Table 1. Effect of nucleotides or H-7 on the inactivation of protein kinase C by FSBA or DTNB

| Addition to assay mixture | % Residual activity | |
|---|---|---|
| | 1 mM FSBA | 0.05 mM DTNB |
| Control | 100 ± 3.5 | 96.3 ± 6.4 |
| None | 47.7 ± 6.0 | 22.3 ± 4.1 |
| ATP (1 mM) | 90.0 ± 11.2 | 71.3 ± 6.1 |
| ADP (1 mM) | 59.3 ± 10.5 | 71.3 ± 3.2 |
| AMP (1 mM) | 56.7 ± 3.5 | 52.6 ± 5.0 |
| H-7 (1 mM) | 71.2 ± 6.0 | 36.6 ± 5.6 |

Protein kinase C was incubated with 1 mM FSBA or 0.05 mM DTNB at 30° in 50 mM HEPES–NaOH, pH 7.5, 2 mM EGTA, as described under Materials and Methods. Residual activity at 30 min (FSBA) or 10 min (DTNB) is given.

By analogy with the cAMP-dependent protein kinase, one can assume that Lys-371 and Cys-502 of rabbit protein kinase C ($\alpha$ type) would lie at or near the ATP binding site [17]. When considering the pattern of protection against FSBA or DTNB, the region binding to H-7 may be Lys-371 and not Cys-502. The selectivity of this compound should facilitate studies on the molecular mechanism of phosphotransferase reactions of protein kinase C.

In summary, protein kinase C purified from rabbit brain is irreversibly inactivated by 5′-*p*-fluorosulfonylbenzoyl-adenosine (FSBA) and 5,5-dithiobis (2-nitro-benzoic acids) (DTNB), compounds postulated to modify residues at or near the active site of the catalytic region, in various protein kinases. Inactivation by FSBA followed pseudo-first order kinetics. The pseudo-first order rate constant for inactivation showed saturation kinetics, thereby indicating a reversible binding of the reagent to the enzyme, prior to inactivation. We examined the effects of Mg-ATP and 1-(isoquinolinylsulfonyl)-2-methylpiperazine (H-7), the latter a selective inhibitor for protein kinase C on the inactivation of the enzyme by these reagents. As Mg-ATP and H-7 protected the enzyme against inactivation by FSBA, H-7 probably binds at or near the ATP-binding site on the protein kinase C molecule. However, H-7 did not protect the enzyme from DTNB and Mg-ATP afforded full protection from DTNB. These data suggest that H-7 may interact selectively with the region labelled by FSBA and does not interfere with the domain labelled by DTNB in the ATP binding site of protein kinase C.

† To whom all correspondence should be addressed.

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*Department of Molecular and Cellular Pharmacology*
*Mie University School of Medicine*
*Edobashi*
*Tsu, Mie 514, and*
**Department of Pharmacology*
*Nagoya University School of Medicine*
*Showa-ku, Nagoya 466*
*Japan*

HISATAKA OHTA
TOSHIO TANAKA
HIROYOSHI HIDAKA*†

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# *In vivo* role of the microsomal ethanol-oxidizing system in ethanol metabolism by deermice lacking alcohol dehydrogenase*

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Two pathways of alcohol dehydrogenase (ADH) independent ethanol metabolism have been reported: the microsomal ethanol-oxidizing system (MEOS) and catalase. The respective role of each pathway in ethanol metabolism is still actively debated [1–7]. To examine the contribution of catalase, many investigators have used 3-amino-1,2,4-triazole (AT), widely reported to be a good catalase inhibitor [8, 9]. AT was found to have little effect on *in vivo* ethanol metabolism in either rats [10–15] or ADH-deficient deermice [1, 3]. This suggested a major role for MEOS rather than catalase in non-ADH ethanol metabolism. By contrast, a recent paper [4] reported that, 6 hr after AT administration in deermice, AT inhibited

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MEOS but not the peroxidatic activity of catalase, and concluded on this basis that the catalase pathway is significant for ethanol metabolism *in vivo*. To address this issue, we reexamined the effect of AT on both pathways at 6 hr in deermice using the methods described in that paper [4]. Since 1-butanol is known to be a competitive inhibitor of MEOS [5] but not a substrate for [16] or an inhibitor of [5, 17] catalase, we also studied the effect of butanol on ethanol elimination *in vivo* and compared it to that of AT.

*Materials and methods*

*Animals.* Deermice genetically lacking hepatic ADH ($Adh^N/Adh^N$) bred in our laboratory from an original stock provided by Dr. M. Felder were used for the experiments. All animals were 3- to 6-month-old males. Animals were fed Lab-Blox (Wayne Food Division, Continental Grain Co., Chicago, IL) and water *ad lib.*

*Effect of 1-butanol on ethanol elimination* in vivo. Ethanol (1.0 g/kg body wt) was injected i.p. as a 10% (w/v) solution followed by 1-butanol as a 3.2% (w/v) solution (at 1 hr, 0.32 g/kg body wt; at 2 hr, 0.16 g/kg body wt) or saline. Air bubble samples were collected every 30 min from 1 hr to 3 hr after ethanol, and ethanol levels were measured by head space gas chromatography as previously described [1]. 1-Butanol levels were measured by head space gas chromatography in blood of a parallel group of animals dosed identically with ethanol and 1-butanol.

*Microsomal oxidation of ethanol and aniline.* Animals were injected with AT (1.0 g/kg body wt, i.p.) or saline 6 hr prior to being killed. Microsome preparation and the measurement of ethanol oxidation and aniline hydroxylation were performed exactly as previously described [4]. Microsomal ethanol oxidation was measured also with head space gas chromatography as described elsewhere [5].

*Peroxidatic activity of catalase.* Determination of peroxidatic activity of catalase was attempted by the method described by Handler *et al.* [4], and also as described by Feytmans and Leighton [14] with these modifications: 1 mg liver was used, glycollate was replaced by glucose plus glucose oxidase sufficient to produce 400 nmol $H_2O_2 \cdot ml^{-1} \cdot min^{-1}$, and a 20 mM methanol concentration was used.

*Results and discussion*

In the presence of 1-butanol, ethanol elimination in ADH negative deermice was inhibited by 50% compared to controls (41.4 ± 1.4 vs 83.4 ± 3.0 $\mu mol \cdot min^{-1} \cdot kg^{-1}$) (Fig. 1). This is the first observation of effective inhibition of ethanol metabolism by an MEOS inhibitor *in vivo*, and suggests a significant role for MEOS in intact animals. Actual blood 1-butanol levels were 2.5 ± 0.1 mM at 1.5 hr and 2.3 ± 0.1 mM at 2.5 hr. The predicted inhibition of ethanol metabolism based on the $K_i$ of 1-butanol for MEOS of 1.6 mM [5] is about 40% at these time points, and similar to the inhibition observed *in vivo*. A recent preliminary report [17] postulated that 1-butanol inhibits *in vivo* $H_2O_2$ generation in the presence of ADH which could affect ethanol peroxidation. However, since our results were obtained in ADH negative animals, such a mechanism could not be operative.

AT has been shown previously to have little effect on ethanol elimination 6 hr after its administration to ADH negative deermice [1, 4, 5]. At that time point, we measured MEOS activity to be 80% of control as judged from the absorbance at 225 nm of acetaldehyde semicarbazone adduct formed. A headspace gas chromatography assay [5] of free acetaldehyde produced by MEOS also revealed the same moderate degree of inhibition (83% of control). Aniline hydroxylase activity was measured to be 63% of control. While not in agreement with the study of Handler *et al.* [4], these results are consistent with other reports of AT effects in rats. Although some degree of inhibition by AT on the microsomal metabolism of various

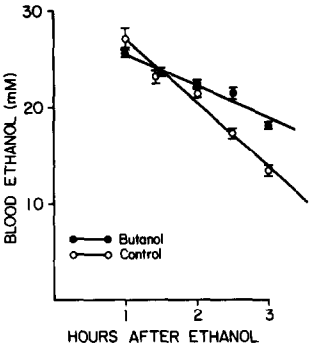


Fig. 1. Effect of butanol on *in vivo* ethanol metabolism of ADH negative deermice. Ethanol (1.0 g/kg) was injected i.p. followed by saline or 1-butanol (at 1 hr, 0.34 g/kg; at 2 hr, 0.17 g/kg). Values are means ± SE, with five animals in each group.

drugs has been shown, this effect was always partial even with large and repetitive AT doses. For instance, after five doses of 1 g/kg AT, aniline hydroxylation remained more than 40% of control [18]. Since MEOS activity probably involves, at least in part, the same cytochrome P-450 as aniline hydroxylase, both activities should show parallel changes. It is thus improbable that MEOS loses almost all activity (to less than 3%) 6 hr after AT as reported by Handler *et al.* [4].

We have observed previously that ethanol peroxidation in deermice is inhibited effectively by AT [5], with a time course similar to that of catalase inhibition observed by others in rats [8, 9, 19]. By contrast, Handler *et al.* [4] found no significant inhibition of methanol peroxidation 6 hr after AT. When we attempted to reproduce their method, we encountered several problems with the measurements of methanol peroxidation as reported [4]. One difficulty stemmed from the high viscosity and short incubation time: the large amount of liver used (200 mg tissue in 1 ml of reaction mixture) could not be mixed rapidly enough with the substrate to obtain reproducible results with a 30-sec end point assay. Additionally, the high blanks resulting from the large amount of tissue also reduced precision in the colorimetric formaldehyde measurements [20]. Furthermore, the concentration of $H_2O_2$ (10 mM) used in this assay [4] is unphysiologically high. Despite these technical problems, the estimate obtained of methanol peroxidation after AT was 40% of control (Table 1). An improved estimation of methanol peroxidation, obtained using a radiometric method, showed only 15% activity remaining after AT (Table 1). Either result is clearly at variance with the report indicating no inhibition of peroxidation at 6 hr [4].

Catalase has been assigned a negligible role in ethanol metabolism based not only on the lack of effect of AT, but also on the very low physiological rate of $H_2O_2$ generation [21]. A recent paper [7] reported, to the contrary, that fatty acids (particularly palmitate) stimulate both $H_2O_2$ generation and ethanol uptake, and suggested a corresponding role for catalase in ethanol metabolism under physiological conditions. It must be pointed out, however, that this phenomenon was observed only with 4-methylpyrazole present. In the absence of 4-methylpyrazole, extra $H_2O_2$ generation with fatty acids in the intact perfused liver has been reported to be small for all fatty acids tested, and even totally absent for palmitate [22]. Indeed, unless 4-methylpyrazole is added, the rate of ethanol metabolism is reduced in the presence of fatty acids [23, 24], and $\beta$-oxidation of fatty acids is inhibited when either ethanol [23] or butanol [17] is metabolized via ADH. Thus, under physiological conditions with ADH present, it is unlikely that $\beta$-oxidation of fatty acids stimulates $H_2O_2$ generation,

Table 1. Hepatic enzyme activities 6 hr after aminotriazole (1 g/kg)

| | Control | Aminotriazole | Assay method (ref.) | % of Control |
|---|---|---|---|---|
| MEOS | 9.0 ± 0.2* | 7.2 ± 0.7* | 4 | 80 |
| [nmol·min$^{-1}$·(mg protein)$^{-1}$] | 10.9 ± 0.4 | 9.1 ± 0.3 | 5 | 83 |
| Aniline hydroxylation | | | | |
| [nmol·min$^{-1}$·(mg protein)$^{-1}$] | 1.6 ± 0.1 | 1.0 ± 0.2 | 4 | 63 |
| Methanol peroxidation | 96 ± 10 | 38 ± 3 | 4 | 40 |
| [$\mu$mol·hr$^{-1}$·(g liver)$^{-1}$] | 317 ± 46 | 47 ± 8 | 14† | 15 |

* Values are means ± SE of five animals.
† The published assay was modified as detailed in Materials and Methods.

or that this peroxisomal pathway represents a significant contribution to ethanol metabolism as proposed by Handler and Thurman [7]. Even in the absence of ADH, the data presented here, as well as previous studies [1, 3, 6], provide *in vivo* evidence which argues against a major role for this pathway.

In conclusion, our study demonstrated effective inhibition of *in vivo* ethanol elimination by the MEOS inhibitor, 1-butanol, but failed to confirm the effects of aminotriazole reported by Handler *et al.* [4] in ADH negative deermice. Moreover, these results agree with other published experiments conducted in the rat, and concur with previous studies of the *in vivo* roles of MEOS and catalase carried out in deermice using completely different methods [3, 6]. Thus, our present as well as past results clearly indicate a significant role of MEOS, but not of catalase, for ethanol metabolism *in vivo* in deermice.

*Alcohol Research and Treatment Center*
*Veterans Administration Medical Center, and*
*Mount Sinai School of Medicine (City University of New York)*
*Bronx, NY 10468, U.S.A.*

SHINZO KATO
JEFFREY ALDERMAN
CHARLES S. LIEBER*

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* Correspondence: C. S. Lieber, M.D., Alcohol Research and Treatment Center, VA Medical Center, 130 West Kingsbridge Road, Bronx, NY 10468, U.S.A.

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## Differences in the induction of carboxylesterase isozymes in rat liver microsomes by xenobiotics*

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Carboxylesterase (EC 3.1.1.1) in liver microsomes catalyzes the hydrolysis of a large number of xenobiotics, including carboxylesters, thioesters, and aromatic amides [1–3], and plays an important role in drug metabolism. Recent studies have focused on the multiplicity of this enzyme, and more than twenty isozymes have been isolated and characterized [3, 4]. Only a few of these isozymes of hepatic microsomal carboxylesterases are induced by exogenous compounds such as phenobarbital [5] and DDT

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