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FACTORS INVOLVED IN THE INHIBITION OF DRUG METABOLISM BY (−)-EMETINE*

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Abstract—The oxidative *N*-demethylation of aminopyrine and of *N*-ethyl morphine as well as the *S*-demethylation of 6-methylmercaptopurine riboside *in vitro* is inhibited by about 50 per cent in liver microsomal preparations from rats pretreated for 24 hr with (−)-emetine dihydrochloride (18 μmoles/kg). Under these conditions, the azo-reduction of neoprontosil is also inhibited, although NADPH-cytochrome *c* reductase and neotretrazolium diaphorase activities are unaffected. The inhibition of drug-metabolizing enzyme activities *in vitro* appears to be related to a lowering of the liver microsomal cytochrome P-450 and cytochrome b_5 content.

When emetine is added directly to control liver microsomal incubations for the assay of *N*- or *S*-demethylation of substrates as above, and aromatic ring hydroxylation of aniline *in vitro*, drug-metabolizing enzyme activities are also inhibited under those circumstances. Emetine appears to be a competitive inhibitor of the *N*-demethylation of aminopyrine. The interaction of emetine and liver microsomal cytochrome P-450 is associated with spectral changes more closely resembling those of aniline (type II) than those of 6-methylmercaptopurine riboside (type I).

DRUG-METABOLIZING activity *in vitro*, as measured by the enzymatic *N*-demethylation of aminopyrine and the azo-reduction of neoprontosil, is inhibited in liver microsomal preparations obtained from rats pretreated with (−)-emetine or (±)-2,3-dehydroemetine.[1] Emetine itself does not appear to be metabolically transformed in the rat[2] and is slowly excreted unchanged in the urine (W. R. Jondorf and R. K. Johnson, unpublished data). When administered to mice, dogs[3] or guinea pigs,[4] emetine tends to be stored in the tissues, and is eliminated slowly in the feces rather than the urine.[3,4] These findings suggest that emetine is unlikely to compete with various other substrates for the hepatic drug-metabolizing enzymes for its own metabolism.

We find though, that the direct addition of emetine to the control rat liver microsomal drug-metabolizing system *in vitro* exerts an inhibitory effect on the *N*-demethylation of aminopyrine without, however, correspondingly inhibiting the azo-reduction of neoprontosil. The present paper is concerned with determining whether these differential effects on demethylation and azo-reduction *in vitro* also hold for other substrates. Furthermore, we set out to examine the kinetics of the inhibitory effect on demethylating activity *in vitro*, and the possible site where emetine might act to interrupt the electron transport chain involved in the mechanisms for drug metabolism.

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MATERIALS AND METHODS

(−)-Emetine dihydrochloride was generously provided by Dr. H. T. Openshaw of Burroughs Wellcome Research Laboratories, Beckenham, Kent, England. Aminopyrine was purchased from Matheson, Coleman & Bell, East Rutherford, N.J. *N*-ethyl morphine was obtained from Merck and Company, Rahway, N.J. Neoprontosil was obtained from the Sterling-Winthrop Research Institute, Rensselaer, N.Y. 6-Methylmercaptopurine riboside, neotetrazolium chloride and horse heart Type III cytochrome *c* were purchased from the Sigma Chemical Company, St. Louis, Mo. All other materials of the highest purity available came from various commercial sources as previously noted.[5,6]

Animals

Female Sprague–Dawley rats (Sprague–Dawley Company, Madison, Wis.) of the same age, and weighing 160 g each, were maintained under standardized stress-free and insecticide-free conditions with access to unlimited Purina Chow rat cubes and tap water. Groups of two or more animals were injected intraperitoneally with freshly made aqueous solutions of (−)-emetine dihydrochloride (18 μmoles/kg) or with distilled water so that the injection volumes were 0·8 ml/160 g body weight. All animals serving as liver donors were fasted in the 24-hr period between pretreatment and death, standardized to extend from 9 a.m.–9 a.m. to deplete hepatic glycogen levels, thus eliminating any differential effects on glycogen storage due to administration of emetine,[7] and minimizing the effects of any biochemical factors subject to diurnal variations. They were then stunned and decapitated in the cold room. The livers were quickly excised and rinsed in ice-cold 0·01 M K_2HPO_4–NaH_2PO_4 phosphate buffer, pH 7·4, containing 1·15% (w/v) KCl. They were then blotted dry, homogenized with 4 vol. of this homogenizing medium and subjected to differential centrifugation under standard conditions[1] for the preparation of microsomal and 105,000 *g* supernatant fractions. The microsomal pellets were carefully redispersed in ice-cold buffered homogenizing medium so that 0·4 ml of microsomal solution was equivalent to 0·375 g wet weight of liver. The microsomal preparations so obtained were used on the day of preparation in combination with 105,000 *g* supernatant material (also equivalent to 0·375 g wet weight of liver) from control rats for the various assays of drug-metabolizing enzyme activity *in vitro*.

For experiments involving measurement of carbon monoxide-binding pigment (cytochrome P-450) in the microsomal preparations, the resuspended microsomal material was again subjected to centrifugation at 105,000 *g* for 1 hr, in order to remove interfering substances. The repelleted microsomal fractions were then carefully resuspended in the buffered homogenizing medium so that the protein concentration was 2 mg/ml. All determinations of protein concentration were performed according to Lowry *et al.*[8] with bovine serum albumin V as the standard.

Assays for enzymatic drug metabolism in vitro

The oxidative *N*-demethylation of aminopyrine and *N*-ethyl morphine, and the S-demethylation[9] of 6-methylmercaptopurine riboside were measured by incubating liver microsomal fractions from emetine-pretreated or control livers (equivalent to 0·375 g wet weight) and 105,000 *g* control supernatant fraction (also equivalent to 0·375 g wet weight) with 2·5 μmoles substrate and a complete supporting system[5] consisting of the

following components: NADP (0·5 μmole); nicotinamide (50 μmoles); glucose 6-phosphate (30 μmoles); magnesium chloride (75 μmoles); 0·5 M K_2HPO_4–NaH_2PO_4 phosphate buffer, pH 7·4 (2 ml); and semicarbazide hydrochloride (5 mg) to trap the formaldehyde formed as a product of metabolism. All incubations (in 4·5 ml volumes) were performed aerobically, in duplicate, in a shaking water bath at 37° for 30 min. Reactions were terminated with saturated barium hydroxide (2·5 ml) and 20% (w/v) zinc sulfate (2·5 ml). Precipitated proteins were removed by centrifugation and aliquots of the clear supernatant solutions were then assayed for formaldehyde by a modified Nash[10,11] method. Recoveries, tissue blanks and protein concentration[8] were determined routinely so that results could be expressed as net average values for formaldehyde metabolite formed (mμmoles) per milligram of microsomal protein in 30 min. In some experiments, (—)-emetine (dihydrochloride) was added directly to the aminopyrine *N*-demethylation assays in varying amounts. Conversely, in other incubations, varying amounts of aminopyrine were added to the system *in vitro* in the presence of constant amounts of (—)-emetine.

The formation of *p*-aminophenol from aniline (hydrochloride) was measured by incubating liver microsomal preparations (equivalent to 0·375 g wet weight tissue) from female rats pretreated with sodium phenobarbital (315 μmoles/kg i.p. daily for 3 days), with 105,000 *g* supernatant preparations from control rat liver (also equivalent to 0·375 g wet weight), 6 μmoles of substrate and a supporting system similar to that for demethylation as above, except for the omission of semicarbazide from the reaction mixtures. Incubations (also carried out in the presence of emetine in the system *in vitro*) were performed aerobically, in triplicate, in a shaking water bath at 37° for 30 min. They were terminated and deproteinized with equal volumes (4·5 ml) of ice-cold 10% (w/v) trichloroacetic acid, and were then assayed for *p*-aminophenol.[12] Recoveries, tissue blanks and protein determinations were run routinely so that results could be calculated as millimicromoles of metabolite formed per milligram of microsomal protein in 30 min.

For the metabolic azo-reduction of neoprontosil, liver microsomal fractions from emetine-pretreated or control rats were incubated in duplicate for 30 min with 105,000 *g* supernatant preparation from control rat liver (also equivalent to 0·375 g wet weight), in a shaking water bath at 37° under an atmosphere of deoxygenated nitrogen or under carbon monoxide, with 10 μmoles substrate and a complete supporting system:[6] NADP (0·5 μmole); nicotinamide (100 μmoles); glucose 6-phosphate (50 μmoles); and 0·05 M K_2HPO_4–NaH_2PO_4 phosphate buffer, pH 7·4 containing 10^{-3}M disodium EDTA (3 ml). Reactions were stopped and deproteinized with equal volumes (5·0 ml) of ice-cold 10% (w/v) trichloroacetic acid. After centrifugation, aliquots of the supernatant solution were analyzed for sulfanilamide by column chromatography and coupling with the Bratton and Marshall[13] reagent as previously described.[6] In some experiments, various amounts of (—)-emetine were added directly to the incubations.

Figures quoted under Results are net average values calculated from duplicate incubations, based on microsomal protein and corrected for nonenzymatic azo-reduction of substrate, and for sulfanilamide recoveries.

NADPH-cytochrome c *reductase assay*

The liver microsomal NADPH-cytochrome *c* reductase activity was determined

by the method of Hernandez *et al.*,[6] also in the presence of varying amounts of (−)-emetine (dihydrochloride) in the system. Enzyme activities were followed by noting the increase in optical density at 550 mμ in 1 cm light-path cuvettes, thermostatically controlled at 25°, in a Beckman DB double-beam spectrophotometer with a Sargeant (model SRL) recorder attachment. The unit of activity is the amount of enzyme protein producing a unit increase in optical density at 550 mμ per min, under the conditions of assay.[6] The amount of cytochrome *c* (horse heart, Type III) reduced per milligram of enzyme protein per minute can then be calculated from the difference in molar extinction coefficient ($18{\cdot}7 \times 10^6$ M^{-1} cm^{-1}) at 550 mμ between the reduced and oxidized forms of cytochrome *c*.[14]

Neotetrazolium diaphorase determination

This was carried out as described by Hernandez *et al.*[6] with liver microsomal enzymes prepared from emetine-pretreated and control rats.

Assay of carbon monoxide-binding pigment (cytochrome P-450)

Microsomal preparations freed from interfering contaminants by recentrifugation were treated with carbon monoxide under the conditions previously described.[15] The quantity of cytochrome P-450 per milligram of microsomal protein could then be calculated from the difference in the optical densities at 450 and 480 mμ and its millimolar extinction coefficient[16] of 91 mM^{-1} cm^{-1}.

Interaction of various substrates and cytochrome P-450

This was measured by following spectral changes on addition of 6-methylmercaptopurine riboside (2·0 mM), aniline (3·6 mM) or emetine (2·7 mM) to liver microsomal preparations from male Sprague–Dawley rats (160 g) pretreated with sodium phenobarbital (315 μmoles/kg i.p.) daily for 3 days. Measurements in the range 350–500 mμ were carried out on a Shimadzu Seisakusho (Kyoto-Japan) multipurpose spectrophotometer, type MPS-50L. Baselines were recorded on this machine by dividing samples of microsomal preparation (1·9 mg/ml of protein) equally into two adjacent cuvettes. Drugs were then added to the sample cuvette to give the final concentrations indicated and the differences in light absorbancy were recorded. These primary recordings were replotted as difference spectra to eliminate irregularities of the baselines by subtracting the baseline recordings from the changed absorbance caused by adding various drugs.[17,18] In a similar manner, the modification of interaction of 6-methylmercaptopurine riboside or aniline and cytochrome P-450 in the presence of emetine (0·3 mM) was determined by adding emetine to the reference and sample cuvettes, and then adding the other drug to the sample cuvette only. Difference spectra could again be plotted, this time indicating whether emetine interfered with the binding of the substrates for microsomal *N*-demethylation or aromatic ring hydroxylation.

Assay of cytochrome b_5

A liver microsomal suspension similar to that used for the determination of cytochrome P-450 (containing 2 mg/ml of protein) was prepared. Samples were divided equally into two matched 1 cm light-path cuvettes and a baseline was recorded in the Beckman spectrophotometer with a recorder attachment, from 500 to 400 mμ. A few milligrams of sodium dithionite ($Na_2S_2O_4$) were then added to the sample cuvette.

The difference spectrum between the oxidized and reduced forms of the cytochrome was then recorded in the same range. The quantity of cytochrome b_5 could then be calculated from the difference in optical density at 423 and 500 mμ and the millimolar extinction coefficient (171 mM^{-1} cm^{-1}) of the reduced cytochrome at 423 mμ.[19,20]

RESULTS

Oxidative demethylation in vitro

As shown in Table 1, pretreatment of rats with emetine inhibits the oxidative demethylation of aminopyrine, as expected,[1] and of two other substrates, *N*-ethylmorphine and 6-mercaptopurine riboside. The addition of emetine directly to the control microsomal incubation system *in vitro* also has an inhibitory effect on demethylating activity *in vitro*. The concentration of emetine required to bring about this latter inhibitory effect is greatly in excess of the amounts of emetine found to be associated with the liver microsomal and 105,000 *g* supernatant fractions 24 hr after pretreatment[21] at 18 *μmoles*/kg.

Metabolism of aniline in vitro

The formation of *p*-aminophenol from aniline *in vitro*, under the conditions outlined in the Methods section, is inhibited in the presence of emetine in the incubation mixtures (Table 1). This is of particular interest, since aniline interacts with liver microsomal cytochrome P-450 in a way that differs from the interaction seen with aminopyrine.[17,22]

Azo-reduction of neoprontosil

This is also inhibited by pretreatment of rats with emetine, as shown in Table 1. However, the inhibitory effects on azo-reduction brought about by direct addition of emetine to the incubations *in vitro* are negligible. Microsomal azo-reductase is known to be affected by carbon monoxide,[15] which suggests that azo-reduction is mediated in part by microsomal cytochrome P-450.

As can be seen in Table 1, emetine pretreatment lowers the total azo-reductase activity (under nitrogen), which includes the cytochrome P-450-dependent pathway of azo-reduction. Emetine pretreatment appears to affect this pathway predominantly, since the decrease in azo-reductase activity after pretreatment is of similar magnitude to the decrease in activity observed when control microsomal incubations for the assay of azo-reductase are carried out in an atmosphere of carbon monoxide. However, emetine pretreatment also inhibits the carbon monoxide-insensitive component of azo-reductase activity. In contrast, direct addition of emetine to the control system *in vitro* affects neither cytochrome P-450-dependent nor carbon monoxide-insensitive components of azo-reductase to any appreciable extent.

Effects on NADPH-cytochrome c *reductase and neotetrazolium diaphorase activities*

Hernandez *et al.*[6] established that purified azo-reductase and NADPH-cytochrome *c* reductase were probably identical, and that azo-reductase and neotetrazolium diaphorase activities were similar in many respects, although azo compounds and neotetrazolium were thought to be reduced at different sites of the same enzyme. These findings tend to be confirmed by effects on those enzymes exerted by direct addition

TABLE 1. EFFECTS OF EMETINE ON VARIOUS PARAMETERS OF DRUG-METABOLIZING ENZYME ACTIVITY *in vitro*

Microsomal preparation	Oxidative demethylation *in vitro* of (mμmoles HCHO formed/mg microsomal protein/30 min)			Ring hydroxylation of aniline *in vitro* (mμmoles *p*-amino phenol formed/mg microsomal protein/ 30 min)	Azo-reduction of Neoprontosil *in vitro* (mμmoles sulfanilamide formed/mg microsomal protein/ 30 min)		NADPH-cytochrome *c* reductase (mμmoles cytochrome *c* reduced/mg protein/ min)	Neotetrazolium diaphorase (mμmoles formazan formed/mg protein/ 10 min)	Cytochrome P-450 (mμmoles P-450/ mg protein)	Cytochrome b_5 (mμmoles b_5/ mg protein)
	Aminopyrine	*N*-ethyl-morphine	6-Methyl-mercaptopurine riboside		Under N_2	Under CO				
Control microsomes	134	130	109	42*	109	63	45	348	0·49	0·43
Emetine—pretreated microsomes†	63 (−53%)‡	29 (−78%)	58 (−47%)		57 (−48%)	43 (−32%)	44 (−2%)	342 (−2%)	0·29 (−41%)	0·26 (−40%)
Control microsomes plus 4 × 10^{-4}M emetine	80 (−40%)	92 (−29%)	78 (−29%)	33* (−21%)	105 (−4%)	67 (+6%)	41 (−9%)	346 (−1%)	0·51 (+4%)	

* Microsomes from rats pretreated with sodium phenobarbital (315 μmoles/kg i.p.) daily for 3 days.
† Microsomes from rats pretreated with (−)-emetine dihydrochloride (18 μmoles/kg i.p.) for 1 day.
‡ Figures in parentheses are percentage changes from control values.

of emetine to the control system *in vitro*, as shown in Table 1. Neither NADPH-cytochrome *c* reductase nor neotetrazolium diaphorase is inhibited by emetine pretreatment or by emetine added directly to the incubations *in vitro*.

Cytochrome P-450 and cytochrome b_5

Both cytochrome P-450 and cytochrome b_5 contents of liver microsomal preparations from emetine-pretreated rats are lower than control, as shown in Table 1. It is now fairly well established that many drugs are oxidatively metabolized by NADPH-dependent enzymes at the liver microsomal level. In these reactions, NADPH is thought to reduce cytochrome *c* reductase,[23] which in turn yields reduced cytochrome P-450,[24] an oxygen acceptor reacting with substrate to yield oxidized drug metabolite and oxidized cytochrome P-450.[17,18,25] Correspondingly, cytochrome b_5 mediates NADH-dependent transformations.[26] We have already seen that emetine pretreatment does not inhibit liver microsomal NADPH-cytochrome *c* reductase activity. It is therefore not unreasonable to relate lowered drug-metabolizing activity *in vitro* after emetine treatment *in vivo* with lower cytochrome P-450 levels in the liver microsomal fractions prepared from the pretreated animals. However, the *N*-demethylation of aminopyrine *in vitro* by control microsomal preparations is inhibited by emetine added directly to the drug-metabolizing system *in vitro*. This type of inhibition is not related to the reduction in the amount of cytochrome P-450, and some other explanation must be sought for this phenomenon.

Competitive inhibition of the N-*demethylation of aminopyrine by emetine* in vitro

The Michaelis constants were determined for various concentrations of emetine and aminopyrine. The results were plotted according to Lineweaver and Burk,[27] as shown in Fig. 1, where the ordinate is the reciprocal of the rate of formaldehyde formation during the initial rate of reaction and the abscissa is the reciprocal of the aminopyrine concentration in the system. It is clear that emetine exerts competitive inhibition on the oxidative *N*-demethylation of aminopyrine. This suggests that emetine binds to the same reactive site on the drug-metabolizing enzymes to which aminopyrine is bound. A Michaelis–Menten constant (K_m) for aminopyrine of $8{\cdot}30 \times 10^{-4}$ M was obtained. The inhibitor constant (K_i) for emetine was calculated to be $4{\cdot}62 \times 10^{-4}$ M from the differences in the slopes of the Lineweaver–Burk plot or from the Dixon[28] plot.

Drug interaction with cytochrome P-450

Figure 2 shows the difference spectra obtained by adding 6-methylmercaptopurine riboside (I), aniline (II) or emetine (III) to liver microsomes prepared from male rats pretreated with phenobarbital as indicated under Methods. The difference spectrum for the mercaptopurine derivative resembles that of a type I spectral change,[17,18,22] also obtainable with aminopyrine. Aniline is a type II compound, where the interaction of drug and cytochrome P-450 gives spectral changes characterized by a minimum at 397 mμ and a maximum at 430 mμ.[17,18,22] The spectral changes brought about by the addition of emetine to the liver microsomal preparations appear to resemble type II, as shown in Fig. 2, where the difference spectrum for emetine (III) has a minimum at 394 mμ and a maximum at 425 mμ.

Figure 3 shows how emetine affects the binding spectrum of 6-methylmercaptopurine riboside and cytochrome P-450. The peak absorption at 389 mμ is affected quite

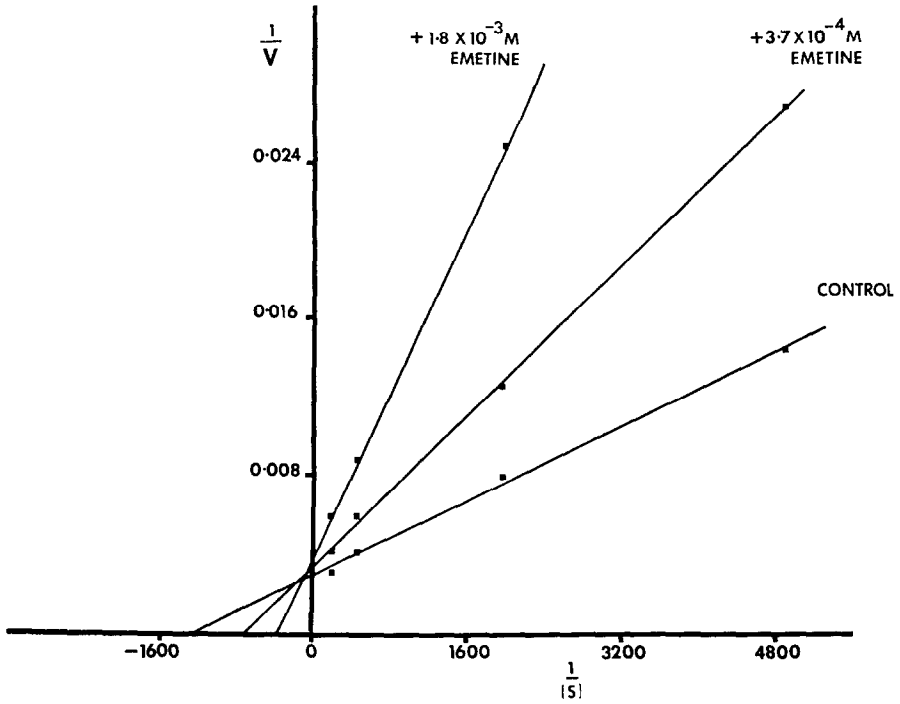

FIG. 1. Effect of added emetine on the oxidative *N*-demethylation of aminopyrine *in vitro*. Details are as described under Methods.

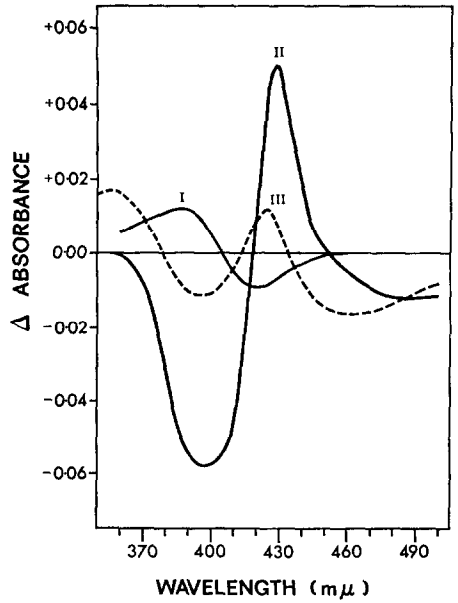

FIG. 2. Difference spectra obtained by the interaction of rat liver microsomal suspensions and 2·0 mM 6-methylmercaptopurine riboside (I), 3·6 mM aniline (II) and 2·7 mM emetine (III). The cuvettes contained approximately 6 mg of microsomal protein/3 ml. Other details are given under Methods.

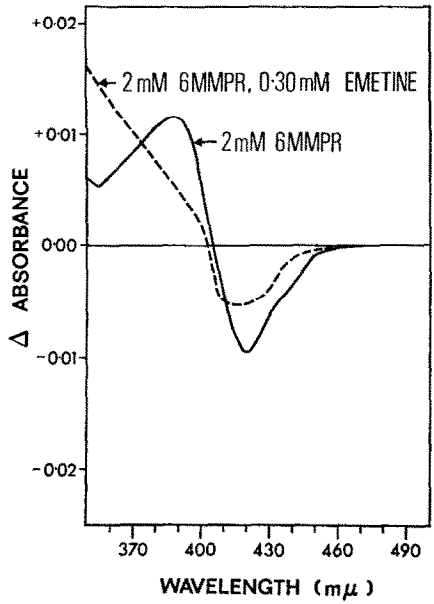

FIG. 3. Modification of difference spectra obtained by interaction of rat liver microsomal suspensions and 2·0 mM 6-methylmercaptopurine riboside (6MMPR) in the presence of 0·3 mM emetine. Procedures are as described under Methods.

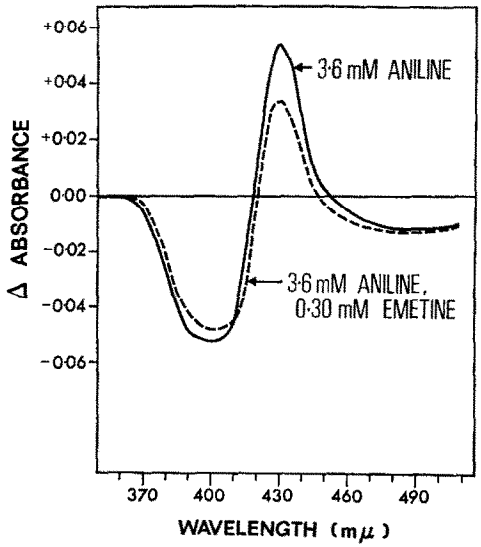

FIG. 4. Difference spectra obtained by interacting 3·6 mM aniline with rat liver microsomal preparations also in the presence of 0·3 mM emetine. Details are given in Methods section.

markedly, suggesting that there are changes in the active complex ascribable to the presence of emetine. In contrast, as shown in Fig. 4, emetine does not noticeably affect the binding of aniline and microsomal cytochrome P-450, since there are no shifts in the absorption peak.

DISCUSSION

Previous studies on the inhibitory effects of emetine pretreatment on drug-metabolizing enzyme activity *in vivo*[1,29] and *in vitro*[1,2,30] were only marginally concerned with the mechanism of inhibition. We have now compared the inhibitory effects on drug metabolism *in vitro* exerted by pretreatment of rats with emetine, and by emetine when added directly to the incubation systems *in vitro*. We find that at 24 hr after pretreatment of rats with emetine (18 μmoles/kg) liver microsomal drug-metabolizing enzymes are inhibited by about 50 per cent. Thus, the oxidative *N*-demethylation of aminopyrine is inhibited as expected,[1] as is the demethylation of another substrate, *N*-ethyl morphine, previously studied by Rubin *et al.*[31] and Gigon *et al.*[32] The *S*-demethylation[9] of 6-mercaptopurine riboside is also inhibited, as shown in Table 1.

There is a corresponding inhibition of the azo-reduction of neoprontosil *in vitro*, affecting both the cytochrome P-450-dependent and carbon monoxide-insensitive pathways of reduction. The finding that emetine pretreatment does not apparently inhibit the closely related NADPH-cytochrome *c* reductase activity is not altogether surprising, since it was previously found that inducing substances may also exert differential effects on azo-reductase and NADPH-cytochrome *c* reductase or neotetrazolium diaphorase.[15]

The inhibition of the drug-metabolizing enzyme activities after pretreatment with emetine may well be related to a reduction of the effective levels of cytochrome P-450 in microsomal preparations from emetine-pretreated rats (Table 1), since cytochrome P-450 is thought to be required for the formation of complexes with drug substrates before liver microsomal oxidative drug metabolism can take place.[17,22,32]

With regard to the direct addition of emetine to the control liver microsomal drug-metabolizing incubation *in vitro*, it would appear that oxidative *N*- and S-demethylation of substrates are inhibited under these circumstances (Table 1). The formation of *p*-aminophenol from aniline *in vitro* is also inhibited by the presence of emetine in the system *in vitro*. However, neither the azo-reduction of neoprontosil nor NADPH-cytochrome *c* reductase and neotetrazolium diaphorase activities are affected.

This type of inhibition *in vitro* is not related to changes in microsomal cytochrome P-450 content. On examining the *N*-demethylation of aminopyrine more closely, it would appear that there was competitive inhibition between emetine, which itself is not metabolized,[2] and aminopyrine, as shown in Fig. 1. The binding of emetine to a reactive site on cytochrome P-450 suggests that emetine may compete with aminopyrine at a critical complex-forming step involving cytochrome P-450.[17,18,22,25]

These effects of emetine *in vitro* differ from the corresponding inhibitory effects on oxidative *N*-demethylation exerted by SKF-525A,[33] the classical inhibitor of drug-metabolizing activity. SKF-525A undergoes *N*-dealkylation[34] and is a competitive inhibitor of the *N*-demethylation of *N*-ethylmorphine with a K_i value of 6×10^{-6} M. This is two orders of magnitude lower than the K_i value obtained for emetine, supporting the finding that SKF-525A is metabolized by the microsomal enzymes as an alternative substrate,[34] yielding metabolites that in turn may be inhibitors of drug metabolism.[35]

Although the spectral changes resulting from interaction of SKF-525A and cytochrome P-450 are type I[22] and those of emetine are type II (Fig. 2), it should be remembered that inhibition of drug metabolism is not necessarily restricted to drugs

exhibiting the same spectral changes on interaction with cytochrome P-450.[22] This is borne out by our studies on the inhibitory effects exerted by emetine *in vitro* on the metabolism of 6-methylmercaptopurine riboside and aniline. The metabolism of both these substrates is inhibited by emetine, although the spectral binding experiments indicate that a type II binding inhibitor such as emetine can alter the binding spectrum of 6-methylmercaptopurine riboside, a type I substrate (Fig. 3), without correspondingly altering the binding spectrum of aniline, a type II binding substrate (Fig. 4).

Further studies may produce evidence that there might well be a better correlation between inhibition of drug-metabolizing enzyme activity and the kinetics of cytochrome P-450 reduction,[32,36] dependent in part on the nature of the complex formed by interaction of substrate, inhibitor and cytochrome P-450.[37]

Since emetine inhibits the metabolism of 6-methylmercaptopurine riboside, it would be interesting to see what effect emetine might have on prolonging the clinical effectiveness of this drug in the treatment of leukemia,[38] particularly since emetine itself has some antileukemic potential.[39,40]

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