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DIFFERENTIAL EXPRESSION OF MICROSOMAL EPOXIDE HYDROLASE GENE BY AZOLE HETEROCYCLES IN RATS

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Abstract—The effects of heterocycles including imidazole (IM), 1,2,4-triazole (TR) and thiazole (TH) on the expression of microsomal epoxide hydrolase (mEH) gene were examined in rats (200 mg/kg body weight/day, i.p.). Hepatic microsomes prepared from rats treated with IM for 3 days failed to exhibit an increase in mEH protein level whereas TR treatment resulted in an approximately 2- to 3-fold elevation in hepatic mEH levels relative to control, as assessed by both SDS–PAGE and immunoblot analyses. In contrast, thiazole-induced hepatic microsomes resulted in a substantial increase in mEH levels (i.e. ~5-fold). Slot and northern blot analyses, probed with an mEH cDNA, showed that the hepatic mEH mRNA levels in the animals treated with IM for 3 days were marginally increased by ~2-fold, as compared with untreated animals, whereas TR caused an ~8-fold increase in hepatic mEH mRNA levels after three consecutive daily treatments. TH treatment resulted in an ~22-fold increase in the mEH mRNA levels, demonstrating that TH is the most efficacious among these three azole heterocycles. Because TH was the most effective in increasing hepatic mEH protein and mRNA levels, the agent was chosen for further evaluation. Time course of mEH gene expression at early times after a single treatment with TH was determined and compared with that caused by pyrazine (PZ), a strong mEH inducer. Hepatic mEH mRNA levels were increased ~1-, 3-, 20- and 16-fold at 3, 6, 12 and 24 hr, respectively, following TH treatment, relative to control, whereas mEH mRNA levels were elevated ~1-, 1-, 22- and 18-fold, respectively, at the same time points after PZ treatment, as monitored by slot RNA hybridization analyses. Northern blot analyses using either total RNA or poly(A)$^+$ RNA fractions exhibited comparable time courses in increasing mEH mRNA levels after TH or PZ treatment with maximal mRNA increases being noted at 12 hr post treatment. Although neither IM or TR failed to affect renal mEH gene expression to a notable extent, TH treatment caused 6- to 8-fold increases in kidney mEH mRNA levels, with a 2-fold increase in mEH protein detected. These results demonstrated that the azole heterocyclic compounds IM, TR and TH differentially induce mEH with TH as the most efficacious azole; and that the changes in mEH levels are primarily associated with increases in mRNA levels.

Key words: epoxide hydrolase; imidazole; triazole; thiazole; enzyme induction

Sulphur and nitrogen-containing heterocycles, such as IM, TR and TH‡, are employed as intermediates in the production of a number of therapeutic agents (e.g. antimycotics and sulphonamides). The nitrogen-containing compounds have also been identified as constituents in tobacco, tobacco smoke and certain roasted foods (e.g. coffee, barley and peanuts) [1, 2].

It has been reported that nitrogen heterocycles exhibited inhibitory effects on cytochrome P450 activity *in vitro* and induced P450 *in vivo* [3, 4]. IM or N-substituted imidazoles interacted with heme group of cytochrome P450 and inhibited P450-catalysed metabolic oxidations [3–5]. In particular, a number of N-1 substituted IM derivatives inhibited cytochrome P450-dependent reactions, suggesting that unhindered nitrogen, such as N-3 in the IM ring, bound to the heme iron of P450. Although IM caused significant increases in cytochrome P450 content in rabbits [3], the compound failed to induce cytochrome P450 in rats [6]. Moreover, IM exhibited a distinct species-dependent induction of CYP2E1 [6]. Hostetler *et al.* [7] also demonstrated that certain imidazole-containing antimycotic agents differentially modulated the expression of several P450 subfamilies.

It has been found that IM derivatives, such as IM antimycotic agents, benzylimidazole, benzimidazole and phenylimidazoles, stimulated mEH activity *in vitro* [8–10], which appeared to be due to an interaction with the enzyme protein itself at a site distinct from the substrate binding region [8, 9]. Studies in this laboratory revealed that the imidazole-containing antifungal agents, including ketoconazole, clotrimazole, miconazole and econazole, induced mEH in rats and that the induction involved large increases in mEH mRNA levels through transcriptional activation [11]. Given the observations that certain imidazole-containing compounds stimulate mEH activity *in vitro* and that IM antimycotic agents induce mEH to a comparable extent, we were interested in whether IM was an active moiety for mEH induction.

Triazole antimycotic agents including itraconazole and fluconazole are the compounds based on 1-substituted TRs, which do not appear to interact

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‡ Abbreviations: mEH, microsomal epoxide hydrolase; IM, imidazole; TR, 1,2,4-triazole; TH, thiazole; PZ, pyrazine.

with human cytochrome P450 [12, 13]. It is believed that the TR antimycotic agents are devoid of potential hepatotoxicity after systemic administration, possibly due to the incorporation of the TR moiety [14]. In view of the application of the TR moiety for development of improved antimycotics and the potential effects of TR on the expression of mEH and cytochromes P450 due to structural similarity with IM, in this research the effect of TR on mEH gene expression was examined and compared with that of IM.

Furthermore, previous studies in this laboratory have shown that TH and PZ, nitrogen and sulphur-containing prototypic heterocycles, efficaciously induced mEH with substantial increases in mEH mRNA levels [15]. Thus, the effects of azoles including IM, TR and TH on the expression of mEH protein and mRNA *in vivo* were compared in order to establish whether these azole heterocycles shared a common basis for mEH induction and whether the structurally-related nitrogen containing compounds modulated mEH protein and mRNA levels to a similar extent. As part of complete evaluation of the effects of these agents on mEH expression, time-dependent changes in mEH mRNA levels at early times, following a single injection of TH or PZ, were determined towards the end of this research. In addition, mEH mRNA levels were quantified in kidney after treatment of rats with TH to study mEH gene expression in extrahepatic tissue.

The present study established that the azole heterocycles differentially induced mEH protein and that the changes in mEH levels by these compounds were primarily associated with increases in mRNA levels at early times.

MATERIALS AND METHODS

Materials. [α-^{32}P]dATP (3000 Ci/mmol) and [γ-^{32}P]ATP (3000 Ci/mmol) were purchased from Amersham (Arlington Heights, IL, U.S.A.). IM, TR, TH and PZ were purchased from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). Biotinylated goat anti-rabbit IgG, streptavidin-conjugated horseradish peroxidase and random prime labelling and 5′-end labelling kits were purchased from BRL (Gaithersburg, MD, U.S.A.).

Animal treatment. Male Sprague–Dawley rats (180–220 g) were treated with either IM, TR, TH, or PZ in aqueous solution (200 mg/kg body weight/day, i.p., 1–3 days) and killed at 24 hr following the last treatment. In a time-dependent study, animals were killed at various time points after a single injection of either TH or PZ. Animals were fasted 18 hr prior to killing. Each data point consisted of samples pooled utilizing groups of animals. Three rats were employed in each treatment group. Results were confirmed using different groups of animals.

Isolation of microsomal proteins. Hepatic or renal microsomes prepared by differential centrifugation were washed in pyrophosphate buffer and stored in 50 mM Tris-acetate buffer (pH 7.4) containing 1 mM EDTA and 20% glycerol. Microsomal preparations were stored at −80° until used. Protein content was determined by the method of Lowry *et al.* [16].

Gel electrophoresis. SDS–PAGE analysis was performed according to Laemmli [17] using a Hoefer gel apparatus.

Production of mEH antibody. Antibody against purified mEH protein was produced in a rabbit, as described previously [18, 19]. Briefly, hepatic mEH protein was purified from TH-induced rat hepatic microsomes. Liver microsomes isolated from rats treated with TH were suspended in 0.2 M potassium phosphate buffer (pH 7.4) containing 0.1 mM EDTA. A 10% tergitol NP-10 was added with stirring to yield a final concentration of 1% and then stirred for an additional 30 min. Unsolubilized microsomes were sedimented by centrifuging for 1 hr at 100,000 *g* and the supernatant was dialysed against 5 mM potassium phosphate buffer (pH 7.25) containing 0.05% NP-10 (buffer A) overnight with two changes. The dialysate was applied to a DEAE-cellulose column (Whatman DE-52) equilibrated with buffer A and the column was washed with buffer A. The eluate was pooled and concentrated using an Amicon ultrafiltration apparatus (Amicon, Beverly, U.S.A.). The homogeneity was determined electrophoretically by SDS–PAGE analysis and Coomassie brilliant blue staining. N-terminal amino acid sequencing on the mEH protein transferred onto Immobilon PVDF membrane following gel electrophoresis was performed using a gas-phase sequencer. The analyses revealed that 16 N-terminal amino acid sequence of the purified mEH protein was exactly identical with that of the published mEH sequence. Single peaks were observed only at each cycle, suggesting that no other protein was co-purified with mEH. Antiserum was produced in a rabbit using the purified mEH protein. Immunochemical specificity of the anti-mEH antibody was also confirmed with an authentic anti-mEH antibody which was kindly provided by Dr C. B. Kasper at the University of Wisconsin, U.S.A.

Immunoblot analysis. Immunoblot analysis was performed according to previously published procedures [11, 20]. Microsomal proteins were separated by 8% SDS–PAGE and electrophoretically transferred to nitrocellulose paper. The nitrocellulose paper was incubated with a rabbit anti-rat mEH antibody. Biotinylated goat anti-rabbit IgG was used as the secondary antibody. Immunoreactive protein was visualized by incubation with streptavidin–horseradish peroxidase, followed by addition of both 4- chloro-1-naphthol and H_2O_2.

Isolation of total RNA and poly (A)$^+$ RNA. Total RNA was isolated using the improved single-step method of thiocyanate–phenol–chloroform RNA extraction according to the methods of Cathala *et al.* [21] and Chomczynski and Sacchi [22], as modified by Puissant and Houdebine [23]. Poly(A) $^+$ RNA was isolated from the total RNA using an oligo(dT)-cellulose column according to the method of Jacobson [24]. cDNA derived from hepatic poly(A)$^+$ RNA obtained from rats treated with PZ was cloned in an M13mp19 phage vector and DNA sequence was confirmed as described previously [11, 20].

Northern blot hybridization. Northern blot was carried out according to procedures described previously [11, 20]. Briefly, poly(A)$^+$ RNA isolated from rat livers was resolved by electrophoresis in a 1% agarose gel containing 2.2 M formaldehyde and

then transferred to supported nitrocellulose paper by capillary transfer. The nitrocellulose paper was baked in a vacuum oven at 80° for 2 hr. The blot was incubated with hybridization buffer containing 50% deionized formamide, 5× Denhardt's solution [0.1% Ficoll, 0.1% polyvinylpyrrolidine and 0.1% bovine serum albumin (Pentex Fraction V)], 0.1% SDS, 200 μg/mL of sonicated salmon sperm DNA and 5× SSPE (1× SSPE: 0.15 M NaCl, 10 mM NaH_2PO_4 and 1 mM Na_2EDTA, pH 7.4) at 42° for 1 hr without probe. Hybridization was performed at 42° for 18 hr with heat-denatured mEH cDNA (1.3 kb), which was random prime-labelled with [α-^{32}P]dATP. Filters were washed in 2× SSC and 0.1% SDS for 10 min at room temperature twice and in 0.1 × SSC and 0.1% SDS for 10 min. at room temperature twice. Filters were finally washed in the solution containing 0.1× SSC and 0.1% SDS for 60 min at 60°.

RNA slot blot hybridization. RNA slot blot analysis was performed using a Hoefer slot blot apparatus according to the manufacturer's protocol. Total RNA or poly(A)$^+$ RNA was serially diluted in 15× SSC (1× SSC: 150 mM NaCl, 15 mM sodium citrate) [11, 20]. Three serially-diluted RNA samples obtained from rat liver or kidney were applied onto slots for optimal quantitation of mEH mRNA increases in the autoradiographic film exposure. Each membrane was baked under vacuum at 80°, prehybridized and then incubated in a hybridization solution containing a ^{32}P-random prime-labelled cDNA probe, as described above for northern blot analysis. Films were exposed at −80° for 1–6 hr using Dupont intensifying screens. The same samples employed for northern blot analyses were also applied to slots. After quantitation of mEH mRNA levels, the membranes were stripped and rehybridized with ^{32}P-end labelled poly(dT)$_{16}$ to quantify the amount of RNA loaded onto the slots. Duplicate northern and slot blot analyses were performed on different mRNA samples.

Scanning densitometry. Scanning densitometry was performed with a Soft Laser Scanning Densitometer (Model, SLR-1D/2D, Bio-Med Instrument Incorporation, Fullerton, U.S.A.). The area of each lane or slot was integrated using Laser and Camera Hard Disk Program, followed by background subtraction ($r = 0.98$–0.99). The quantitation of the mRNA loaded on the slot blot was accomplished by hybridization of stripped membranes with ^{32}P-end labelled poly(dT)$_{16}$ and the relative change in mRNA was determined from normalization of hybridization signal to the mRNA loaded onto the slots.

RESULTS

SDS–PAGE analysis of hepatic microsomes

The SDS–PAGE of hepatic microsomes isolated from rats treated with IM for 3 days (200 mg/kg/day, i.p.) failed to show an increase in intensity of a band migrating in the region of mEH (Fig. 1A). Hepatic microsomes isolated from rats treated with TR exhibited a minimal increase in band intensity migrating in mEH region. Treatment of animals with TH showed a notable elevation of band intensity in the region of mEH (Fig. 1A). In order to quantify mEH levels immunochemically, western immunoblot analyses were carried out in a subsequent experiment.

Immunoblot blot analysis for mEH levels

mEH Induction was monitored by measuring mEH protein levels immunochemically. Hepatic microsomes isolated from rats treated with IM failed to show an increase in mEH level, whereas hepatic microsomes prepared from TR-treated rats exhibited an ~2-fold increase in mEH band intensity relative to control (200 mg/kg, i.p.) (Fig. 1B). In contrast, treatment of animals with TH resulted in a marked induction of mEH (Fig. 1B). Scanning laser densitometry of the blots revealed an ~5-fold increase in mEH levels in TH-induced hepatic microsomes, as compared with untreated microsomes, which was consistent with this group's previous observations [15]. Thus, the rank order of mEH induction by these azole heterocycles were TH > TR > IM. To determine whether the differential mEH expression by the azole heterocycles was accompanied by comparable changes in mRNA levels, RNA hybridization analyses were performed.

Effects of azoles on hepatic mEH mRNA expression

Northern blot analysis revealed that the level of mEH mRNA were differentially increased by the azole heterocycles, exhibiting that the rank order of mEH mRNA expression was TH > TR > IM (Fig. 2A). Northern blot analysis also demonstrated the integrity of employed RNA and specificity of the probe as detected by the single bands (Fig. 2A).

Slot blot hybridization analysis was also carried out for quantitative purposes, revealing that hepatic mEH mRNA levels in the animals treated with IM for 3 days were only marginally increased (i.e. ~2-fold) relative to control, whereas TR caused an ~8-fold increase in mEH mRNA levels after three consecutive daily treatment (Fig. 2B). Treatment of rats with TH resulted in an ~22-fold increase in the levels of mEH mRNA, as determined by scanning laser densitometry of the blots, suggesting that TH was the most efficacious compound among the azole heterocycles examined (Fig. 2B). Thus, the rank order in increasing mEH mRNA levels at 3 days after daily treatment with each of the heterocycles was the same as that assessed by northern blot analysis. These results strongly support the fact that the efficacy in increases in hepatic mEH mRNA levels by the heterocycles was consistent with that in mEH protein levels.

Time course of hepatic mEH mRNA increases

As part of an extended evaluation of the effects of azoles on mEH expression, a study was designed to examine time courses in mEH mRNA expression at early times following a single dose of TH injection (i.e. 3, 6, 12 and 24 hr post treatment) and the effect of TH was compared with that caused by PZ. Northern RNA blot analyses performed with hepatic total RNA revealed substantial time-dependent increases in mEH mRNA levels from 6 to 24 hr time points (Fig. 3A). Hepatic mEH mRNA levels in total RNA were elevated ~1-, 3-, 20- and 16-fold at 3, 6, 12 and 24 hr, respectively, following TH

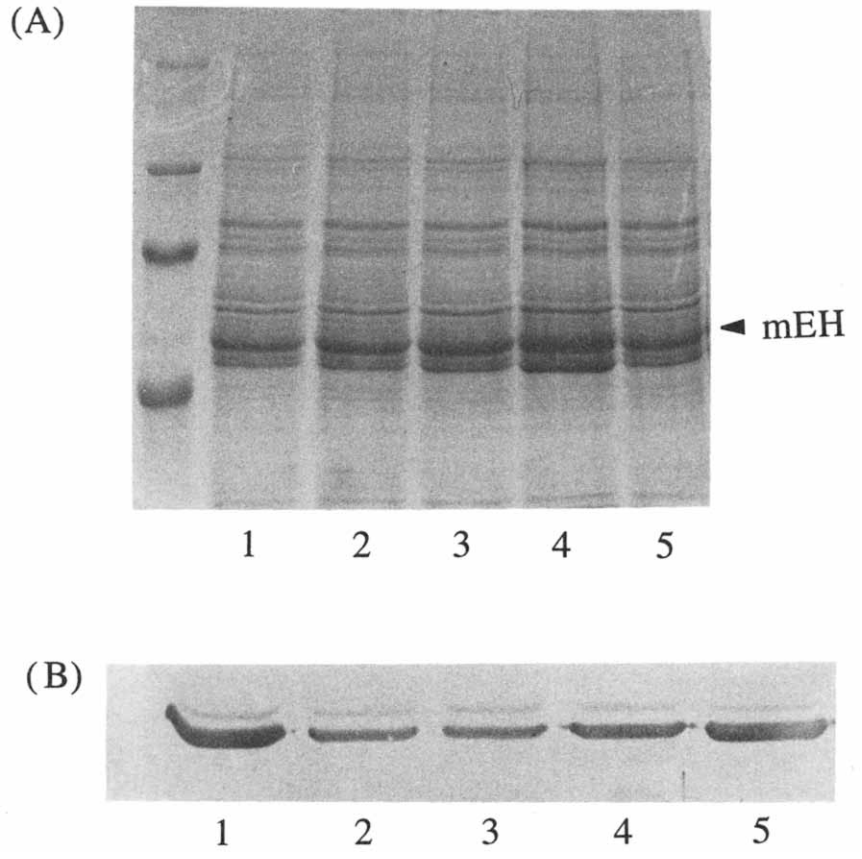

Fig. 1. SDS–PAGE and immunoblot analyses of rat hepatic microsomes. Panel A: SDS–PAGE analysis of rat hepatic microsomes isolated from untreated (lanes 1 and 5), IM- (lane 2), TR- (lane 3) or TH- (lane 4) treated rats (200 mg/kg body weight/day, i.p., 3d). Each lane was loaded with 15 μg of rat liver microsomes. This gel stained with Coomassie brilliant blue exhibited a marked increase in the band intensity migrating in the region of mEH protein following treatment of animals with thiazole. Molecular weight standards shown above are ovalbumin (43 kDa), bovine serum albumin (68 kDa), phosphorylase B (97.4 kDa) and myosin (H-chain) (200 kDa). Panel B: Immunoblot analysis of rat hepatic microsomes with rabbit anti-rat mEH antibody. This blot exhibited mEH levels in hepatic microsomes isolated from untreated (lane 2), IM- (lane 3), TR- (lane 4), or TH- (lane 1 and 5) treated rats (200 mg/kg body weight/day, p.o., 3d). Each lane was loaded with 5 μg of rat liver microsomes. This blot exhibited apparent significant increases in mEH protein following treatment with TH, whereas TR caused a moderate increase.

treatment, relative to control, as assessed by slot blot analysis. mEH mRNA levels were increased ~1-, 1-, 22- and 18-fold, respectively, at the same time points, following PZ treatment (Fig. 3A). Thus, the rates of mEH mRNA increased by either TH or PZ were greatest between 6 and 12 hr time points, with maximal increases being noted 12 hr post treatment.

When hybridization was performed with the poly(A)$^+$ RNA fractions, the minor thin bands of larger mRNA which were detected in total RNA fractions disappeared (Fig. 3B), suggesting that the thin mRNA bands may have been non-adenylated pre-mRNA species. Comparable time courses were noted in both RNA fractions. In order to quantify the amount of RNA loaded onto the slots, membranes were stripped and reprobed with ^{32}P-end labelled poly(dT)$_{16}$.

Effects of azoles on renal mEH expression

Immunoblot analysis showed that either IM or TR treatment failed to induce mEH levels in kidney to a notable extent, whereas treatment of rats with TH for 3 days resulted in an ~2-fold increase in kidney mEH levels (Fig. 4A). The levels of renal mEH mRNA were also quantified (Fig. 4B and C). Although rats treated with either IM or TR failed to exhibit increases in renal mEH mRNA (data not shown), treatment of rats with TH resulted in 6- to 8-fold increases in mEH mRNA levels in kidney 1–3 days post treatment (Fig. 4B and C).

DISCUSSION

Microsomal epoxide hydrolase catalyses the hydration of epoxide reactive intermediates, being

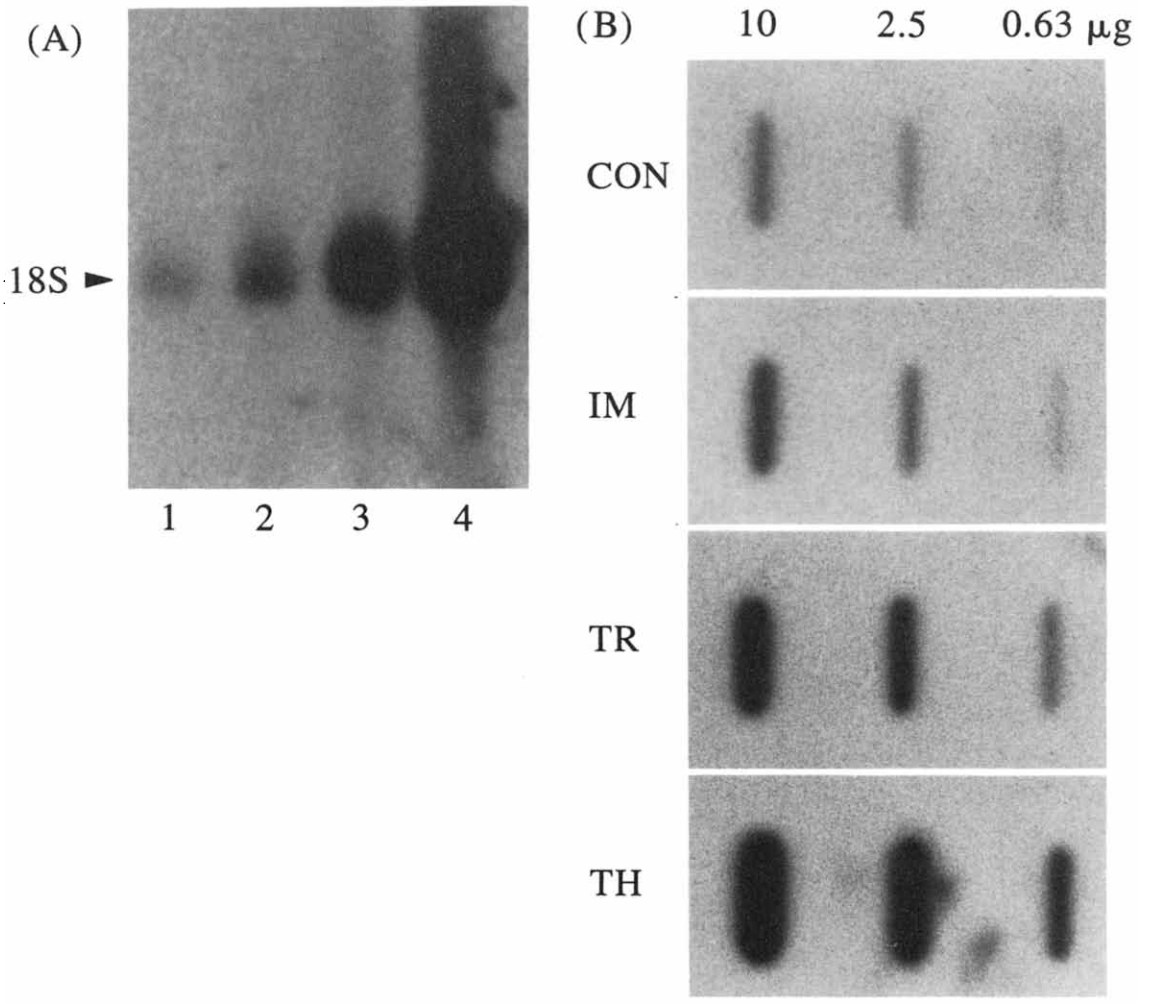

Fig. 2. RNA blot analyses of hepatic mEH mRNA levels after azole treatment. Panel A: Northern blot analysis of total RNA obtained after treatment with azole heterocycles. Lanes 1–4 represent mEH mRNA levels in 20 μg of hepatic total RNA isolated from untreated rats or rats treated with either IM, TR or TH for 3 days, respectively. Panel B: Slot blot analyses of mEH mRNA in hepatic total RNA after treatment of rats with azole heterocycles (200 mg/kg body weight/day, i.p., 3d). Total RNA (10, 2.5 and 0.63 μg, respectively) isolated from untreated rats or rats treated with either IM, TR or TH for 3 days was blotted and probed with an 1.3 kb mEH cDNA. The rank order in increasing mEH mRNA levels was TH > TR > IM.

primarily associated with detoxication [25, 26]. It has been reported that mEH is inducible by phenobarbital, 3-methylcholanthrene, *N*-acetylaminofluorene, *trans*-stilbene oxide and alkylnitrosamines [27–30].

Studies suggested that imidazole-containing antimycotic agents interacted with the enzyme protein itself *in vitro*, activating the hydrolysis of styrene oxide in hepatic microsomes [8–10]. Further, *in vivo* studies accomplished in this laboratory showed that these IM antifungal drugs affected the expression and regulation of mEH gene [11]. Nuclear run-on analyses provided evidence that increases in mEH mRNA by the antimycotic agents were primarily associated with transcriptional activation of this gene [11]. Because these agents have the IM moiety in their structure in common, and improved azole antifungal agents, such as itraconazole and fluconazole contain TR(s) in their chemical structures as a functional group, the present studies were initiated to evaluate the role of prototypic heterocycles, IM and TR, on the expression of mEH and its mRNA levels.

Previous studies showed that the levels of mEH mRNA were increased 15–20-fold at 3 days following either TH or PZ treatment, as compared with those in untreated animals [15], suggesting that the increase in mEH protein levels by these xenobiotics was accompanied by the accumulation of mEH mRNA. Given the results of substantial elevation of both mEH protein and mRNA levels after either TH or PZ treatment [15], and the similarity in chemical structures among the azoles including IM, TR and TH, the effects of these compounds on mEH expression were comparatively evaluated in this research.

The present study revealed that treatment of rats with IM failed to show an increase in mEH protein

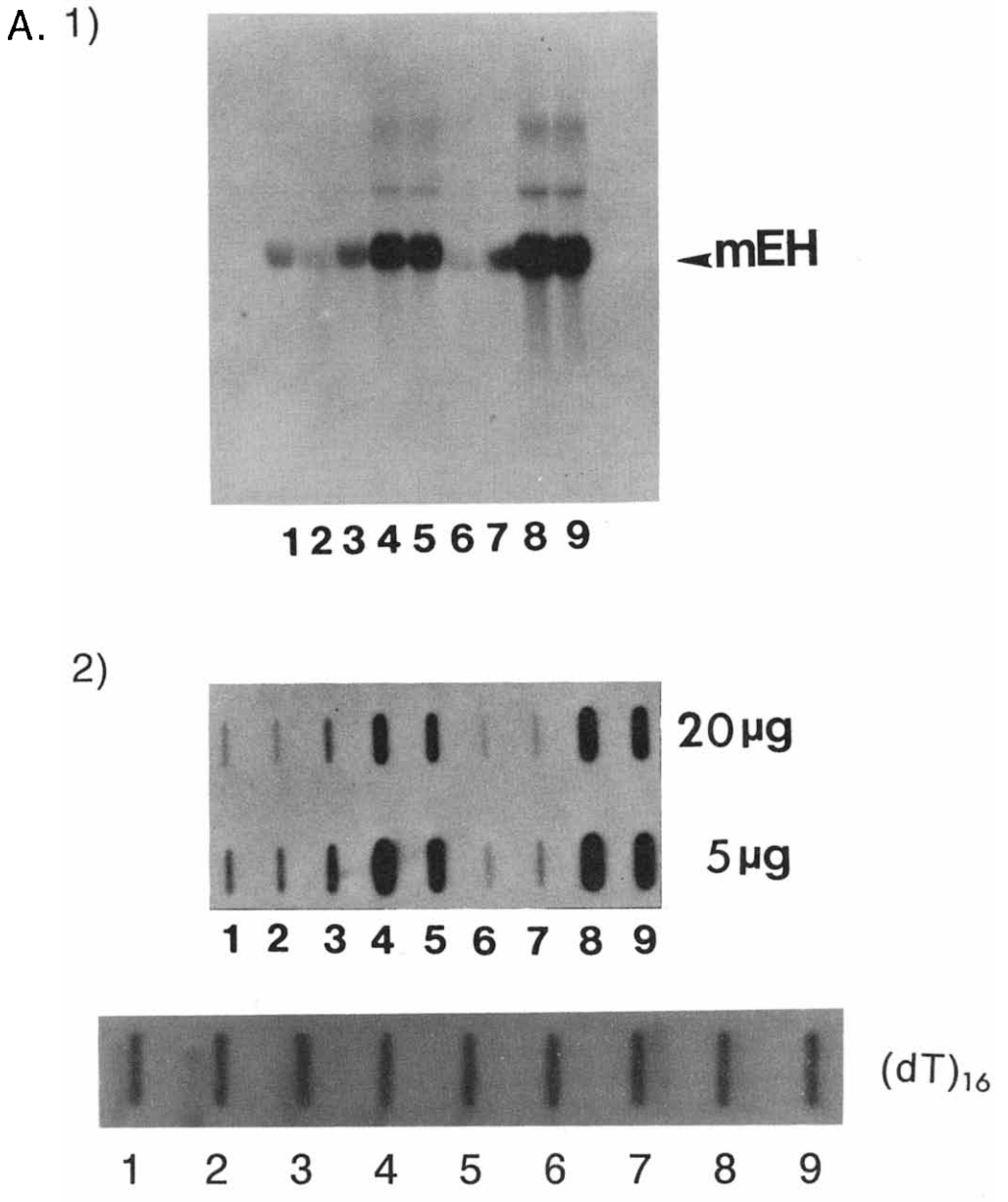

Fig. 3.

levels with only a minimal increase in mEH mRNA levels observed at 3 days after treatment. TR, which has a nitrogen atom instead of carbon at number 5 position of the IM ring, caused a somewhat larger increase in mEH mRNA levels (i.e. ~8-fold) with a detectable increase in mEH protein level being noted (i.e. ~2-fold). TH, which has a sulphur atom instead of nitrogen at number 1 position of the IM ring, appeared to be the most efficacious among the azoles examined. These data suggest that induction of mEH protein by the azoles at 3 days post treatment occurred in parallel with elevation of mRNA levels by the compounds. Atomic weights of HN—C, HN—N and S—C in IM, TR and TH are 27, 29 and 44, respectively. Interestingly, the calculated correlation coefficient between the atomic weights and relative mRNA increases by the azoles was 0.98. According to these data, it is highly likely that *in vivo* induction of mEH and elevation of mEH mRNA by the azoles were associated with the sizes of the substituted atoms in the azole ring. In other words, as the size(s) of the substituted atom(s) in the azole ring become larger, the extent of mEH induction was greater. This is further supported by the previous observation that IM antimycotic agents induced mEH with large increases in mEH mRNA

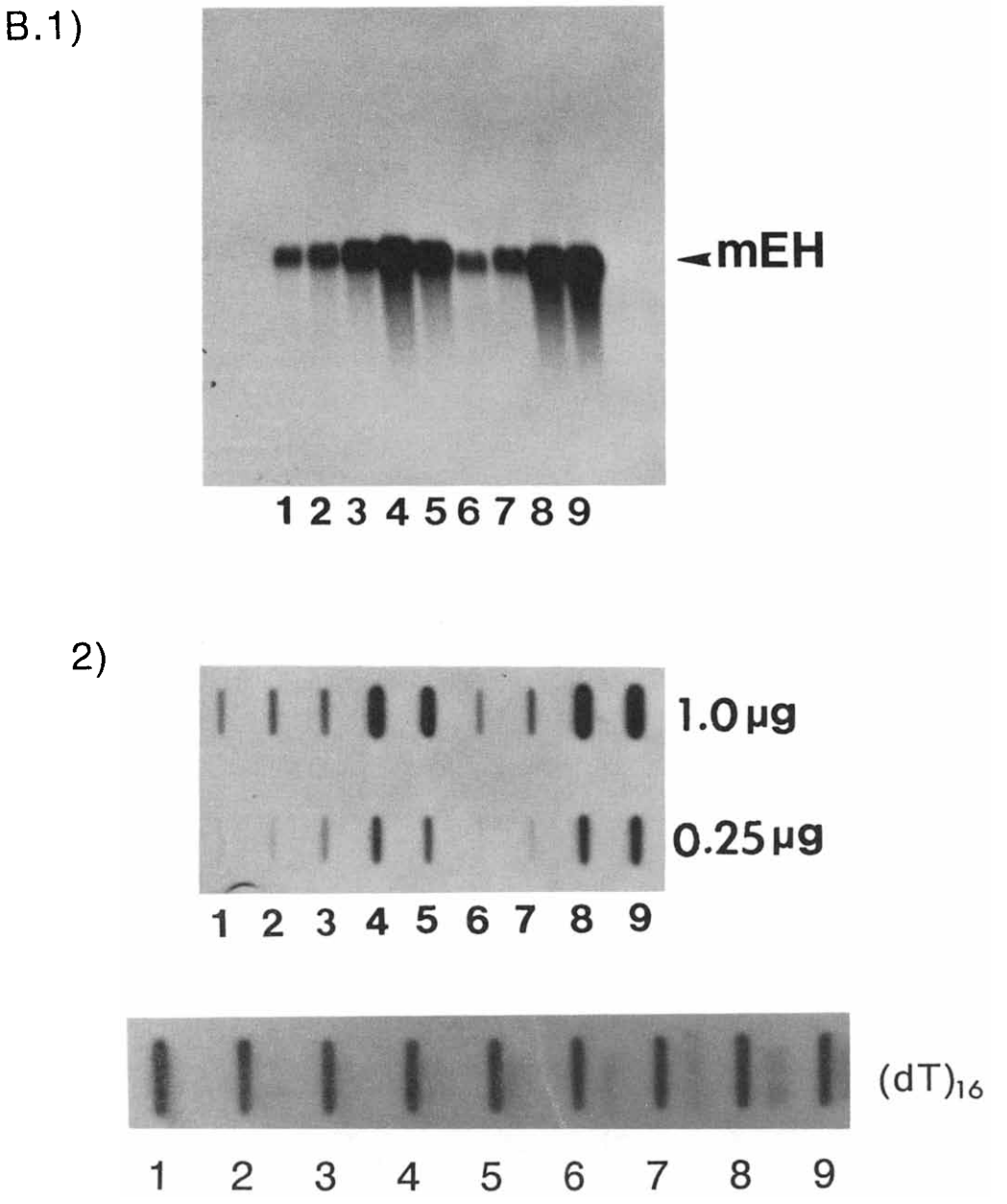

Fig. 3. RNA hybridization analyses for mEH RNA after thiazole or pyrazine treatment. Panel A: Northern and slot blot analyses for mEH RNA in total RNA fractions. For northern blot analysis 3A(1), each lane contained 20 μg of hepatic total RNA. Lane 1 in the northern blot analysis is control (i.e. untreated rats). Lanes 2–5 represent mEH levels in the liver at 3, 6, 12 and 24 hr, respectively, after treatment with TH, lanes 6–9 are associated with samples obtained at the same time points after PZ treatment. Samples were also applied to slots 3A(2) for mEH mRNA hybridization (20 and 5 μg of RNA in the upper and lower row, respectively). The stripped membranes were hybridized with ^{32}P-end labelled poly(dT)$_{16}$ to quantify the amount of mRNA loaded onto the slots. Panel B: Northern and slot blot analyses for mEH RNA in poly(A)$^+$RNA fractions. For northern blot analysis 3B(1), 2 μg of hepatic poly(A)$^+$RNA isolated at the identical time points as above were probed with a ^{32}P-labelled mEH cDNA. Slot blots 3B(2) were associated with 1 and 0.25 μg of poly(A)$^+$RNA in the upper and lower rows, respectively. After hybridization with the mEH cDNA probe, equal loading of RNA onto the slots were confirmed by rehybridization with ^{32}P-labelled poly(dT)$_{16}$ after stripping of the membranes.

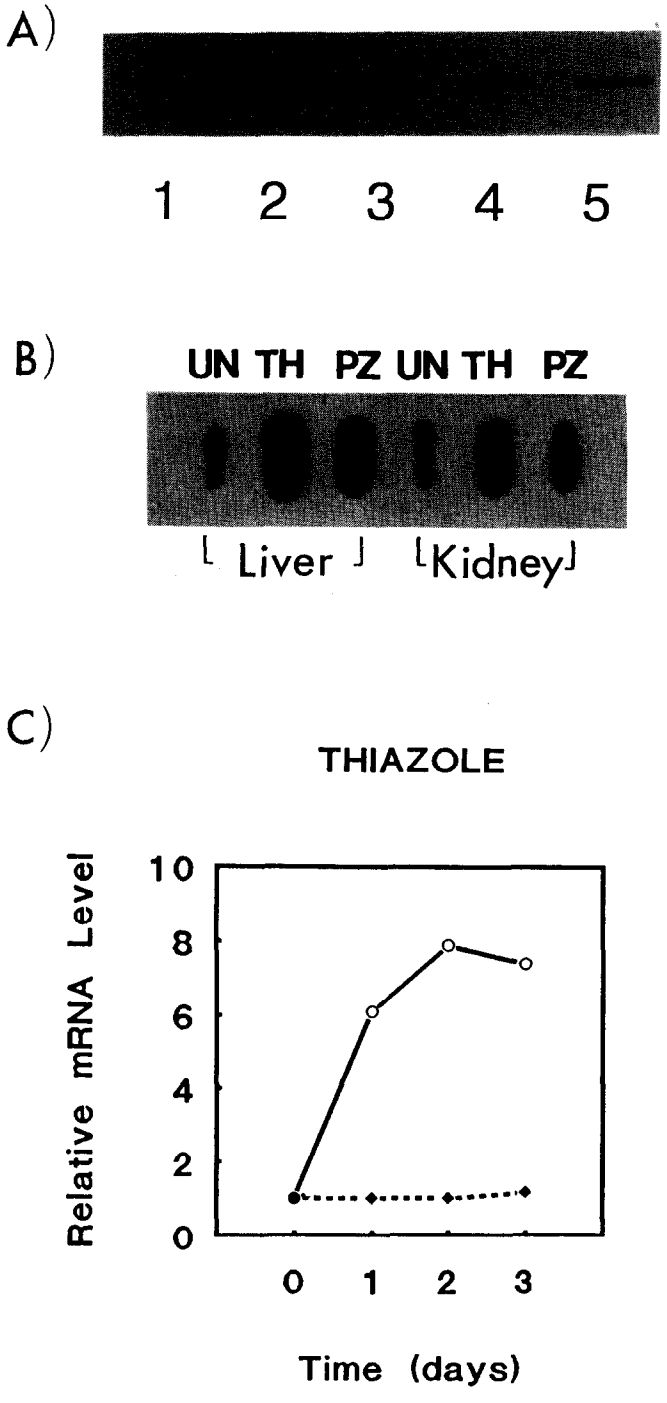

Fig. 4. mEH protein and mRNA levels in kidney. Panel A: Immunoblot shows mEH levels in kidney microsomes isolated from untreated (lane 2), IM- (lane 3), TR- (lane 4) or TH- (lanes 1 and 5) treated rats (200 mg/kg body weight/day, p.o., 3d). Panel B: Slot blot analysis of mEH mRNA content in rats treated with TH or PZ. The total RNA (20 μg) isolated from liver and kidney at 24 hr following treatment with TH or PZ (200 mg/kg body weight, i.p.) was blotted to nitrocellulose membranes and hybridized with ^{32}P-labelled mEH cDNA probe. Panel C: Relative mEH mRNA levels in kidney after TH treatment.

levels to the comparable extent that TH caused [11]. It was probably due to the fact that the antimycotics have bulky substituted groups in common at N-1 of IM. Both the present and previous studies demonstrated that azole heterocycles served as mEH inducers differentially and that mEH induction was greatest by the molecules which had either the N-1 atom or proximal atom(s) to N-1 in azole ring substituted with a larger atom(s) such as N = N or sulphur, or with a bulky substituent.

Studies in other laboratories have also shown that the epoxide hydrolase activity toward styrene oxide increased after treatment of animals with certain heterocyclic compounds, including coumaran, trimethylene oxide, trimethylene sulphide and benzofuran, which have strained bond angles to a nucleophilic heteroatom [31]. Those studies along with the results of this research support the hypothesis that the induction of mEH by heterocycles is attributed to bonding force of compounds and/or sizes of atoms in heterocycles, possibly in conjunction with other chemical properties such as electronegativity [32].

In addition, the time courses of mEH mRNA increases were determined at early times following a single injection of TH in this study. TH was chosen for a time-related study among the azoles examined because of its relatively strong efficacy. The time-dependent changes in mEH mRNA levels after TH treatment were compared with those caused by PZ because both compounds elevated mEH levels to a similar extent 3 days after treatment [15]. Time courses for mEH gene expression at early times by either TH or PZ were also comparable to each other with maximal increases being noted at 12 hr. The levels of mEH mRNA by these heterocycles increased as a function of time and the rates of increase in mEH mRNA levels were similar to those caused by either nitrosamines [30] or IM antimycotic agents [11], which suggested that these heterocyclic compounds may share common molecular mechanisms for mEH induction.

Previous studies in this laboratory revealed that renal mEH protein and mRNA levels failed to show an age-dependent change in either sex during maturation, although hepatic mEH protein and RNA levels significantly elevated or suppressed in males and females, respectively [20]. Thus, there was a distinct tissue-specific mEH expression. In this study, TH treatment caused increased mEH mRNA levels to a much lesser extent in kidney than in liver, and that either IM or TR failed to show a notable increase in renal mEH mRNA levels, representing that these xenobiotics were much less effective in inducing mEH in kidney.

In summary, these results demonstrated that the azole heterocycles differentially modulate mEH levels and that IM affected mEH gene expression minimally unless the N-1 nitrogen in IM had a substituted group (e.g. IM antimycotic agents). This study also showed that TRs increased hepatic mEH protein and mRNA levels to a moderate extent while TH appeared to be the most efficacious agent among the azoles examined, and that TH and PZ exhibited comparable time courses in the expression of the mEH gene at early times.

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