patients receiving amiodarone concomitantly with other drugs [10] Amiodarone potentiated the anticoagulant effect of warfarin, probably by interfering with the metabolism of this compound [11–15]. Co-administration of amiodarone also increases the serum concentrations of digoxin [16–20], quinidine [21, 22], procainamide [22] and aprindine [23] In rats, administration of amiodarone depresses several monooxygenase activities [24]; the mechanism of this effect was not determined [24]

Amiodarone contains a diethylamino group Several tertiary amines are transformed by cytochrome P-450 into metabolites, probably nitrosoalkanes (R—N=O), which form stable complexes with the iron(II) of cytochrome P-450 [25, 26] Such cytochrome P-450Fe(II)–metabolite complexes exhibit a Soret peak around 455 nm, are unable to bind CO and are biologically inactive. Addition of potassium ferricyanide to the microsomes, however, oxidizes the heme iron to the ferric state, this disrupts the complex and liberates uncomplexed cytochrome P-450 which, once reduced, is again able to bind CO or oxygen and to oxidize substrates We report here the formation of such an inactive cytochrome P-450–metabolite complex after administration of amiodarone in rats, mice and hamsters

MATERIALS AND METHODS

Animals and treatments. Male Sprague–Dawley rats, Crl· CD®(SD)BR, weighing 180–220 g and male Swiss mice, Crl: CD-1(ICR)BR, weighing 32–35 g were purchased from Charles River France (Saint-Aubin-lés-Elbeuf, France). Male Golden Syrian hamsters, weighing 90–110 g were purchased from Fichet (Ormesson, France) Animals were fed normal standard diets given *ad libitum* (Autoclavé 113, UAR, France for rats and M-25 biscuits, Extralabo, Provins, France for mice and hamsters).

Amiodarone hydrochloride and *N*-desethylamiodarone were generous gifts from Clin Midy, Montpellier, France. Dexamethasone phosphate was kindly provided by Merck, Sharp & Dohme, Paris, France Pregnenolone-16 α-carbonitrile was generously given by the Upjohn Company, Kalamazoo, Michigan Some animals were treated with phenobarbital (100 mg/kg i.p.), 3-methylcholanthrene (20 mg/kg i.p.), dexamethasone phosphate (50 mg/kg i p.) or pregnenolone-16 α-carbonitrile (50 mg/kg i p.), daily for 3 days, and were killed 24 hr after the last dose. Amiodarone hydrochloride (50–150 mg/kg), dissolved in water (1 ml for 50 mg), was administered i p for 1–7 days In some experiments, the drug was given alone for 1–7 days. In other

experiments, a single dose of amiodarone hydrochloride was administered to animals pretreated for 3 days with various inducers In still other experiments, both the inducer and amiodarone hydrochloride were administered concomitantly for 3 days

Preparation of microsomes Animals were killed by cervical dislocation and the liver was removed Liver fragments were homogenized in 3 vol of 0 15 M KCl 0 01 M sodium–potassium phosphate buffer (pH 7 4) The liver homogenate was centrifuged at 10,000 *g* for 10 min The 10,000 *g* supernatant was centrifuged at 100,000 *g* for 60 min Some microsomal pellets were resuspended in buffer and again centrifuged at 100,000 *g* (washed microsomes)

Uncomplexed, complexed and total cytochrome P-450 The amount of uncomplexed, complexed, and total cytochrome P-450 was determined as previously reported in a study with troleandomycin [27] With a first batch of microsomes, we determined cytochrome P-450 according to Omura and Sato [28]. Because complexed cytochrome P-450 cannot bind CO [27], this technique measures uncomplexed cytochrome P-450 only [27] With a second batch of microsomes, we looked for the presence of a cytochrome P-450 Fe(II)–metabolite complex absorbing around 455 nm We added 50 μM potassium ferricyanide in the reference cuvette to destroy the complex, if any, in this cuvette The difference spectrum around 455 nm (in this case, 453 nm) measured the Soret peak of the complex in the sample cuvette and was used to calculate complexed cytochrome P-450 with a molar extinction coefficient of 75 mM $^{-1}$ cm $^{-1}$, previously reported for similar complexes [25 27] To determine total cytochrome P-450, we now added potassium ferricyanide to the sample cuvette also, so that the complex, if any, was now destroyed in both cuvettes We then repeated the procedure of Omura and Sato [28] Because complexed cytochrome P-450, when present, had been converted to uncomplexed cytochrome P-450, this technique now measured total cytochrome P-450 [27]

Other microsomal enzymes and monooxygenase activities NADPH-cytochrome *c* reductase activity was measured as previously reported [29] Microsomal protein concentration was measured by the technique of Lowry *et al* [30] [2-^{14}C] Hexobarbital hydroxylase activity was measured by the method of Kupfer and Rosenfeld [31] Aminopyrine or troleandomycin *N*-demethylase activity was determined as previously reported by Mazel [29] Benzo(*a*)pyrene hydroxylase activity was measured as described by Kuntzman *et al* [32] 7-Ethoxycoumarin deethylase activity was measured according to Greenlee and Poland [33] Unless otherwise indicated, the concentration of the substrate was 0 25 mM for hexobarbital, 0 5 mM for aminopyrine, 0 3 mM for troleandomycin, 0 2 mM for benzo(*a*)pyrene and 0 5 mM for 7-ethoxycoumarin In some tests 50 μM potassium ferricyanide was added to the microsomes before their addition to the incubation mixtures, accordingly, the final concentration of potassium ferricyanide in the incubation mixtures was 0 5–12 5 μM

In vitro formation of a 453 nm-absorbing complex The *in vitro* formation of a complex absorbing at 453 nm was measured with a suspension of washed hepatic microsomes containing 4 mg of microsomal protein per ml, 4 mM NADPH and 1 5 mM EDTA Cuvettes divided into 2 compartments were used The microsomal suspension was placed in one compartment, while ethanol was placed in the other compartment Both cuvettes were heated at 37 The base-line was recorded and memorized Amiodarone hydrochloride or *N*-desethylamiodarone (0 1 or 0 25 mM) was then added in 20 μl of ethanol to the microsomal suspension in the sample cuvette and to ethanol in the reference cuvette Successive spectra were repeatedly recorded for 15 min from 350 to 480 nm, on an Aminco DW-2C spectrophotometer

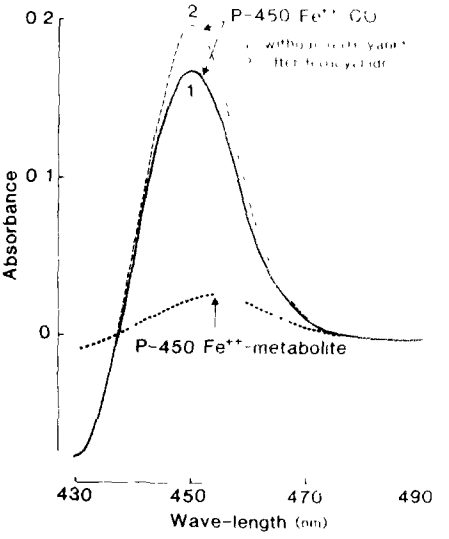

Fig 1 Presence of a cytochrome P-450-metabolite complex in a rat treated with amiodarone alone for 3 days Rats were treated with amiodarone hydrochloride (100 mg kg daily) for 3 days and were killed 24 hr after the last dose A microsomal suspension containing microsomes from 62 mg of liver per ml was prepared and divided into 2 cuvettes The lower curve shows the Soret peak of the complex, as observed after addition of 50 μM potassium ferricyanide in the reference cuvette only (and disruption of the complex in this cuvette) The 2 upper curves show the CO-difference spectrum of dithionite-reduced microsomes measured either without, or after adding 50 μM potassium ferricyanide to the microsomal suspension A representative experiment is shown

RESULTS

In vivo formation of a cytochrome P-450(Fe(II))–amiodarone metabolite complex

Administration of amiodarone resulted in the *in vivo* formation of a cytochrome P-450(Fe(II))–metabolite complex in rats The complex exhibited a Soret peak at 453 nm (Fig 1) which disappeared upon addition of potassium ferricyanide Disruption of the complex with potassium ferricyanide increased the CO-binding spectrum of microsomes secondarily reduced with sodium dithionite and then exposed to carbon monoxide (Fig 1)

In rats treated for 3 days with various daily doses of amiodarone hydrochloride (Fig 2) complexed

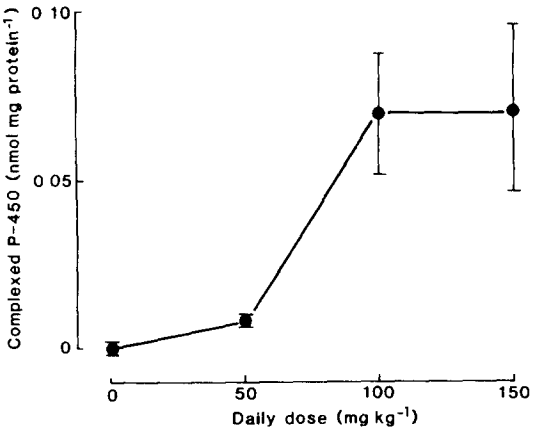

Fig 2 Effect of the dose of amiodarone on the *in vivo* formation of a cytochrome P-450–metabolite complex in rats Rats were treated for 3 days with various daily doses of amiodarone hydrochloride (50,100 or 150 mg/kg daily) and were killed 24 hr after the last dose The amount of complexed cytochrone P-450 present in liver microsomes was determined from its Soret peat at 453 nm Results are means ± S E M for 8 rats

cytochrome P-450 present in hepatic microsomes increased with the dose, reaching an apparent maximum for a dose of 100 mg/kg of amiodarone hydrochloride (150 μmol/kg of amiodarone). This dose was selected for further studies

When this dose of amiodarone was repeated for seven days in rats, the amount of complexed cytochrome P-450 increased linearly with time, reaching 0 15 nmol per mg of microsomal protein after 7 days of treatment (Fig. 3), it then slowly decreased after cessation of the administration of amiodarone (Fig 3).

The amount of complexed cytochrome P-450 recovered in microsomes from rats killed 24 hr after

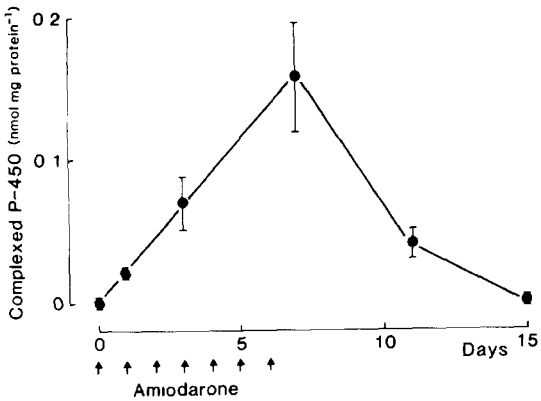

Fig 3 Time-course for the appearance and disappearance of a cytochrome P-450–metabolite complex after administration of amiodarone in rats Rats received amiodarone hydrochloride (100 mg/kg daily) for 7 days and were killed either during this treatment (24 hr after the preceding dose) or 5 or 9 days after the last dose of amiodarone The amount of hepatic microsomal cytochrome P-450–metabolite complex was determined from its Soret peak at 453 nm Results are means ± S E M for 8 rats

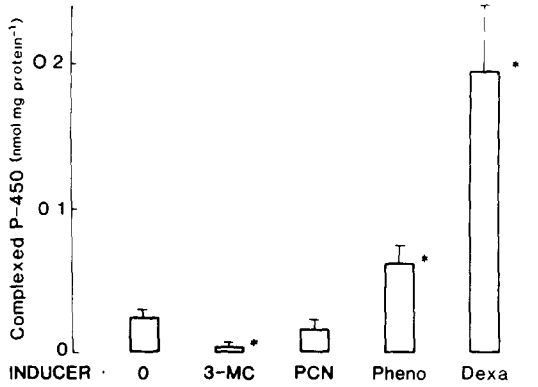

Fig 4 Effects of various inducers on the *in vivo* formation of a cytochrome P-450–metabolite complex after a single dose of amiodarone in rats Rats were pretreated for 3 days with various inducers 3-methylcholanthrene (3-MC) 20 mg/kg i p daily, pregnenolone 16 α-carbonitrile (PCN), 50 mg/kg i p daily, phenobarbital (Pheno), 100 mg/kg i p daily, or dexamethasone phosphate (Dexa), 50 mg/kg i p daily, 24 hr after the last dose of the inducer, rats received amiodarone hydrochloride (100 mg/kg i p) and were killed 24 hr later The amount of complexed cytochrome P-450 present in liver microsomes was measured from its Soret peak at 453 nm Results are means ± S E M for 6 rats. The asterisks indicate a significant difference from values in non pretreated rats (t-test for independent data), $P < 0\ 05$

a single dose of amiodarone was decreased by pretreatment of the animals with 3-methylcholanthrene (Fig 4); it was not modified by pretreatment with pregnenolone-16α-carbonitrile, was slightly increased by pretreatment with phenobarbital and was markedly increased by pretreatment with dexamethasone (Fig. 4) Even in the latter rats, however, the amount of complexed cytochrome P-450 present after such a single dose of amiodarone remained quite small, being only 0 2 nmol/mg of microsomal protein (Fig 4)

If, however, both amiodarone and the inducer were now given concomitantly for 3 days, the amount of cytochrome P-450 complexed *in vivo* became quite noticeable (Table 1) Dexamethasone had the most potent effect, enhancing 10-fold the formation of the complex (Table 1). In rats receiving both dexamethasone and amiodarone for 3 days, complexed cytochrome P-450 reached 0 78 nmol per mg of microsomal protein, or 40% of total cytochrome P-450 (Table 1) In contrast, concomitant administration of 3-methylcholanthrene decreased the formation of the complex; pregnenolone-16α-carbonitrile had no effect while phenobarbital slightly increased the formation of the complex (Table 1)

The formation of the complex appeared strikingly different in several rodent species (Fig 5) After administration of amiodarone given alone for 3 days, the complex was similar in rats and in mice, but 2 5-times greater in hamsters, reaching 0 17 nmol per mg of microsomal protein or 13% of total cytochrome P-450 in the latter species (Fig 5) However, whereas the concomitant administration of dexamethasone markedly increased the formation of the complex in rats and in mice, it did not modify it in hamsters (Fig. 5) It is noteworthy that the effect of dex-

Table 1 Microsomal enzymes in rats treated for 3 days with inducers and/or amiodarone

Treatment	Liver weight/ body weight (%)	Microsomal protein (mg/g liver)	NADPH-cytochrome *c* reductase (nmol/min/mg protein)	Uncomplexed cytochrome P-450 (nmol/mg protein)	Total cytochrome P-450 (nmol/mg protein)	Complexed cytochrome P-450 (nmol/mg protein)	Complexed cytochrome P-450 (% of total P-450)
None	4 4 ± 0 1	37 ± 1	69 ± 13	0 95 ± 0 07	0 95 ± 0 07	N D	0
Amiodarone	4 2 ± 0 1	38 ± 3	53 ± 4	0 66 ± 0 07*	0 73 ± 0 06**	0 07 ± 0 02	10
3-MC	4 6 ± 0 1	34 ± 1	64 ± 3	1 64 ± 0 04	1 62 ± 0 02	N D	0
3-MC + Amiodarone	4 9 ± 0 2	34 ± 1	71 ± 3	1 78 ± 0 07	1 85 ± 0 07	0 03 ± 0 01	2
PCN	4 9 ± 0 1	34 ± 1	79 ± 3	1 36 ± 0 03	1 33 ± 0 04	N D	0
PCN + Amiodarone	5 0 ± 0 1	32 ± 1	97 ± 2	1 53 ± 0 10	1 62 ± 0 11**	0 09 ± 0 02	6
Pheno	5 2 ± 0 2	41 ± 1	136 ± 7	2 20 ± 0 09	2 25 ± 0 07	N D	0
Pheno + Amiodarone	5 0 ± 0 1	45 ± 1	120 ± 3	2 05 ± 0 08	2 25 ± 0 12**	0 17 ± 0 02	8
Dexa	7 3 ± 0 2	30 ± 1	177 ± 8	1 20 ± 0.04	1 17 ± 0 05	N D	0
Dexa + Amiodarone	6 7 ± 0 4	44 ± 2	136 ± 7	1 26 ± 0 15	2 10 ± 0 22*,**	0 78 ± 0 15	40
Dexa + Pheno	7 7 ± 0 4	35 ± 1	163 ± 4	1 95 ± 0.06	1 94 ± 0 05	N D	0
Dexa + Pheno + Amiodarone	7 5 ± 0 3	34 ± 2	140 ± 5	2 34 ± 0 09	2 93 ± 0 11*,**	0 62 ± 0 07	20

Rats were treated for 3 days with inducers and/or amiodarone, the various compounds being given concomitantly The inducers were 3-methylcholanthrene (3-MC 20 mg/kg daily), pregnenolone 16 α-carbonitrile ('PCN' 50 mg/kg daily), phenobarbital ("Pheno", 100 mg/kg daily), or dexamethasone phosphate (Dexa' 50 mg/kg daily) Rats were killed 24 hr after the last dose(s) The amounts of uncomplexed cytochrome P-450 was determined by the CO-binding spectrum of dithionite-reduced microsomes Total cytochrome P-450 was similarly determined, after first adding 50 μM potassium ferricyanide to the microsomes The amount of complexed cytochrome P-450 was determined by its Soret peak at 453 nm, with a molar extinction coefficient of 75 mM^{-1} cm^{-1} Results are means ± S E M for 8 rats N D , not detected

* Significantly different from that in rats not treated with amiodarone (*t*-tested for independent data), $P < 0.05$

** Significantly different from uncomplexed cytochrome P-450 (*t*-test for dependent data), $P < 0.01$

Fig 5 Comparison of the *in vivo* formation of a cytochrome P-450–amiodarone metabolite complex in various rodent species Animals were treated for 3 days with amiodarone hydrochloride (100 mg/kg i p daily), given alone or in combination with dexamethasone (50 mg/kg i p daily) Animals were killed 24 hr after the last dose(s). The amount of cytochrome P-450–metabolite complex present in liver microsomes was determined from its Soret peak at 453 nm Results are means ± S E M for 8 animals

amethasone phosphate given alone (50 mg/kg i.p. daily for 3 days) on microsomal cytochrome P-450 varied in different species: cytochrome P-450 was slightly increased in rats (Table 1) and in mice (from 0 70 ± 0 05 nmol/mg microsomal protein to 1.08 ± 0.07, mean ±S.E M. for 8 mice); in contrast, it was decreased in hamsters (from 0.94 ± 0.05 nmol/mg microsomal protein to 0.52 ± 0.04, mean ± S.E.M. for 8 hamsters).

In vitro *formation of a cytochrome P-450*Fe(II)–*amiodarone metabolite complex*

Incubation of microsomes from dexamethasone-treated rats with 1.5 mM EDTA, 4 mM NADPH and 0 1 mM amiodarone at 37° resulted in the slight formation of a cytochrome P-450Fe(II)–metabolite complex absorbing at 453 nm: 37 ± 8 pmol/5 min/mg microsomal protein, mean ± S.E.M. for 6 experiments The 453 nm-peak was usually fully developed after 5 min of incubation and appeared stable for the next 10 min. This peak disappeared, however, upon addition of 100 μM potassium ferricyanide to the microsomes. The 453 nm-absorbing peak was not formed when either NADPH or amiodarone was omitted or when piperonyl butoxide (4 mM) was added to the microsomal suspension in both cuvettes. Increasing the concentration of amiodarone to 0.25 mM did not significantly enhance the *in vitro* formation of the 453 nm-absorbing complex (not shown)

The *in vitro* formation of the 453 nm-absorbing complex was even smaller with microsomes from phenobarbital rats 17 ± 2 pmol/5 min/mg microsomal protein, mean ± S E.M for 6 experiments, it was not detectable with microsomes from control rats.

Similar observations were made when microsomes were incubated with the secondary amine, *N*-desethylamiodarone. the amount of formed cytochrome P-450–metabolite complex was 40 ± 9 pmol/5 min/mg protein with microsomes from rats pretreated by dexamethasone, and 26 ± 4 with microsomes from rats pretreated with phenobarbital, mean ± S E.M for 6 experiments. No complex was found with microsomes from untreated rats

Effects of amiodarone on monooxygenase activities in vivo *and* in vitro

The liver weight/body weight ratio, microsomal protein content, NADPH-cytochrome *c* reductase activity, and total cytochrome P-450 levels were not significantly modified after administration of amiodarone given alone for 3 days (Table 1). This treatment, however, slightly decreased some monooxygenase activities (Table 2). In these rats, in which complexed cytochrome P-450 represented only 10% of total cytochrome P-450 (Table 1), the addition of potassium ferricyanide to the microsomes did not significantly enhance monooxygenase activities (Table 2). In contrast, in rats receiving both amiodarone and dexamethasone, in which complexed cytochrome P-450 represented 40% of total cytochrome P-450 (Table 1), the addition of potassium ferricyanide selectively increased some monooxygenase activities (Table 2). While hexobarbital hydroxylase and aminopyrine or troleandomycin demethylase activities were not modified, benzo(*a*)-pyrene hydroxylase activity, 7-ethoxycoumarin deethylase activity and the *in vitro* formation of the cytochrome P-450Fe(II)–amiodarone metabolite complex in the presence of 0.1 mM N-desethylamiodarone were increased by 30, 40 and 270%, respectively (Table 2). Addition of potassium ferricyanide also tended to increase the *in vitro* formation of the complex in the presence of 0 1 mM amiodarone, the difference, however, was not statistically significant (Table 2).

In vitro, amiodarone (0 15 mM) inhibited by 20–70% monooxygenase activities in microsomes from dexamethasone-treated rats (Table 3) An almost similar inhibition was observed in microsomes from control rats (Table 3)

Table 2 Monooxygenase activities in rats treated for 3 days with dexamethasone and/or amiodarone

Treatment	Hexobarbital hydroxylase		Aminopyrine demethylase		Troleandomycin demethylase		Benzo(*a*)pyrene hydroxylase		7-Ethoxycoumarin deethylase		In vitro formation of a cytochrome P-450-metabolite complex with			
											Amiodarone		N-desethylamiodarone	
	Without Ferricyanide	With Ferricyanide	Without Ferricyanide	With Ferricyanide	Without Ferricyanide	With Ferricyanide	Without Ferricyanide	With Ferricyanide	Without Ferricyanide	With Ferricyanide	Without Ferricyanide	With Ferricyanide	Without Ferricyanide	With Ferricyanide
	(nmol min mg protein)		(nmol min mg protein)		(nmol min mg protein)		(nmol min mg protein)		(nmol min mg protein)		(pmol 5 min mg protein)			
Control	3.1 ± 0.2	2.9 ± 0.1	1.7 ± 0.3	1.8 ± 0.3	0.36 ± 0.04	0.33 ± 0.02	0.35 ± 0.02	0.32 ± 0.02	0.82 ± 0.08	0.84 ± 0.11	N.D.	N.D.	N.D.	N.D.
Amiodarone	2.1 ± 0.2*	2.2 ± 0.2	1.3 ± 0.3	1.3 ± 0.3	0.26 ± 0.03	0.28 ± 0.04	0.23 ± 0.01*	0.24 ± 0.01*	0.66 ± 0.10	0.67 ± 0.13	7 ± 3	12 ± 6	13 ± 5	14 ± 2
Dexamethasone	2.2 ± 0.1	2.2 ± 0.1	3.2 ± 0.4	3.2 ± 0.4	1.06 ± 0.08	1.17 ± 0.07	0.30 ± 0.01	0.29 ± 0.01	1.25 ± 0.24	1.24 ± 0.26	37 ± 6	23 ± 4	39 ± 6	29 ± 9
Dexamethasone + Amiodarone	1.1 ± 0.1*	1.1 ± 0.2*	2.5 ± 0.4	2.7 ± 0.4	1.73 ± 0.13	1.80 ± 0.07	0.27 ± 0.02	0.36 ± 0.04*†	1.02 ± 0.10	1.40 ± 0.19*†	25 ± 5	44 ± 10	11 ± 4	31 ± 6 *

Rats were treated for 3 days with dexamethasone phosphate (50 mg/kg daily) and/or amiodarone hydrochloride (100 mg/kg daily) and were killed 24 hours after the last dose(s). Part of the microsomal suspension was treated with 50 μM potassium ferricyanide before its addition to the various incubation mixtures. The *in vitro* formation of a cytochrome P-450-metabolite complex with 0.1 mM amiodarone or 0.1 mM N-desethylamiodarone was determined at 5 min. Results are means ± S.E.M. for 8 rats. N.D. not detected (less than 5 pmol/5 min/mg protein).

* Significantly different from that in rats not treated with amiodarone (*t*-test for independent data) $P < 0.05$.

† Significantly different from that without potassium ferricyanide (*t*-test for dependent data) $P < 0.05$.

Table 3 *In vitro* inhibitory effects of amiodarone on monooxygenase activities

Treatment	Hexobarbital hydroxylase		Aminopyrine demethylase		Benzo(*a*)pyrene hydroxylase		7-Ethoxycoumarin deethylase	
	Without Amiodarone	With Amiodarone	Without Amiodarone	With Amiodarone	Without Amiodarone	With Amiodarone	Without Amiodarone	With Amiodarone
	(nmol min mg protein)							
Control	2.85 ± 0.15	2.37 ± 0.13*	0.85 ± 0.11	0.58 ± 0.06	0.21 ± 0.03	0.08 ± 0.01	0.46 ± 0.05	0.21 ± 0.02
Dexamethasone	1.94 ± 0.15	1.53 ± 0.13*	1.37 ± 0.11	0.91 ± 0.03*	0.29 ± 0.02	0.12 ± 0.02	0.89 ± 0.21	0.25 ± 0.05

Some rats were treated for 3 days with dexamethasone phosphate (50 mg/kg daily). Monooxygenase activities were measured in the presence or the absence of 0.15 mM amiodarone. The concentration of the various substrates was kept constant at 0.25 mM. Results are means ± S.E.M. for 4 experiments.

* Significantly different from that without amiodarone (*t*-test for dependent data) $P < 0.05$.

DISCUSSION

Our results show that amiodarone is transformed by cytochrome P-450 into a metabolite which forms a 453-nm absorbing complex with the iron(II) of cytochrome P-450 in rats. Indeed, administration of amiodarone *in vivo*, or incubation with hepatic microsomes, NADPH and amiodarone *in vitro* resulted in the appearance of a Soret peak at 453 nm (Fig 1, Tables 1 and 2). Disruption of this inactive cytochrome P-450 Fe(II)–amiodarone metabolite complex, by addition of potassium ferricyanide to the microsomes, restored the ability of cytochrome P-450 to bind carbon monoxide or molecular oxygen, and to oxidize substrates (Fig. 1, Table 2). As a consequence, addition of potassium ferricyanide increased several monooxygenase activities in microsomes from rats treated with amiodarone and dexamethasone (Table 2)

The very low formation of complex *in vivo* after administration of a single dose amiodarone (Fig. 4) and its absence *in vitro* with microsomes from control rats show that cytochrome P-450 isozymes present in untreated rats have probably a low ability to form this complex Two factors, however, markedly increased the amount of cytochrome P-450-metabolite complex A first factor was the induction of cytochrome P-450 isozymes more apt to form the complex The formation of the complex *in vitro* and *in vivo* was increased slightly by phenobarbital-pretreatment and markedly by dexamethasone-pretreatment, but was not modified by pretreatment with pregnenolone-16α-carbonitrile (Table 2, Fig 4) This may indicate that the isozyme(s) involved in the formation of the cytochrome P-450-amiodarone metabolite complex may yet differ from that purified cytochrome P-450 isozyme which is induced by both dexamethasone and pregnenolone-16α-carbonitrile [34, 35]

A second factor which dramatically enhanced the amount of complex present in the liver was the repetition of amiodarone administration (Fig. 3) Unlike previous findings with macrolides [36–42], there was no clear-cut evidence for an inducing effect of amiodarone, as judged by activities of hepatic microsomal enzymes (Table 1) The progressive increase in the amount of complexed cytochrome P-450 observed upon repeated administration of amiodarone (Fig 3) may be mainly related to the long survival of complexed cytochrome P-450, leading to its progressive accumulation in the liver This would also explain the slow disappearance of complexed cytochrome P-450 upon cessation of amiodarone administration (Fig 3) Indeed, a slow disappearance rate has been previously suspected for other cytochrome P-450Fe(II)–metabolite complexes [42] or in the presence of other heme iron ligands [43]. However, the lysosomal storage of amiodarone (a cationic amphiphilic drug) and its consequent slow elimination from the body [44] may have been contributory

Interestingly, induction of some cytochrome P-450 isozyme(s) by the administration of some inducers and the repetition of amiodarone administration had multiplying effects on the amount of complex present in the liver Indeed, in rats receiving both amiodarone and dexamethasone for 3 days (Table 1), the amount of complexed cytochrome P-450 was much higher than in rats pretreated with dexamethasone but receiving only a single dose of amiodarone (Fig 4), or in rats receiving only amiodarone for 3 days (Table 1).

It is clear that the formation of the inactive cytochrome P-450-metabolite complex is but one of the several mechanisms decreasing the activity of cytochrome P-450 This is well apparent *in vitro* where amiodarone markedly inhibited monooxygenase activities in control microsomes (Table 3), although no cytochrome P-450-metabolite complex could be detected with such microsomes (see results) As a general rule, drugs forming cytochrome P-450–metabolite complexes are thought to inhibit cytochrome P-450 in at least two ways: first by competing with other substrates for reversibly binding to cytochrome P-450 and, eventually, by forming the inactive cytochrome P-450–metabolite complex [25, 45]. Other mechanisms may contribute to cytochrome P-450 hypoactivity. For example, it is conceivable, albeit still unproven, that the presumed nitrosoalkane metabolite, unless stabilized through the formation of an iron(II)–metabolite complex, may react with, and covalently bind to, the apoprotein itself [46] Indeed, conjugation with glutathione and/or covalent binding to the SH-groups of proteins is known to occur with several nitrosoarenes [47–49] and might also occur with the nitrosoalkanes (or their nitrone precursors) formed during the metabolism of macrolides [50, 51].

So far, all species tested (rats, mice and hamsters) did form the cytochrome P-450–metabolite complex *in vivo* (Fig 5), albeit in variable amounts, and with different responses to dexamethasone. In the case of macrolide antibiotics, the initial findings in rats [36–39] have been subsequently corroborated by similar results in humans [27, 52]. It is therefore tempting to speculate that amiodarone may likewise form a cytochrome P-450–metabolite complex in humans, which may contribute to the many drug interactions known to occur with this antiarrhythmic agent [10–23]. Studies in humans are now undertaken to delineate the clinical significance of the present findings.

We conclude that amiodarone is transformed by cytochrome P-450 into a metabolite which forms an inactive cytochrome P-450Fe(II)–metabolite complex in rats, mice, and hamsters. In rats, the amount of complex present in the liver is small after a single dose of amiodarone but is markedly increased when the doses of amiodarone are repeated or when dexamethasone is added, reaching 40% of total cytochrome P-450 when both enhancing factors are combined

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