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Preliminary Observation of Interactions between Methimazole and Hexobarbital in Mice

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Abstract

The effect of pretreatment with methimazole, an antithyroid drug, on hexobarbital metabolism was assessed by measurement of sleeping time, hepatic microsomal metabolism and relative rates of plasma and brain levels of hexobarbital in mice. When methimazole (10, 30, 60, 90, 120 and 150 mg/kg, i.p.) was given 10 min prior to the determination, methimazole increased sleeping time in a dose- and time-dependent manner. In addition to, methimazole inhibited the hepatic metabolism of hexobarbital with competitive inhibition in the 9,000 x g supernatant incubation mixture. Methimazole also interacted directly with cytochrome P-450 as measured by difference spectra and caused a type I spectral change. No metabolic intermediate complex could be demonstrated for drug. Furthermore, measurement of the concentration of hexobarbital in the brain immediately upon recovery from hypnosis revealed no differences in any of the groups. The present findings indicate that methimazole alter the duration of action of hexobarbital by inhibition of microsomal enzymes.

Keywords: methimazole, hexobarbital-induced sleeping time, hexobarbital metabolism, mice

Introduction

Numerous studies have demonstrated that during the hepatic mixed-function oxidase-catalyzed metabolism of thiosulfur-containing compounds becomes covalently bound to the microsomal macromolecules[1-4]. Accompanying the binding of sulfur, which was shown to be NADPH dependents was, a decrease in cytochrome P-450 detectable as the carbon monoxide complex and a decreased ability of these microsomes to metabolize drugs [1-3,5]. This decrease in cytochrome P-450 and ability to metabolize drug appeared to be roughly

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proportional to the amount of sulfur bound to the microsomes. Inhibition processes may lead to drug interactions with coadministered drugs as a result of the decreased oxidation capacity of the liver. Thus, many sulfhydryl containing compounds interact with P-450 enzymes to inhibit the biotransformation of coadministered drugs and cause the accumulation of these drugs in the body[4,6-9].

During the course of another investigation, we observed that mice pretreated with methimazole[10], an antithyrotoxicosis drug, and anesthetized with hexobarbital slept much longer than mice treated with hexobarbital alone. This observation suggested a possible interaction of the two drugs on microsomal drug metabolism or on the sensitivity of receptors in the central nervous system[11].

Hexobarbital is almost completely transformed by hepatic microsomes in experimental animals and man[11,12], while barbital is excreted largely unchanged in the urine[13].

Methimazole is metabolized by the liver, but the subcellular locus or this biotransformation has not been established[14,15].

The present preliminary study was undertaken to clarify the relationship between the in vivo and in vitro effects of the clinically important methimazole, a drug commonly used to treat hyperthyroidism, on the sleeping time, elimination and microsomal metabolism of hexobarbital in mice. The effect of methimazole on barbital sleeping time was studied because of the possibility of interaction at the receptors.

Materials and Methods

Animals Male ddY mice, 5 weeks of age, were obtained from Japan SLC (Hamamatsu, Japan). The animals were housed under conditions of controlled temperature (23±1°C), humidity (55±5%) and lighting (7 : 00 a.m. to 7 : 00 p.m.), and were provided with standard food pellets (CE-2, Nihon clea, Tokyo, Japan) and water *ad libitum* and for at least days prior to experiments. Animals were housed in facilities accredited by the Japan Association of Laboratory Animals Care and the research protocols were approved by the Guide-line for Animal Experimentation for Tohoku College of Pharmacy.

Drug treatment Methimazole (Nakalai Tesque, Kyoto) was administered subcutaneously after dissolving in 0.9% saline. To induce cytochrome P-450 activity, mice were injected intraperitoneally with phenobarbital (80 mg/kg, in 0.9% saline) for 2 consecutive days. All the concentrations of compounds were prepared immediately before use so that 0.1 ml/10 g body weight provided the doses desired for mice.

Determination of sleeping time Sleeping time was determined after intraperitoneal administration of hexobarbital (100 mg/kg) or sodium barbital (300 mg/kg). The barbiturates were administered 5, 10, 15, 20, 25 and 30 min after pretreatment with methimazole and saline. Control and experimental groups were studied side by side to obviate possible differences in environmental conditions or influences of normal circadian rhythm. Sleeping time as defined as the time interval from loss of reflexes to reappearance to the righting reflex. Methimazole alone produced no observable narcosis.

Determination of the decay of hexobarbital in plasma and brain Mice in groups of 18-19 received either 60 mg/kg of methimazole or normal saline. Ten min later, hexobarbital

(100 mg/kg, i.p.) was administered. At predetermined intervals after hexobarbital treatment, blood and brain were analyzed for hexobarbital [16]. The mice in other groups (five to seven animals each) received similar pretreatment but the animals were allowed to awaken before killing and the brain was assayed for hexobarbital as described by Ishikawa et al.[17].

Determination of drug metabolism and cytochrome P-450 in vitro Mice were killed by decapitation between 9 and 10 a.m, and livers of ten mice were pooled for each determination. Livers were removed, weighed, perfused with 1.15% KCl and homogenized with 3 volume of ice-cold 1.15% KCl in a glass tube homogenizer with a moter-driven Teflon pestle. The homogenate was centrifuged for 20 min at 9,000 x g, and the resulting supernatant fraction served as the enzyme source for the measurement of the activity of hexobarbital oxidase.

The 9,000 x g supernatant was used for the hexobarbital metabolism experiments as described by Ishikawa et al.[17] with the following modifications. Incubation mixtures contained: 9,000 x g supernatant fraction equivalent to 330 mg liver, 1.2 μmol NADPH, 25 μmol $MgCl_2$, 20 μmol glucose 6-phosphate, 600 μmol phosphate buffer (pH. 7.4), 1 ml methimazole (5×10^{-4}, 1×10^{-3} or 1.5×10^{-2} mM), or 1 ml of 0.1 M phosphate buffer (pH 7.4). The final incubation volume was 3 ml. The hexobarbital concentrations were 0.125, 0.25, and 0.5 μmol/3 ml. The rate of hexobarbital disappearance was estimated by the method described previously[17]. The rate obtained was presented by the method of Lineweaver and Burk. The mean of at least three determinations was used to average and plot each point.

To separate microsomes, the 9,000 x g supernatant fraction was centrifuged at 105,000 x g for 60 min at 4°C and the microsomal pellet was kept at −80°C until use. Microsomal pellets were resuspended in 0.1 M phosphate buffer (pH 7.4) at a concentration of approximately 5 mg protein/ml.

Aminopyrine N-demethylase was assayed as the amount of formaldehyde formed using the method of Nash [18] with the incubation mixture described earlier[17]. Aniline hydroxylase was assayed as the rate of p-aminophenol production by hepatic microsomes as described previously[19]. Cytochrome P-450 was determined from the carbon monoxide difference spectrum of dithionite-treated microsomes assuming a value of 91/mM/cm as the molar extinction coefficient between 450 and 490 nm for cytochrome P-450[20] and the levels are expressed as nmol cytochrome P-450/mg protein. The protein concentration was determined by the method of Bradford[21] using bovine serum albumin as the standard.

Statistical analysis Data were statistically analyzed by the Student's t-test. Differences with $P<0.05$ were considered to be significant.

Results

Effect of methimazole on barbiturate-induced narcosis Hexobarbital sleeping time is one of the simplest and most widely used in vivo test of cytochrome P-450-mediated drug metabolism. An investigation of the effect of methimazole pretreatment on hexobarbital sleeping time indicated that methimazole inhibited the activity of the hepatic mixed-function oxidase system, since methimazole prolonged the hexobarbital sleeping time. In a preliminary study, hexobarbital-induced sleeping time was significantly longer in animals treated

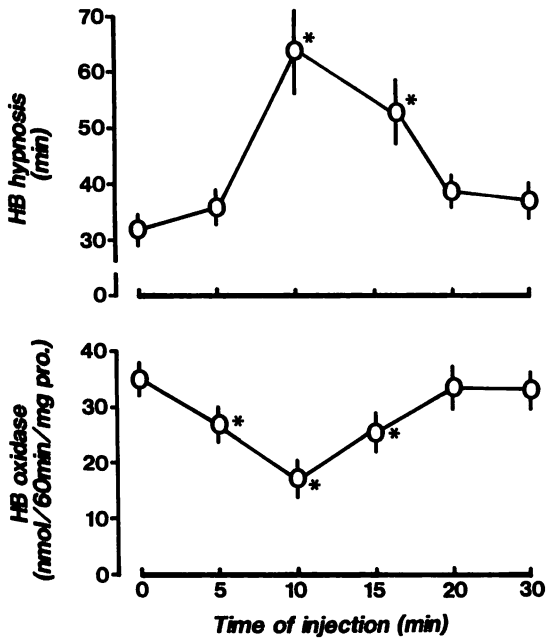

Fig. 1 Time course of methimazole effects on hexobarbital hyposis and hepatic microsoaml metabolism in mice.
Upper panel: Mice were given hexobarbital (100 mg/kg, intraperitoneally) at various times after methimazole (60 mg/kg, intraperitoneally). Each point represents the mean±S.E. of the sleeping time of 10 mice.
Lower panel: Mice were killed at the indicated time points after the injection of methimazole (60 mg/kg, intraperitoneally) and enzyme sources were prepared from their livers. Each point represents the mean±S.E. of 3-5 value obtained from the pooled mixture of the liver of two mice.
A significant change ($P<0.05$) in the sleeping time or metabolism from control is designated by an asterisk. Abbreviations; HB: hexobarbital, pro.: protein

with methimazole (30 mg/kg, i.p., 10 min) than in those that received only saline. Measurement of the concentration of hexobarbital in the brain immediately upon recovery from hypnosis revealed no differences in the brain (data not shown).

Thus, the time- and dose-dependence of this prolongation were examined. First, to determine if these activities are attributed to the microsomal enzyme, the ability of prolongation of methimazole on the hexobarbital-induced sleeping time was compared that of hexobarbital metabolism. The sleeping time induced by hexobarbital increased with an interval to 10 min in the time of administration of methimazole (60 mg/kg, i.p.). Thereafter, there was a decrease with further increases in the time of administration of methimazole. Namely, maximum prolongation was observed at 10 min, at which time the hexobarbital-induced sleeping time was prolonged by methimazole to about 230% of that of the control (Fig. 1). The prolongation of sleeping time was dose-dependent in saline-treated control mice and phenobarbital-stimulated mice (Fig. 2). The relative prolongation in sleeping time produced by methimazole was greater in the stimulated group than normal mice. The same pattern of inhibition of hexobarbital metabolism was also observed when the activity of hexobarbital oxidase assayed instead of the sleeping time. These findings indicate that most, if not all, of the prolongation of hexobarbital-induced sleeping by methimazole is localized in the inhibition of hexobarbital metabolism.

On the other hand, barbital sleeping time was increased by the pretreatment with methimazole (60 mg/kg, i.p.) in mice, and onset time was shortened by the pretreatment with methimazole in a dose-dependent manner (Table 1). The acute toxicity of barbital (250 and 300 mg/kg, i.p.) was increased in the methimazole (60 mg/kg, i.p.)-pretreated group.

Effect of methimazole on in vitro hexobarbital metabolism Based on the significant reduction in microsomal metabolism of hexobarbital previously described, we determined

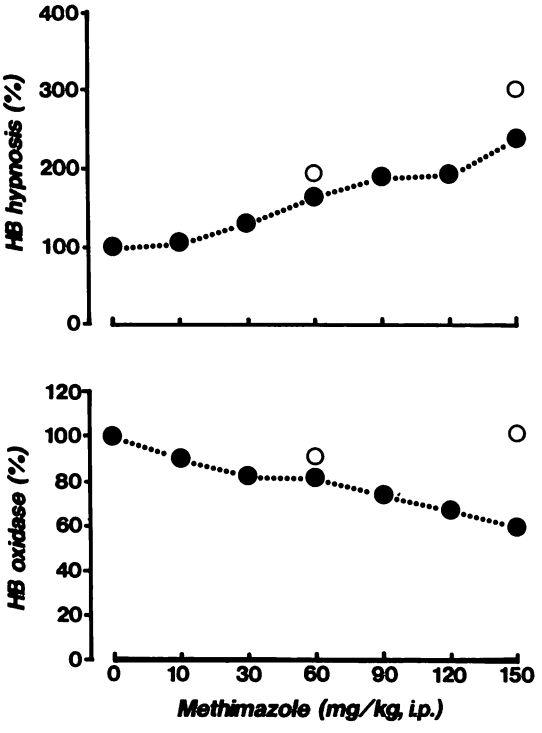

Fig. 2 Dose response of methimazole effects on hexobarbital hypnosis and hepatic microsomal metabolim in unstimulated and phenobarbital-stimulated mice.
Mice were stimulated by phenobarbital injection (80 mg/kg intraperitoneally) 2 times every 24 hr (○). Unstimulated mice received saline instead of phenobarbital (●). Methimazole (intraperitoneally) was injected 24 hr after final injection of phenobarbital. Hexobarbital hypnosis and metabolism were determined at 10 min after the intraperitoneal injection of methimazole. The control was administered saline.
Each point represents the percentage of the 10 (for hexobarbital hypnosis) or 3-5 (for metabolism) determinations compared with corresponding saline-treated group. The experimental condition and analysis are as in Fig. 1.

Table 1 Effect of methimazole on sleeping time and acute mortality-induced by barbital in mice

Treatment	Hypnosis (min)		Lethality
	Onset time	Sleeping time	
Barbital 200 mg/kg			
Saline	34.0±5.11		0/10
Methimazole 60 mg/kg	22.8±2.04[a)]		0/10
Barbital 250 mg/kg			
Saline	25.3±1.84	21.0±3.55	0/10
Methimazole 60 mg/kg	21.1±1.48[a)]	99.8±18.11[a)]	1/10
Barbital 300 mg/kg			
Saline	26.6±2.88		4/10
Methimazole 60 mg/kg	18.3±0.71[a)]		6/10

Barbital was injected intraperitoneally 10 min after injection of saline (intraperitoneally) or methimazole (60 mg/kg intraperitoneally). Lethality in mice was observed continuously for 24 hr. Each value represents the mean±S.E. of the sleeping time of 10 mice. [a)] Significantly different from respective control values ($P<0.05$).

whether a comparable reduction in Michaelis constants occurred upon direct addition of methimazole to control microsomal incubations (Fig. 3).

The following values were obtained from the Lineweaver-Burk plots of the data obtained for methimazole: Vmax=10 μmoles per 60 min per mg protein; $Km=3.3\times10^{-4}$ M; and $Ki=1.3\times10^{-2}$ M. It is evident that methimazole is a potent inhibitor of hexobarbital

Table 2 Effect of methimazole on hexobarbital levels in brain and plasma upon recovery from hypnosis in mice

Treatment	Hexobarbital concentration		Brain/plasma ratio
	Brain (nmol/ml)	Plasma (nmol/g)	
Normal			
Saline	0.335±0.0301	0.063±0.0267	5.3
Methimazole 60 mg/kg	0.328±0.0059	0.047±0.0051[a)]	6.9
Phenobarbital			
Saline	0.281±0.0423	0.044±0.0014	6.3
Methimazole 60 mg/kg	0.299±0.0274	0.062±0.0091[a)]	4.8

Mice received saline or methimazole (60 mg/kg intraperitoneally) and 10 min later hexobarbital (100 mg/kg intraperitoneally). Upon recovery from hypnosis the animals were killed and brain and plasma levels of hexobarbital were determined. Each value is the mean±S.E. of 5-8 animals. [a)] Significantly different from respective control values ($P<0.05$).

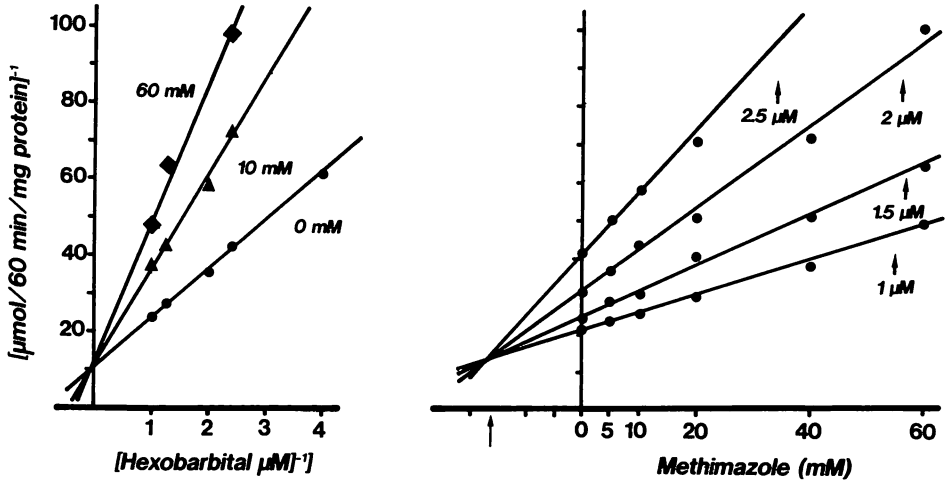

Fig. 3 Effect of in vitro methimazole addition on hexobarbital metabolism.
Left panel: For the determination of Lineweaver-Burk plot of the in vitro effect of methimazole on hexobaribatal metabolism, the 9,000 x g supernatant fractions were isolated from untreated mice and the rate of hepatic metabolism was determined in either the presence or absence of varying concentration of methimazole. Each point represents the mean of 3 to 8 separate determinations of the 9,000 x g supernatant fraction, each composed of livers from at least 4 male mice. Right panel: K_I values were determined from Dixon plots.

metabolism and the inhibition appeared to be competitive.

Influence of methimazole on the extent of plasma protein binding of hexobarbital Methimazole did not interact to decrease the extent of binding of hexobarbital (Fig. 4).

Effect of methimazole on in vitro aminopyrine and aniline metabolism and cytochrome P-450 To determine mechanistically how methimazole alters hexobarbital metabolism in the mice, an experiment was conducted in which methimazole was added in vitro to microsomes which had been obtained from normal mice. The purpose of the experiment

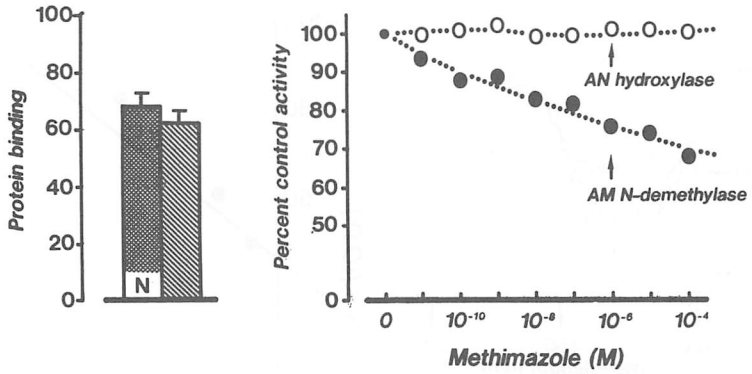

Fig.4 Influence of methimazle on the extent of hexobarbital plasma protein binding and hepatic metabolism of aminopyrine and aniline
Left panel: Methimazole (12.5 μg/ml) was added to experimental plasma; equivalent volumes of distilled water were added to control plasma (N). Hexobarbital (100 μg/ml) was added to plasma samples. The extent of protein binding was determined by ultrafiltration, using a previously described method (22).
Right panel: Liver microsomes from mice were used to determine the inhibitory effect of methimazole on the aminopyrine (●) and aniline (○). Each point represents the mean of three experiments. Control activities equaled 126.5±11.4 nmol formaldehyde produced/20 min/mg protein and 65.1±2.6 nmol p-aminophenol produced/20 min/mg protein.

was to determine if methimazole could exert a direct inhibitory effect on hepatic microsomal metabolism or cytochrome P-450 levels. Methimazole was added in vitro in a concentration range from 10^{-11} M to 10^{-4} M. The data presented in Fig. 4 indicate that methimazole added in vitro significantly reduced the rate of metabolism of aminopyrine in a dose-dependent manner. However, the maximal inhibition achieved was only 36%. Since methimazole in the highest concentration of 10^{-4} M did not produce a reduction of 32% in drug metabolism, methimazole would probably be classified as a relatively weak inhibitor of the mono-oxygenase system. On the other hand, addition of methimazole to this in vitro system did not alter aniline hydroxylase. This may be due to the fact that aminopyrine N-demethylase and aniline hydroxylase exhibit different types of binding to cytochrome P-450, aminopyrine N-demethylase exhibiting type I binding and aniline hydroxylase exhibiting type II binding.

In addition, it should be noted that the in vitro addition of methimazole did not alter the cytochrome P-450 level or produce any conversion of cytochrome P-450 to the inactive form, P-420 (data not shown).

Effect of methimazole on the biological decay of hexobarbital in blood and brain The rate of hexobarbital disappearance in blood was decreased in the methimazole-treated animals. The half-life of hexobarbital increased from 22 min in the control group to 35 min in the methimazole-treated group (data not shown). Similarly, the hexobarbital half-life in the brain increased from 19 min in the control group to 28 min in the methimazole-treated animals.

Binding difference spectra of microsomal cytochrome P-450 To determine whether methimazole interacted directly with the cytochrome P-450 enzymes in liver microsomes prepared from mouse, spectra binding studies were performed. The difference spectra were

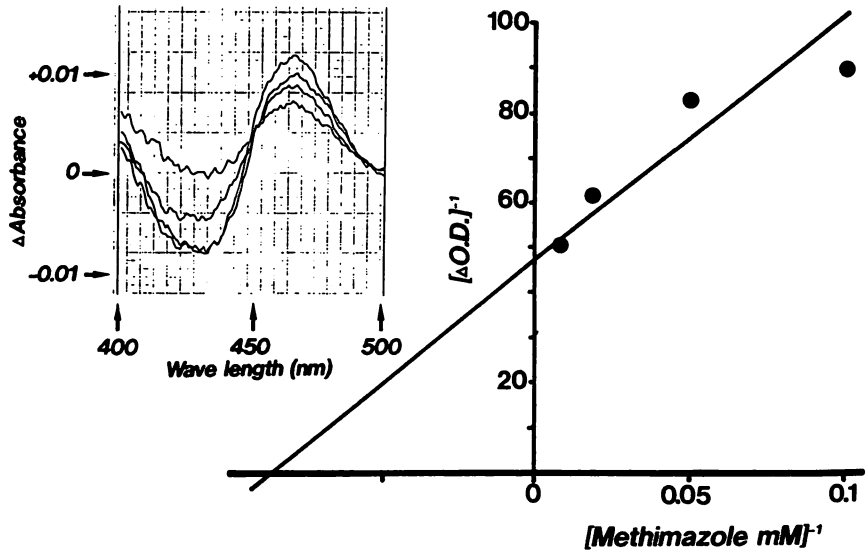

Fig. 5 The spectral interaction of methimazole with mice microsomes
Left panel: The binding spectra of methimazole with cytochrome P-450 were measured in a suspension of microsomes containing about 1.5-2 mg/ml microsomal protein in phosphate buffer.
Right panel: Reciprocal plot of the changes in absorbance produced by the addition of various concentrations of methimazole.

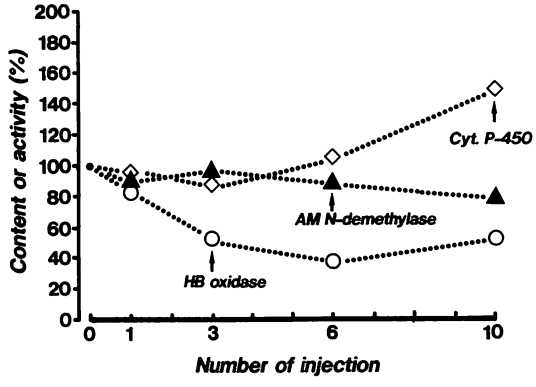

Fig. 6 The effect of successive administration of methimazole on the hepatic drug metabolism in mice
Methimazole (60 mg/kg) was injected intraperitoneally every 24 hr for ten days. Mice were killed at the indicated time points after the injection of methimazole and microsomes were prepared from their livers. Control animals received saline instead of methimazole. Each point represents the mean±S.E. of 3-5 values obtained from the pooled mixture of the liver of two mice. Abbreviation; HB: hexobarbital, AM: aminopyrine, cyt. P-450: cytochrome P-450.

recorded at a final methimazole concentration of 10 μM and 20 μM. The absorbance maximum of the methimazole-cytochrome P-450 complex is located at 390 nm and the absorbance minimum at 424 nm (Fig. 5). Thus, by convention, methimazole can be classfied as a type I compound. The facts that methimazole is hydroxylated, binds to cytochrome P-

450, and demonstrates a Type I binding spectrum all suggest that methimazole may competitively inhibit metabolism of other type I substrates such as hexobarbital. Competition between inhibitors and substrates can be a mechanism by which drug metabolism is impaired both in vitro and in vivo[23,24].

Effects of daily methimazole administration on hepatic drug metabolism Fig. 6 summarizes the results from this investigation. Administration of methimazole to ddy mouse for 10 days produced approximately a 42% increase in hepatic cytochrome P-450 levels, and a 65% decrease in microsomal hexobarbital hydroxylase activity. Aminopyrine N-demethylase did not significantly change during the entire study. This may be due to the fact that hexobarbital hydroxylase and aminopyrine N-demethylase show different types of binding to cytochrome P-450.

Discussion

The hexobarbital sleeping time or zoxazolamine paralysis time is one of the simplest and most widely used in vivo tests of cytochrome P-450 (or P-448)-mediated drug metabolism[11-13].

Loss of righting reflex is frequently used as a pharmacodynamic index of the hypnotic or general anesthetic effect of drugs on rodents. It has been customary to determine the duration of the loss of righting reflex as a convenient, indirect measure of the relative rate of elimination of a central nervous system depressant drug from the body. Some investigators also have determined the concentration of such drugs in the plasma or brain at the offset of action (i.e., upon return of the righting reflex) to confirm the assumption that the variable under study (typically one that modifies the elimination kinetics of the central nervous system depressant drug) has no effect on the drug concentration required to produce loss of righting reflex. A different experimental strategy is required when loss of righting reflex is used to determine the possible effects of various compounds on the drug concentration-pharmacologic activity relatioship. This strategy should permit a clear distinction to be made between the effects of drugs or other physiological perturbations on the pharmacodynamics and the pharmacokinetics of a drug. Although this purpose may be served best by the drug-recepter interaction studies, direct in vivo measurement of pharmacologic effects is required to determine if a drug may have affected the events that lead from drug-receptor interaction to the expression of a therapeutically relevant pharmacologic response. Were one to find significant differences between two groups of animals with respect to the concentration of hexobarbital in the brain at the time of offset of loss of righting reflex, it would not be justifiable to conclude that these groups differ in pharmacodynamic characteristics. Hypothetically, the concentration difference could be due to different binding of the drug to nonspecific sites in the brain (perhaps as a consequence of increased circulating concentrations of endogenous binding inhibitors in one of the groups due to renal dysfunction or other diseases) or to differences with respect to concentrations of pharmacologically active drug metabolites. Use of onset of action as the pharmacodynamic end point and cerebrospinal fluid as the drug concentration reference site (when this is justifiable on the basis of appropriate experimental results) minimizes or excludes a number of potential nonpharmacodynamic

perturbations that may otherwise not be recognized and that could cause misinterpretation of data. Some of these potential variables will be briefly mentioned here with special references to hexobarbital.

We determined the effect of methimazole pretreatment on both in vivo and in vitro oxidative drug metabolism in the mice. The findings suggested that methimazole pretreatment inhibits both in vivo and in vitro hexobarbital. Both hexobarbital and zoxazolamine are metabolized by the microsomal mixed-function oxidase system. However, hexobarbital metabolism is more related to cytochrome P-450 than to cytochrome P-448 dependent enzyme activity[22] and conversely zoxazolamine metabolism is more related to cytochrome P-448 than to P-450[23].

Methimazole increased hexobarbital-induced sleeping time both in a time- and dose-related manner, but did not affect the time to narcosis initiation. The duration of action of drugs in the body is largely controlled by the efficiency of mechanism for biotransformation, excretion and sequestration. This increase in sleeping time may be attributed to several factors such as, (I) methimazole may increase brain sensitivity to hexobarbital; (II) methimazole may decrease the metabolism of hexobarbital; (III) methimazole may potentiate hypothermic, and (IV) methimazole may increase brain hexobarbital uptake. The inhibition of hexobarbital metabolism by methimazole explains the mechanism by which the duration of action and half-life of hexobarbital are increased. The duration of action and plasma concentrations of several drugs have been shown to be related to the activity of drug-metabolizing enzymes[11,12,16]. Evidence which tends to rule out impairment of excretory processes was present in the experiment on barbital sleeping time. Barbital is excreted largely in an unchanged form in the urine[13].

Methimazole prolonged the barbital sleeping time in mice. This experiment does not rule out the possibility of an increase in barbiturate receptor sensitivity. However, if receptor sensitivity was increased, one would expect that methimazole pretreatment would increase both hexobarbital sleeping time and zoxazolamine paralysis time. Further evidence against an alteration in receptor sensitivity is that the brain levels of hexobarbital at awakening were not significantly different in the methimazole-treated group even though sleeping time was approximately 2.1 times longer than that in the control mice. The increase in hypnosis by hexobarbital produced by methimazole pretreatment could be due to the decreased rate of biotransformation, since this would allow a given concentration of the barbiturate to persist longer at the receptor site. Hexobarbital was chosen for these studies since it was known to show 40-50% binding to plasma protein[26], so that fluctuations in either direction could be detected. As described above, methimazole had no effect on the plasma protein binding of hexobarbital.

Another possibility which may be considered is that of displacement of barbiturate from central nervous system binding sites by methimazole. Methimazole is known to cross freely the blood-brain barrier[14,15]. Such a mechanism would be compatible with the facts which are available at the present time; in ability to demonstrate increased brain levels of hexobarbital and potentiation of depressant effect of hexobarbital by methimazole. If bound inactive hexobarbital were displaced from central nervous system proteins, this could permit redistribution within the brain with no change in total barbiturate content of the brain.

Methimazole appears to belong to the class of drugs which increases the duration of action of barbiturate by interfering with the rate of biotransformation but which do not reinduce sleep. Other drugs which exert these effects are β-diethylaminoethyl diphenylpropylacetate hydrochloride (SKF-525A)[27], 2, 4-dichloro-6-phenoxyethyldiethylamine hydrobromide (Lilly-18947)[28], and dimercaprol[29]. Chlorpromazine and reserpine prolong the action of barbiturates by the potentiation, i.e., they do not alter the uptake of drugs by they do reinduces sleep[12]. The inhibition of hexobarbital metabolism by methimazole was shown to be of the competitive type. Rubin et al.[30] showed that several chemically unrelated drugs inhibited the biotransformation of each other competitively. Methimazole was a weak inhibitor of hexobarbital metabolism in vitro. The Ki value was small than, those reported for SKF-525A (6.0×10^{-6} M)[31] and chlorpromazine (5.6×10^{-5} M)[30] in the inhibition of hexobarbital and aminopyrine metabolism.

The binding of methimazole to microsomal cytochrome P-450 seen spectrometrically is confirmatory of an interaction of methimazole with cytochrome P-450 of similar affinity observed for the inhibition of in vitro hexobarbital metabolism. Methimazole caused a type I change in the difference spectra[32], and methimazole with type I spectrum ($K_D=90\ \mu M$) likely interacts with a hydrophobic region of the molecule[33]. Although the K_D values for methimazole appear to be slightly higher than this K_I values for inhibition or K_m values for hexobarbital oxidation, the binding of the methimazole to cytochrome P-450 indicates that they can act directly with cytochrome P-450 and therefore can prevent the binding and subsequent metabolism of hexobarabital. Methimazole is a structurally related 1-substituted imidazole antithyroide drug. Wilkinson et al.[34] recognized the inhibitory properties of imidazole and imidazole derivatives towards microsomal enzyme activities, and showed that they were closely related to the ability of these compounds to bind to cytochrome P-450. The findings suggest that with methimazole the same phenomena may occur in hepatocytes. The inhibition of hexobarbital oxidase by methimazole is clearly not due to the formation of metabolic-intermediate complexes as no spectral evidence for such complexes could be found when methimazole was incubated with microsomes and NADPH.

Since hexobarbital sleeping time was significantly increased in methimazole-treated mice, but zoxazolamine paralysis time was not, cytochrome P-450 dependent drug metabolizing enzyme systems may be inhibited by methimazole treatment. Methimazole treatment thus has a specific effect on drug metabolism similar to doxapram[35] which has been observed to increase hexobarbital sleeping time in rodents. This may be due to the fact that hexobarbital and zoxazolamine exhibit different types of binding to cytochrome P-450, hexobarbital exhibiting type I binding and zoxazolamine exhibiting type II binding[32,33].

Thus our findings indicate that methimazole pretreatment modifies the activities of hepatic drug metabolizing enzyme as established in vivo by changes in hexobarbital sleeping time and in vitro by the observed decrease in biotransformation of hexobarbital by the hepatic microsomal preparations obtained from methimazole-treated mice. Our finding of decreased hexobarbital metabolism in the methimazole-pretreated mice is in good agreement with the results of Hunter and Neal[4], who reported that benzphetamine metabolism was reduced by a single methimazole administration. However, the decrease in aminopyrine N-demethylase and aniline hydroxylase was not statistically significant suggesting that not all

the drug metabolizing enzymes are equally affected.

As methimazole may modify the metabolism and effectiveness or toxicity of concomitantly administered drugs, these findings may have clinical significance. This has been demonstrated in animal studies, where we observed that the nephrotoxicity of cisplatin was reduced in methimazole-treated mouse when compared to non-treated controls[36].

The inhibitory effects of methimazole on the biotransformation of other substrates should be examined and the practice of administering antithyroid drug in combination with other drugs should be reviewed.

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