Glutarimide antibiotics and drug metabolism in the rat

The mRNA-dependent incorporation of [^{14}C]-labelled L-phenylalanine into protein *in vitro* by rat liver microsomes is increased by more than 100% in preparations from animals pretreated for 24 h with the glutarimide antibiotics cycloheximide (1 mg kg^{-1}), acetoxycycloheximide (0·1 mg kg^{-1}), streptovitacin A (1 mg kg^{-1}) or streptimidone (50 mg kg^{-1}) (Jondorf, Simon & Avnimelech, 1966, 1967; Jondorf, 1968; Jondorf & Filler, 1968). Although cycloheximide pretreatment gives rise to increased amino-acid incorporation by liver microsomes, there is an attendant decrease in hepatic drug metabolizing activity as measured by *N*-demethylation of aminopyrine (Cochin & Axelrod, 1959) and azo-reduction of neoprontosil (Hernandez, Gillette & Mazel, 1967) *in vitro* (Jondorf & others, 1966).

We now report our observations on whether such a dissociation of effects could also be observed in rats pretreated with the other glutarimide antibiotics previously examined and we have extended the observations of Miller, Johnson & others (1969) to ascertain whether co-administration of an inducer of protein synthesis and drug metabolism such as sodium phenobarbitone (Gelboin, 1971) would cause inhibition or induction to predominate.

Groups of two or more female Sprague-Dawley rats (160 g) were injected intraperitoneally with water or with freshly made aqueous solutions (or aqueous ethanol for streptimidone) of glutarimide antibiotics at the doses already mentioned. Sodium phenobarbitone (80 mg kg^{-1}) was administered to other groups of rats either alone or in combination with one of the antibiotics at the chosen dose so that total injection volume for all animals was 1·0 ml 100 g^{-1} body weight. Food was withheld from all animals in the 24 h between drug treatment and death by stunning and decapitation. Livers excised immediately thereafter, were processed for the preparation of microsomal and 105 000 *g* supernatant fractions by homogenization with ice cold 1·15% w/v KCl solution and differential centrifugation (Miller, Johnson & others, 1970). Different microsomal preparations were incubated with control 105 000 *g* supernatant fraction, the complete supporting system and substrates for the assay of *N*-demethylation (aminopyrine) and azo-reduction (neoprontosil) according to Jondorf & others

Table 1. *Liver microsomal* N-*demethylation of aminopyrine and azo-reduction of neoprontosil* in vitro. 24 h after pretreating female Sprague-Dawley rats with cycloheximide (1 mg kg^{-1}), acetoxycycloheximide (0·1 mg kg^{-1}), streptovitacin A (1 mg kg^{-1}) or streptimidone (50 mg kg^{-1}) alone or simultaneously with sodium phenobarbitone (80 mg kg^{-1}).

	nmol metabolite mg^{-1} microsomal protein 0·5 h^{-1}			
Treatment	Formaldehyde	Change %	Sulphanilamide	Change %
Control	137 ± 21	—	79·2 ± 4·9	—
Cycloheximide	90 ± 11	−34%	53·3 ± 4·2	−33%
Acetoxycycloheximide	39 ± 10	−71%	48·0 ± 4·0	−39%
Streptovitacin A	60 ± 16	−56%	44·3 ± 2·6	−44%
Streptimidone	75 ± 6	−45%	47·8 ± 2·0	−40%
Phenobarbitone	400 ± 40	+193%	107 ± 8·0	+35%
Phenobarbitone + cycloheximide	101 ± 18	−26%	54·2 ± 8·0	−32%
Phenobarbitone + acetoxycycloheximide	71 ± 21	−48%	44·4 ± 1·1	−44%
Phenobarbitone + streptovitacin A	107 ± 27	−22%	58·8 ± 5·8	−33%
Phenobarbitone + streptimidone	84 ± 6	−39%	47·5 ± 3·0	−40%

Values listed are the mean ± the standard error of the mean of 3–8 experiments. (Change %) refers to percentage change from control which is set at 100%.

(1966) as modified by Miller & others (1970). Microsomal protein assays (Lowry, Rosebrough & others, 1951) were made so that results could be expressed as nmol metabolite (formaldehyde or sulphanilamide) mg^{-1} microsomal protein $0{\cdot}5\ h^{-1}$ (Table 1).

From Table 1 it is evident that cycloheximide is not the only glutarimide antibiotic that exerts an inhibitory effect on drug metabolizing enzyme activity 24 h after pretreatment of rats at a dose known to stimulate microsomal protein synthesis *in vitro* (Jondorf & others, 1966). A similar dissociation of effects on microsomal protein synthesis and drug metabolism is revealed on measuring oxidative and reductive drug metabolizing activity 24 h after administration of acetoxycycloheximide ($0{\cdot}1\ mg\ kg^{-1}$), streptovitacin A ($1\ mg\ kg^{-1}$) or streptimidone ($50\ mg\ kg^{-1}$). At such pretreatment levels, which are inversely related to the potency of the antibiotics previously determined in the rat liver protein synthesizing system (Jondorf & others, 1966, 1967; Jondorf, 1968; Jondorf & Filler, 1968), there are inhibitory effects on drug metabolism of some 50%. In rats challenged simultaneously with an inducer of drug metabolizing activity such as sodium phenobarbitone, and one of the glutarimide antibiotics at the dose level chosen, the inhibitory effects of the antibiotics predominate. These results differ from data obtained in somewhat analogous experiments with puromycin (Conney & Gilman, 1963) where puromycin merely blocked induction without affecting existing enzyme levels. Our present results do however resemble those obtained in a related series of experiments with (−)-emetine and (±)-2,3-dehydroemetine (Miller & others, 1970) and provide further evidence for the striking similarity in biological activities displayed by glutarimide antibiotics and ipecac alkaloids, first stressed by Grollman (1966).

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