Hepatic Drug-Metabolizing Enzyme Activity in Rats Pretreated with (-)-Emetine or (±)-2,3-Dehydroemetine

By H. H. MILLER, R. K. JOHNSON, J. D. DONAHUE AND W. R. JONDORF
Department of Pharmacology, The George Washington University School of Medicine,
Washington, D.C. 20005, U.S.A.

(Received 27 November 1969)

1. Pretreatment of female rats with (-)-emetine or (±)-2,3-dehydroemetine (at 18 µmol/kg body wt. for 24 h) prolongs the hexobarbital-induced sleeping-time of the treated animals. 2. This effect is not observed on pretreating animals with other compounds closely related to (-)-emetine, such as (-)-isoemetine or (+)-O-methylpsychotrine. 3. Liver microsomal drug-metabolizing enzyme activity *in vitro* as measured by *N*-demethylation of aminopyrine and azo-reduction of Neoprontosil is inhibited in rats pretreated with (-)-emetine or with (±)-2,3-dehydroemetine. 4. These inhibitory effects on drug metabolism *in vitro* are not observed in corresponding experiments involving pretreatment of rats with (-)-isoemetine or (+)-O-methylpsychotrine. 5. Co-administration of emetine or 2,3-dehydroemetine and sodium phenobarbital or 1,1-dichloro-2-*o*-chlorophenyl-2-*p*-chlorophenylethane to rats abolishes or greatly diminishes the stimulation of drug-metabolizing enzyme activity *in vitro* usually obtained by the administration of phenobarbital or 1,1-dichloro-2-*o*-chlorophenyl-2-*p*-chlorophenylethane alone. 6. Further, in rats pretreated with sodium phenobarbital and subsequently injected with emetine or 2,3-dehydroemetine the pre-stimulated drug-metabolizing enzyme activity *in vitro* is diminished. 7. The inhibitory effects on drug-metabolizing enzyme activity after pretreatment with (-)-emetine or (±)-2,3-dehydroemetine do not appear to be related to NADPH generation.

Liver microsomal preparations obtained from rats pretreated with sodium phenobarbital have a greater capacity than the corresponding control preparations for incorporating labelled amino acid into protein *in vitro* (Kato, Jondorf, Loeb, Ben & Gelboin, 1966). The liver microsomal stimulation of protein-synthesizing activity after treatment with phenobarbital is also associated with increased liver microsomal drug-metabolizing enzyme activity *in vitro* (Conney, 1967). In similar experiments involving (-)-emetine (Jondorf & Szapary, 1968) or (±)-2,3-dehydroemetine (Jondorf, Drassner, Johnson & Miller, 1969a), liver microsomal preparations isolated from rats pretreated with these compounds had greatly enhanced amino acid-incorporating activities *in vitro* compared with the controls at 24 h after pretreatment. At this time point, as already briefly reported (Jondorf, Johnson & Drassner, 1969b; Jondorf, Johnson & Donahue, 1969c), pretreatment with emetine or 2,3-dehydroemetine exerted an inhibiting effect on drug-metabolizing enzyme activity in liver microsomal preparations *in vitro*. We therefore tested whether co-administration to rats of an inducer of drug-metabolizing

enzyme activity, such as phenobarbital, and an inhibitor, such as emetine or 2,3-dehydroemetine, would lead to an overall inhibition of drug-metabolizing activity as measured *in vitro* by using aminopyrine (Jondorf, Simon & Avnimelech, 1966) and Neoprontosil (Hernandez, Gillette & Mazel, 1967) as substrates. We further tested emetine and 2,3-dehydroemetine inhibitory effects against a different type of inducer, *o,p'*-DDD* (Boyland & Jondorf, 1962; Straw, Waters & Fregly, 1965; Kupfer & Peets, 1966), to see whether the emetine type of compound could neutralize the stimulating effects on drug-metabolizing activity brought about by other drugs used in human therapy.

We also set out to determine whether structure-activity relationships among emetine derivatives that had been established for anti-amoebic activity and for effects exerted by these compounds on protein synthesis (Grollman, 1966; Jondorf *et al.* 1969a) were valid for inhibitory effects observed on drug-metabolizing enzyme activity in the rat. Isoemetine and O-methylpsychotrine were selected

* Abbreviation: *o,p'*-DDD, 1,1-dichloro-2-*o*-chlorophenyl-2-*p*-chlorophenylethane.

Fig. 1. Structural formula of emetine showing the four centres of asymmetry at positions 2, 3, 11b and 1'. Isoemetine differs from emetine only in the stereochemistry at C-1'. 2,3-Dehydroemetine has the same configuration as emetine at C-1'. *O*-Methylpsychotrine (1',2'-dehydroemetine) has no centre of asymmetry at C-1', but has the same configuration as emetine at positions 2, 3 and 11b. The active centre of the emetine molecule (Grollman, 1966) is thought to involve the correct stereospecific alignment at C-1' and a secondary amine structure at N-2'.

for comparison with emetine and 2,3-dehydroemetine (Fig. 1) for this purpose. The results obtained by studying effects on drug metabolism taken in conjunction with previous studies using *N*-methyl-emetine (Jondorf, Johnson & Donahue, 1969d) are consistent with the suggestion that the 'active centre' determining biological potency of the emetine molecule involves the correct stereochemical alignment at the asymmetric carbon atom in the 1'-position in the molecule, and a free secondary amine structure at the 2'-position (Grollman, 1966).

MATERIALS

(-)-Emetine dihydrochloride, (-)-isoemetine dihydrobromide and (+)-*O*-methylpsychotrine were obtained as gifts from Burroughs Wellcome Research Laboratories, Beckenham, Kent, U.K., with the generous co-operation of Dr H. T. Openshaw and Dr N. Whittaker. (±)-2,3-Dehydroemetine dihydrochloride was a gift from Hoffmann-La Roche Inc., Nutley, N.J., U.S.A., through the courtesy of Dr W. E. Scott. *o,p'*-DDD was generously provided by the Cancer Chemotherapy National Service Center of the U.S. Public Health Service, Bethesda, Md., U.S.A. Sodium phenobarbital was purchased from Merck and Co., Rahway, N.J., U.S.A. Aminopyrine, one of the substrates for measuring drug-metabolizing enzyme activity *in vitro*, was purchased from Matheson, Coleman & Bell, East Rutherford, N.J., U.S.A. Two other substrates, Neoprontosil and sodium hexobarbital, were obtained from the Sterling-Winthrop Research Institute, Rensselaer, N.Y., U.S.A. All other materials of the highest purity available were purchased from various commercial suppliers as before (Jondorf *et al.* 1966).

METHODS

Animal treatment. Female Sprague-Dawley rats (Sprague-Dawley Co., Madison, Wis., U.S.A.) of the same age, and weighing 160g each, were kept until needed under standardized stress-free conditions in an insecticide-free room maintained at 24°C and adjusted to alternating dark and illuminated periods each lasting 12h. The rats had access to unlimited Purina Chow (Ralston Purina Co., St Louis, Mo., U.S.A.) rat cubes and tap water.

In general, groups of two or more rats were injected intraperitoneally with freshly made aqueous solutions of drug or with water, so that the injection volume was 0.5ml/100g body wt. In experiments involving the administration of more than one drug, the total injection volume was increased to 1.0ml/100g body wt. for both experimental and control animals. The usual dosages of drugs administered were 18μmol/kg body wt. for emetine (Jondorf & Szapary, 1968), 2,3-dehydroemetine (Jondorf *et al.* 1969a), isoemetine or *O*-methylpsychotrine, and 315μmol/kg body wt. for sodium phenobarbital. In some experiments sodium phenobarbital was administered daily for 3 days at this dosage. Some slight modifications of these procedures were made in all experiments involving pretreatment with *o,p'*-DDD (312μmol/kg, i.e. 100mg/kg body wt.). Since younger animals are apparently more responsive to this drug (Kupfer & Peets, 1966), *o,p'*-DDD (dissolved in corn oil) was administered to 80g female Sprague-Dawley rats from the same supplier. In these experiments, control animals received corresponding intraperitoneal injections of corn oil.

All animals for experiments *in vitro* were kept without food in the 24h period between drug administration and death to deplete liver glycogen contents, thereby eliminating any differential effects on liver glycogen storage resulting from pretreatment with emetine (Diamant, 1958). They were then killed by stunning and decapitation in the cold-room, according to a schedule designed to minimize any effects that diurnal variations in biochemical parameters might exert on the experiments. The livers were immediately excised and rinsed twice in ice-cold 1.15% (w/v) KCl solution. They were blotted dry and then homogenized with 4 vol. of medium in a motor-driven coaxial Teflon-glass homogenizer (clearance 0.84mm) rotating at 4000rev./min for ten complete vertical excursions before being subjected to differential centrifugation (Hogeboom, 1955) at 600g (10min), 15000g (15min) and 105000g (60min) at 0–4°C, for the preparation of microsomal pellets and 105000g supernatant fractions. The microsomal pellets were rinsed several times with fresh ice-cold homogenization medium and were then carefully redispersed in this medium so that 0.4ml of the opalescent microsomal solution was equivalent to 0.375g wet wt. of liver. The microsomal preparations obtained in this way were used in combination with 105000g supernatant preparations (also equivalent to 0.375g wet wt. of liver) from control rats, for the assay of drug-metabolizing activity *in vitro* as indicated below. The subcellular materials were always used on the day of preparation.

Drug-metabolizing enzyme activity *in vitro*. For measuring the oxidative *N*-demethylation of aminopyrine, microsomal fractions from pretreated or control livers equivalent to 0.375g wet wt. of liver were incubated

aerobically, in duplicate, in a shaking water bath at 37°C for 30 min with 105 000g control supernatant preparation also equivalent to 0.375g of liver, 2.5 μ mol of aminopyrine, and a complete supporting system (Jondorf *et al.* 1966) and semicarbazide to react with any formaldehyde formed metabolically. Reactions were stopped with equal volumes (4.5 ml) of saturated lead subacetate solution. Subsequently the precipitated protein was removed by centrifugation and portions of the clear supernatant material were aspirated and assayed for formaldehyde by the Cochin & Axelrod (1959) modification of the Nash (1957) method. Recoveries, tissue blanks and protein concentration (Lowry, Rosebrough, Farr & Randall, 1951) were determined for each preparation so that the results could be expressed as net average values for formaldehyde formed (in nmol)/mg of microsomal protein in 30 min. Bovine serum albumin fraction V (Armour Pharmaceutical Co., Kankakee, Ill., U.S.A.) served as the standard in all protein determinations.

For measuring the azo-reduction of Neoprontosil, microsomal preparations from livers of pretreated or control rats were incubated with control 105 000g hepatic supernatant material (equivalent to 0.375g wet wt. of liver) in duplicate, in the shaking water bath at 37°C under an atmosphere of O₂-free N₂ with 10 μ mol of substrate and the complete supporting system of Hernandez *et al.* (1967). Reactions were stopped after 30 min with equal volumes (5.0 ml) of ice-cold 10% (w/v) trichloroacetic acid. After centrifugation, the sulphanilamide formed as a metabolite from Neoprontosil was separated by column chromatography (Hernandez *et al.* 1967) of the deproteinized supernatant material and was then assayed by diazotization and coupling with *N*-(1-naphthyl)ethylenediamine (Bratton & Marshall, 1939). All results quoted in the Results section are net average values calculated from duplicate incubations, based on microsomal protein content and corrected for non-enzymic azo-reduction of Neoprontosil, and for sulphanilamide recoveries.

Reduction of NADP⁺ in the support system for the drug-metabolizing in vitro. The formation of NADPH was measured by the method of Horecker & Kornberg (1957) by using the 105 000g supernatant fraction from normal untreated rat liver as the source of the glucose 6-phosphate dehydrogenase enzyme. Reaction mixtures contained the following components in total volumes of 3.0 ml: 0.04 M-glycylglycine buffer, pH 7.5, 0.5 ml; 0.1 M-MgCl₂, 0.3 ml; 1 μ M-NADP⁺ in 0.3 ml of buffer; 0.02 M-glucose 6-phosphate, 0.1 ml; 105 000g supernatant fraction, 0.1 ml; water, 1.7 ml or 1.4 ml depending on whether ($\pm$)-2,3-dehydroemetine in various amounts (in 0.3 ml) of buffer was added to the system. The formation of NADPH was observed by reading the E_{340}^{40} value in quartz cuvettes at various time-points at 37°C in a thermostatically controlled Beckman DU u.v. spectrophotometer with a Gilford model 2000 attachment. Protein determinations (Lowry *et al.* 1951) of the supernatant fractions were performed so that the results could be expressed as pmol of NADPH/min per mg of protein.

Drug-metabolizing activity in vivo. The time-interval between the loss and the return of the righting reflex after treatment of animals with sodium hexobarbital (Quinn, Axelrod & Brodie, 1958; Brodie, Maickel & Jondorf, 1958) is recognized as an indicator of hepatic drug-metabolizing activity *in vivo*. Alterations in the duration of the hexo-

barbital-induced sleeping-time may be brought about by pretreatment of animals with other drugs. These alterations can be related to changes in drug-metabolizing enzyme activity, if it is assumed that the pretreatment does not sensitize the animals to the barbiturate in any way.

Groups of ten carefully matched female Sprague-Dawley rats (50g body wt.), of the same age from the same breeder, were injected intraperitoneally with water or with emetine (or one of its related compounds) at a dosage of 18 μ mol/kg body wt. The injection volume (0.5 ml/50g rat) was intended to minimize error through leakage at the site of injection. All rats were starved for 24 h before they were treated intraperitoneally with sodium hexobarbital in freshly made aqueous solution (388 μ mol/kg, i.e. 100 mg/kg body wt.). The duration of the loss of the righting reflex was then measured for the various groups of animals.

RESULTS

Comparison of effects due to pretreatment with various compounds related to emetine in vivo on liver microsomal drug metabolism in vitro. On incubating the liver microsomal fractions prepared from groups of two rats pretreated for 24 h with emetine or one of its derivatives, with 105 000g supernatant fraction obtained from the livers of control animals, and substrates for measuring oxidative *N*-demethylation or azo-reduction, as outlined in the Methods section, we obtained the results shown in Table 1. It is evident that pretreatment with either emetine or 2,3-dehydroemetine at 18 μ mol/kg for 24 h exerts an inhibiting effect of about 50% on drug-metabolizing enzyme activity *in vitro*. However, pretreatment of rats with isoemetine or *O*-methylpsychotrine had no such inhibiting effect *in vitro* on demethylation of aminopyrine or azo-reduction of Neoprontosil by the liver microsomal fractions subsequently prepared from animals given injections with these other compounds related to emetine. Even when the rats were pre-injected with doses of isoemetine or *O*-methylpsychotrine as high as 144 μ mol/kg there was no noticeable inhibitory effect on drug-metabolizing enzyme activity *in vitro* as measured by *N*-demethylation and azo-reduction.

Influence of pretreatment with emetine or related compounds on drug metabolism in vivo as measured by duration of the hexobarbital-induced sleeping-time. Kale, Joglekar & Balwani (1966) showed that emetine pretreatment would prolong the hexobarbital-induced loss of righting reflex in rats, and decrease their ascorbic acid synthesis as measured by urinary excretion. Both of these are parameters of decreased liver microsomal drug-metabolizing enzyme activity (Quinn *et al.* 1958; Conney & Burns, 1959). Table 2 shows the effects of sodium hexobarbital administration (100 mg/kg) on the average sleeping-times of groups of ten carefully selected

Table 1. *Comparative effects of pretreatment of rats with emetine and related compounds on liver microsomal drug-metabolizing enzyme activity in vitro*

All incubations from individually processed livers were carried out in duplicate as described in the Methods section, with hepatic microsomal preparations from the variously pretreated 24 h starved female rats and 105 000g supernatant fraction from control animals together with substrates and appropriate cofactors. Results are given as average values $\pm$ s.e.m. Values in parentheses are percentage changes from control values, which are set at 100%.

Pretreatment	Dosage (μ mol/kg)	No. of animals	Metabolite formed (nmol/30 min per mg of microsomal protein)	
			From aminopyrine	From Neoprontosil
Control	—	7	157 $\pm$ 5	202 $\pm$ 6
Emetine	18	4	72 $\pm$ 16 (–54%)	97 $\pm$ 4 (–52%)
Isoemetine*	18	4	164 $\pm$ 32 (+ 4%)	190 $\pm$ 6 (– 6%)
2,3-Dehydroemetine	18	3	75 $\pm$ 19 (–52%)	92 $\pm$ 14 (–54%)
O-Methylpsychotrine*	18	4	173 $\pm$ 16 (+11%)	187 $\pm$ 20 (– 7%)

* Similar results are obtained at pretreatment dosage of 144 μ mol/kg.

Table 2. *Effect of 24 h pretreatment (intraperitoneal) groups of 50 g female Sprague-Dawley rats with various compounds related to emetine on the duration of the hexobarbital-induced sleeping-time*

Sodium hexobarbital was administered intraperitoneally at a dosage of 388 μ mol/kg (i.e. 100 mg/kg) 24 h after pretreatment with the alkaloids or with water. Results are given as average sleeping-time $\pm$ s.e.m., for ten animals in each treatment. Values in parentheses represent percentage changes from control values.

Pretreatment	Dosage (μ mol/kg)	Sleeping-time (min)
Control	—	62 $\pm$ 3
Emetine	18	101 $\pm$ 6 (+63%)
Isoemetine	18	64 $\pm$ 6 (+ 3%)
2,3-Dehydroemetine	18	93 $\pm$ 3 (+50%)
O-Methylpsychotrine	18	69 $\pm$ 4 (+11%)

50 g female rats 24 h after pretreatment with emetine, isoemetine, 2,3-dehydroemetine or O-methylpsychotrine at 18 μ mol/kg. Emetine and 2,3-dehydroemetine prolong the sleeping-time under these circumstances by about 50%, but the structural analogues isoemetine and O-methylpsychotrine have no such prolonging effect on the sleeping-time at the dosage used. We conclude from this type of experiment that pretreatment of rats with either emetine or 2,3-dehydroemetine has an inhibiting effect on liver microsomal drug metabolism also *in vivo*. However, the administration of the closely related analogues isoemetine or O-methylpsychotrine at 18 μ mol/kg 24 h before hexobarbital has no appreciable influence on drug-metabolizing activity *in vivo*. This is not altogether surprising in view of what is known about structure-activity relationships among emetine derivatives in other systems (Grollman, 1966; Jondorf *et al.* 1969a).

Table 3. *Effect on NADPH formation of increasing the concentration of 2,3-dehydroemetine (dihydrochloride) added directly to the NADP-reducing glucose 6-phosphate dehydrogenase support system for liver microsomal drug metabolism in vitro.*

The assays were performed as described in the Methods section. Results are given as mean $\pm$ s.e.m.

Concentration of 2,3-dehydroemetine (mM)	NADPH formed (pmol/min per mg of protein)
Control	39.7 $\pm$ 1.5
0.01	40.4 $\pm$ 2.8
0.1	37.5 $\pm$ 2.1
1.0	38.2 $\pm$ 2.1

Effect of 2,3-dehydroemetine on the reduction of NADP⁺ by glucose 6-phosphate dehydrogenase. A study by Watkins & Guess (1968) on the inhibitory effect exerted by emetine on oxidative phosphorylation in isolated embryonic chick heart cells suggested that emetine inhibited the formation of NADH from NAD⁺. This implied that emetine inhibited a dehydrogenase. If this were true, then, by analogy, the effects exerted by emetine or 2,3-dehydroemetine on NADPH-dependent drug-metabolizing enzyme activity (Gillette, Brodie & La Du, 1957) might be explained by inhibition of glucose 6-phosphate dehydrogenase-mediated formation of NADPH from NADP⁺.

However, as indicated in Table 3, the addition of various amounts of 2,3-dehydroemetine to the control hepatic 105 000g supernatant fraction used in the drug-metabolizing experiments *in vitro* as the source for glucose 6-phosphate dehydrogenase has no effect on the generation of NADPH from NADP⁺. Similar results were obtained with emetine.

We conclude from this that the inhibitory effects

Table 4. *Effect of combined pretreatment (intraperitoneal) of 80g female rats with o,p'-DDD and various compounds related to emetine on liver microsomal N-demethylation and azo-reduction in vitro*

o,p'-DDD (in corn oil) was administered at 312 $\mu\text{mol/kg}$ body weight. All other drugs were administered in aqueous solution at 18 $\mu\text{mol/kg}$. Animals not receiving drugs were given sham injections of corn oil and/or water. Values for *N*-demethylation of aminopyrine and azo-reduction of Neoprontosil are averages for duplicate determinations $\pm$ s.e.m. Values in parentheses are percentage changes from control values. Experimental conditions are as described in the Methods section.

Pretreatment	No. of animals	Metabolite formed (nmol/30min per mg of microsomal protein)	
		From aminopyrine	From Neoprontosil
Control	4	197 $\pm$ 9	159 $\pm$ 5
<i>o,p'</i> -DDD	3	389 $\pm$ 6 (+ 97%)	231 $\pm$ 28 (+45%)
<i>o,p'</i> -DDD + emetine	3	225 $\pm$ 35 (+ 14%)	137 $\pm$ 21 (-14%)
<i>o,p'</i> -DDD + 2,3-dehydroemetine	3	248 $\pm$ 23 (+ 26%)	123 $\pm$ 26 (-23%)
<i>o,p'</i> -DDD + isometine	3	394 $\pm$ 34 (+100%)	229 $\pm$ 25 (+44%)
<i>o,p'</i> -DDD + <i>O</i> -methylpsychotrine	3	377 $\pm$ 26 (+ 91%)	210 $\pm$ 7 (+32%)

on drug-metabolizing activity *in vitro* exerted by 2,3-dehydroemetine and emetine are not brought about by interference in the generation of NADPH from NADP⁺, for in this type of experiment the concentration of 2,3-dehydroemetine is greatly in excess of the concentrations known to be associated with the liver microsomal and supernatant sub-cellular fractions 24h after pretreatment of rats at a dosage of 18 $\mu\text{mol/kg}$ (Miller & Jondorf, 1970).

Co-administration of o,p'-DDD and various analogues of emetine. *o,p'*-DDD when administered to 160g female rats had relatively little effect on some of the parameters of liver microsomal drug-metabolizing enzyme activity 24h later. However, quite pronounced stimulatory effects on *N*-demethylation of aminopyrine and reductive cleavage of Neoprontosil *in vitro* were obtained with liver microsomes from 80g female rats pretreated with *o,p'*-DDD at 100mg/kg for 24h, as shown in Table 4. When emetine or 2,3-dehydroemetine (18 $\mu\text{mol/kg}$) are co-administered with *o,p'*-DDD, the inhibitory effects exerted by these compounds on drug-metabolizing activity tend to predominate. This is shown most clearly in the azo-reduction of Neoprontosil.

The co-administration of isometine or *O*-methylpsychotrine (also at 18 $\mu\text{mol/kg}$) and *o,p'*-DDD, in contrast, does not diminish the stimulatory effects of *o,p'*-DDD on liver microsomal *N*-demethylating and azo-reduction activities *in vitro*.

Co-administration of sodium phenobarbital and emetine or 2,3-dehydroemetine. Table 5 shows the effects on liver microsomal *N*-demethylation of aminopyrine *in vitro* brought about by pretreatment of rats with sodium phenobarbital and emetine or 2,3-dehydroemetine alone or in combination. The results refer to different injection schedules, which were devised to try and differentiate between effects exerted by emetine or 2,3-dehydroemetine on

Table 5. *Effect of combined administration (intraperitoneal) of sodium phenobarbital and emetine or 2,3-dehydroemetine to 160g female rats on liver microsomal N-demethylation of aminopyrine and azo-reduction of Neoprontosil in vitro*

PB₁ refers to administration of sodium phenobarbital (315 $\mu\text{mol/kg}$) for 1 day. PB₃ indicates daily administration for 3 days. With PB₃ treatment, emetine (EM) or 2,3-dehydroemetine (DHE) are co-administered (18 $\mu\text{mol/kg}$) with phenobarbital on the third day. Values for *N*-demethylation and azo-reduction are averages of quadruplicate determinations from groups of two animals. Values in parentheses are percentage changes from control values. All animals not receiving drug are sham-injected with water daily.

Pretreatment	Metabolite formed (nmol/30min per mg of microsomal protein)	
	From aminopyrine	From Neoprontosil
1-day control	146	166
PB ₁	365 (+150%)	222 (+34%)
PB ₁ + EM	94 (-36%)	98 (-41%)
PB ₁ + DHE	157 (+8%)	113 (-32%)
EM	86 (-41%)	82 (-51%)
DHE	70 (-52%)	78 (-53%)
3-day control	136	181
PB ₃	750 (+451%)	303 (+67%)
PB ₃ + EM	481 (+254%)	229 (+27%)
PB ₃ + DHE	451 (+232%)	209 (+15%)
EM	71 (-48%)	99 (-45%)
DHE	72 (-47%)	74 (-59%)

incipient stimulation of drug-metabolizing activity by phenobarbital, and on already existing phenobarbital-induced stimulation of *N*-demethylating activity.

In the type of experiment where animals are treated with phenobarbital for 1 day, the co-

administration of emetine or 2,3-dehydroemetine blocks the incipient stimulation of *N*-demethylating activity. Emetine or 2,3-dehydroemetine alone, as we have shown above (Table 1), inhibits drug-metabolizing activity in this type of 1-day experiment by about 50%.

When animals are pretreated with phenobarbital daily for 3 days, much greater stimulation of liver microsomal drug-metabolizing activity is observed, as expected. This stimulation of *N*-demethylating activity is diminished but not eliminated in rats where emetine or 2,3-dehydroemetine are co-administered with phenobarbital on the third day of the phenobarbital treatment. Both emetine and 2,3-dehydroemetine exert inhibiting effects on the drug-metabolizing enzyme activity as measured by formaldehyde formation from aminopyrine *in vitro*, even in preparations from animals that were pre-stimulated with phenobarbital.

The pattern is essentially similar for azo-reduction, as measured by the enzymic formation of sulphanilamide from Neoprontosil in liver microsomal incubations *in vitro*. The stimulation brought about by phenobarbital by 1-day pretreatment with phenobarbital is not so great in terms of percentage increase, but the co-administration of emetine or 2,3-dehydroemetine blocks the incipient stimulating effect on azo-reduction by phenobarbital and decreases this activity to values below that of the control. Emetine or 2,3-dehydroemetine alone inhibits sulphanilamide formation from Neoprontosil by about 50% (see Table 1). With 3 days of phenobarbital treatment there is a greater stimulation of azo-reduction than at 1 day. The pre-stimulated animals respond to emetine or 2,3-dehydroemetine co-administered with phenobarbital on the third day, and the phenobarbital effects are diminished by over 50%.

DISCUSSION

(-)-Emetine and (±)-2,3-dehydroemetine are specific remedies in the treatment of infections with *Entamoeba histolytica* (Woolfe, 1963, 1965) and may find use in the treatment of certain antifolate-sensitive leukaemias (Jondorf, 1969; W. R. Jondorf, B. J. Abbott, N. H. Greenberg & J. A. R. Mead, unpublished work). Both compounds affect protein synthesis in a number of systems *in vitro* (Grollman, 1966, 1968; Jondorf & Szapary, 1968; Jondorf *et al.* 1969a). Previous studies on the tissue distribution of emetine in rabbits (Parmer & Cottrill, 1949), guinea-pigs (Schwartz & Herrero, 1965) and rats (Gimble, Davison & Smith, 1948; Davis, Dodds & Tomich, 1962) indicated that emetine was retained in some tissues for considerable periods of time, and was not excreted to any appreciable extent in the urine (Gimble *et al.* 1948; Schwartz & Herrero, 1965).

This suggested that emetine might not be metabolized. In this connexion, Kale *et al.* (1966) have found that emetine had some inhibiting effects on certain parameters of liver microsomal drug-metabolizing enzyme activity. We have been able to confirm that emetine is not metabolized by *O*-demethylation in a rat liver microsomal drug-metabolizing system *in vitro* (Jondorf *et al.* 1969d), and we have already briefly reported that both emetine and 2,3-dehydroemetine inhibit drug-metabolizing activity *in vitro* by liver microsomal preparations from animals pretreated with either of these compounds (Jondorf *et al.* 1969b,c).

We have now shown that liver microsomal drug-metabolizing enzyme activity, as measured by *N*-demethylation of aminopyrine and azo-reduction of Neoprontosil *in vitro*, and duration of hexobarbital-induced sleeping time *in vivo*, is inhibited in rats pretreated with (-)-emetine or (±)-dehydroemetine at 18 µmol/kg for 24 h. In corresponding experiments with rats pretreated with (-)-isoemetine or (±)-*O*-methylpsychotrine (Fig. 1) at the same dosage and for the same period of time, no inhibitory effects on liver microsomal drug-metabolizing enzyme activity were observed (Tables 1 and 2). Further, no changes in drug-metabolizing activity were noted in animals that had received isoemetine or *O*-methylpsychotrine at a pretreatment dosage of 144 µmol/kg, which would be well within the lethal range for emetine (Jondorf & Szapary, 1968) or 2,3-dehydroemetine (Jondorf *et al.* 1969a).

We conclude from this that structure-activity relationship among derivatives of emetine, first suggested by Grollman (1966) in connexion with his studies on amoebicidal effects and inhibition of protein synthesis *in vitro*, are also consistent with the degree of effectiveness in inhibiting the rat liver microsomal drug-metabolizing systems. According to Grollman (1966), the structural requirements for amoebicidal activity and inhibitory effects on protein synthesis in compounds related to emetine are first, the correct stereospecific alignments at the asymmetric C-1' position (Fig. 1) and an unsubstituted secondary amine at the N-2' position in the molecule. Naturally-occurring emetine and synthetic 2,3-dehydroemetine satisfy these requirements. Both compounds are active in Grollman's (1966) systems. On the other hand, isoemetine lacks the correct configuration at C-1', and in the *O*-methylpsychotrine molecule C-1' is no longer a centre of asymmetry, nor is the nitrogen at the 2'-position a secondary amine (Fig. 1). Neither isoemetine nor *O*-methylpsychotrine is an effective inhibitor of protein synthesis (Grollman, 1966). In our own previous studies on the comparative effects exerted by various compounds related to emetine on some parameters of protein biosynthesis in rat liver (Jondorf & Szapary, 1968; Jondorf *et al.*

1969a,d) we found that pretreatment of rats with emetine, 2,3-dehydroemetine or cephaeline at $18\mu\text{mol/kg}$ had a stimulatory effect on the amino acid-incorporating activity *in vitro* of liver microsomal fractions prepared from the treated rats 24h later. Under these conditions isoemetine and *O*-methylpsychotrine had no effect on protein-synthesizing activity of the rat liver microsomal system (Jondorf *et al.* 1969a). *N*-Methylemetine pretreatment had some intermediate stimulatory effect on the protein-synthesizing system (Jondorf *et al.* 1969d), which demonstrated that the requirement for a free secondary amine structure at the 2'-position (Grollman, 1966), though important, was not quite so decisive as the correct configuration at the 1'-position.

It is not altogether unexpected that isoemetine and *O*-methylpsychotrine would be inactive as inhibitors of drug-metabolizing activity in the rat. What is surprising, however, is that the biochemically active compounds should exert inhibitory effects on drug-metabolizing activity and yet stimulate amino acid-incorporating activity at the liver microsomal level in the rat, at the same dosage and at the same time-interval after pretreatment. We have previously noted such divergent microsomal effects in rats after pretreatment with cycloheximide (Jondorf *et al.* 1966) or related glutarimide antibiotics (Miller, Johnson, Donahue & Jondorf, 1969).

In trying to explain the mechanism of the inhibitory effects on drug-metabolizing enzyme activity, we examined the effects of adding 2,3-dehydroemetine directly to the *N*-demethylating system *in vitro* (R. K. Johnson, P. Mazel, J. D. Donahue & W. R. Jondorf, unpublished work). Under these conditions there is a concentration-dependent inhibition of *N*-demethylation of aminopyrine. However, on pretreatment of female Sprague-Dawley rats with 2,3-dehydroemetine at $18\mu\text{mol/kg}$ for 24h and measuring the subcellular distribution of this compound in the liver (Miller & Jondorf, 1970), we find that the amounts of drug associated with the combined microsomal and 105 000g supernatant fractions equivalent to 0.375g of liver used in the assays here are too minute to explain any inhibitory effects on drug metabolism.

Another possibility arose from a consideration of the work of Appelt & Heim (1965), who had shown that emetine inhibited the NAD^+ -dependent oxidation of substrates by rat heart homogenates without marked interference in the rate of NAD^+ synthesis. Similarly, Watkins & Guess (1968) had suggested that emetine inhibited NADH formation, thus uncoupling oxidative phosphorylation and decreasing ATP production in embryonic chick heart. It was conceivable that the inhibitory effects on drug-metabolizing activity in rat liver might be due to some emetine- or 2,3-dehydroemetine-linked

inhibition of glucose 6-phosphate dehydrogenase-dependent generation of NADPH from NADP^+ . However, as shown in Table 3, this is not the case, and the inhibitory effects exerted by 2,3-dehydroemetine or emetine are not connected with the impaired generation of the NADPH -cofactor required for drug metabolism *in vitro* (Gillette *et al.* 1957; Brodie *et al.* 1958; Brodie, Gillette & La Du, 1958).

With respect to the combined pretreatment of rats with compounds related to emetine and drugs such as *o,p'*-DDD (Boyland & Jondorf, 1962; Straw *et al.* 1965; Kupfer & Peets, 1966) or sodium phenobarbital (Gillette, 1963; Conney, 1967), known to stimulate drug-metabolizing enzyme activity, we find that emetine and 2,3-dehydroemetine each prevent the expected enhancement of drug-metabolizing enzyme activity at the liver microsomal level *in vitro* (Tables 4, 5). Under the appropriate circumstances these anti-amoebic drugs not only prevent the stimulation of the drug-metabolizing enzymes but inhibit pre-existing stimulation of the drug-metabolizing activity by liver microsomes (Table 5).

These would appear to be very important effects in view of the large number of drugs and environmental factors known to stimulate drug metabolism in various species (Conney, 1967). Inhibitors of drug metabolism are relatively rare (Netter, 1961; Gillette, 1963) and the knowledge that emetine and 2,3-dehydroemetine can inhibit liver microsomal drug-metabolizing enzyme activity will undoubtedly prove useful with regard to the molecular pharmacology of microsomal drug metabolism since the 'active centre' of the emetine molecule is known (Grollman, 1966; Jondorf *et al.* 1969a), and this ought to provide a further stimulus for studying the enzymic site of the inhibition.

This work was taken in part from a thesis presented by H. H. M. to the Department of Pharmacology, The Graduate School of Arts and Sciences, The George Washington University, Washington, D.C., U.S.A. in compliance with the regulations for the M.S. degree. The work was generously supported by the U.S. Public Health Service (Grants GM-13749 and 5T01-GM26), by Burroughs Wellcome (U.S.A.) Ltd. and by Hoffmann-La Roche Inc. We thank Professor H. G. Mandel for putting all the resources of his department at our disposal, and Dr P. Mazel for advice on the Neoprontosil assay.

REFERENCES

- Appelt, G. D. & Heim, H. C. (1965). *J. pharm. Sci.* **54**, 1621.
- Boyland, E. & Jondorf, W. R. (1962). *Br. J. Cancer*, **16**, 489.
- Bratton, A. C. & Marshall, E. K. (1939). *J. biol. Chem.* **128**, 537.

- Brodie, B. B., Gillette, J. R. & La Du, B. N. (1958). *A. Rev. Biochem.* **27**, 427.
- Brodie, B. B., Maickel, R. P. & Jondorf, W. R. (1958). *Fedn Proc. Fedn Am. Socs. exp. Biol.* **17**, 1163.
- Cochin, J. & Axelrod, J. (1959). *J. Pharmac. exp. Ther.* **125**, 105.
- Conney, A. H. (1967). *Pharmac. Rev.* **19**, 317.
- Conney, A. H. & Burns, J. J. (1959). *Nature, Lond.*, **148**, 363.
- Davis, B., Dodds, M. G. & Tomich, E. G. (1962). *J. Pharm. Pharmac.* **14**, 249.
- Diamant, E. J. (1958). *J. Pharmac. exp. Ther.* **122**, 465.
- Gillette, J. R. (1963). In *Progress in Drug Research*, vol. 6, p. 11. Ed. by Jucker, E. Basle: Birkhäuser Verlag.
- Gillette, J. R., Brodie, B. B. & La Du, B. N. (1957). *J. Pharmac. exp. Ther.* **119**, 532.
- Gimble, A. I., Davison, C. & Smith, P. K. (1948). *J. Pharmac. exp. Ther.* **94**, 431.
- Grollman, A. P. (1966). *Proc. natn. Acad. Sci. U.S.A.* **56**, 1867.
- Grollman, A. P. (1968). *J. biol. Chem.* **243**, 4089.
- Hernandez, P. H., Gillette, J. R. & Mazel, P. (1967). *Biochem. Pharmac.* **16**, 1859.
- Hogeboom, G. H. (1955). In *Methods in Enzymology*, vol. 1, p. 16. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Horecker, B. L. & Kornberg, A. (1957). In *Methods in Enzymology*, vol. 3, p. 879. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Jondorf, W. R. (1969). *Med. Ann. Dist. Columbia*, **38**, 73.
- Jondorf, W. R., Drassner, J. D., Johnson, R. K. & Miller, H. H. (1969a). *Archs Biochem. Biophys.* **131**, 163.
- Jondorf, W. R., Johnson, R. K. & Donahue, J. D. (1969c). *Biochem. J.* **113**, 10p.
- Jondorf, W. R., Johnson, R. K. & Donahue, J. D. (1969d). *Archs Biochem. Biophys.* **134**, 233.
- Jondorf, W. R., Johnson, R. K. & Drassner, J. D. (1969b). *Fedn Proc. Fedn Am. Socs. exp. Biol.* **28**, 418.
- Jondorf, W. R., Simon, D. C. & Avnimelech, M. (1966). *Molec. Pharmac.* **2**, 506.
- Jondorf, W. R. & Szapary, D. (1968). *Archs Biochem. Biophys.* **126**, 892.
- Kale, A. K., Joglekar, A. V. & Balwani, J. H. (1966). *J. Postgrad. Med.* **12**, 88.
- Kato, R., Jondorf, W. R., Loeb, L. A., Ben, T. & Gelboin, H. V. (1966). *Molec. Pharmac.* **2**, 171.
- Kupfer, D. & Peets, L. (1966). *Biochem. Pharmac.* **15**, 573.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
- Miller, H. H., Johnson, R. K., Donahue, J. D. & Jondorf, W. R. (1969). *Abstr. 4th int. Cong. Pharmac., Basle*, p. 191.
- Miller, H. H. & Jondorf, W. R. (1970). *J. Pharm. Pharmac.* (in the Press).
- Nash, T. (1957). *Biochem. J.* **55**, 416.
- Netter, K. J. (1961). In *Proc. 1st int. Pharmac. Meet.*, vol. 6, p. 213. Ed. by Brodie, B. B. & Erdős, E. G. New York: Macmillan Co. (Pergamon Press).
- Parmer, L. G. & Cottrill, C. W. (1949). *J. Lab. clin. Med.* **34**, 818.
- Quinn, G. P., Axelrod, J. & Brodie, B. B. (1958). *Biochem. Pharmac.* **1**, 152.
- Schwartz, D. E. & Herrero, J. (1965). *Am. J. trop. Med.* **14**, 78.
- Straw, J. A., Waters, I. W. & Fregly, M. J. (1965). *Proc. Soc. exp. Biol. Med.* **118**, 391.
- Watkins, W. D. & Guess, W. L. (1968). *J. pharm. Sci.* **57**, 1968.
- Woolfe, G. (1963). In *Experimental Chemotherapy*, vol. 1, p. 355. Ed. by Schnitzer, R. J. & Hawking, F. New York: Academic Press, Inc.
- Woolfe, G. (1965). In *Progress in Drug Research*, vol. 8, p. 11. Ed. by Jucker, E. Basle: Birkhäuser Verlag.