Half-life time of charge-transfer complex in NAP-chymotrypsin in the presence of 2,2,2-trifluoroethanol, 2-chloroethanol, 2-bromoethanol, 1-propanol or ethanol

	Trifluoroethanol		Chloroethanol		Bromoethanol		Propanol		Ethanol	
Concentration of alcohol (M)	3.20	5.33	1.60	2.13	0.27	0.40	3.20	5.33	6.00	6.67
Half-life time (min)	1.5	<0.5	1.5	<0.5	1.5	<0.5	10.0	2.5	25.0	17.5

3.20 M, 2-chloroethanol above 1.60 M, and 2-bromoethanol above 0.27 M. However, the time was 10 min for 1-propanol at 3.20 M and 25 min for ethanol at 6.00 M. Figure 1 and table 1 show that the halogeno-ethanols changed the structure around the active sites more rapidly than ethanol and 1-propanol. Urea was similar to ethanol and 1-propanol, and changed it slowly as shown in figure 1. The surviving complex with the alcohols and urea at infinite time is given as a function of their concentration in figure 2. The concentration required to break-up the complex in half was 1.0 M for 2-chloroethanol, 0.2 M for 2-bromoethanol, 1.5 M for 2,2,2-trifluoroethanol, 2.5 M for 1-propanol, and 6.0 M for ethanol. The halogeno-ethanols, therefore, have an ability to cause a more effective and rapid change in the structure around the active sites of α-chymotrypsin than 1-propanol and ethanol; the latter group is similar to urea in that ability. We suppose that the difference in the ability of the alcohols and urea to inhibit α-chymotrypsin described in the introductory statement is due to differences in their ability to change the structure around its active sites. The similarity of urea to 1-propanol and ethanol seems to be contradictory to the differences in the denaturation process of a protein in urea and in organic solvents such as alcohols; denaturation in urea appears as a transition from the native to the denatured unfolded state[4-6], but putting a protein into a high concentration of organic solvents results in a structural ordering reaction through a partially unfolded state[4,6]. However, the apparent contradiction can be solved by the following possibility, which was proposed in the previous report[1]. The change in the structure around the active sites caused by 1-propanol and ethanol, as well as urea, is mediated through an extensive conformational change, and that caused by the halogeno-ethanols occurs possibly at first as a local structural change.

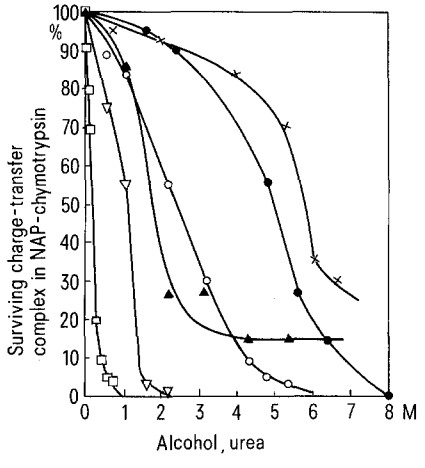

Fig. 2. Change in the surviving charge-transfer complex in NAP-chymotrypsin at infinite time with the concentration of alcohols (▲——▲, 2,2,2-trifluoroethanol; ▽——▽, 2-chloroethanol; □——□, 2-bromoethanol; ○——○, 1-propanol; ×——×, ethanol) and urea (●——●).

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Monoacylcadaverines as substrates for both monoamine oxidase and diamine oxidase; low rates of activity

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Summary. Monoacetylcadaverine and monopropionylcadaverine were found to be substrates for both rat liver monoamine oxidase and hog kidney diamine oxidase, but all the K_m-values for the oxidases were very high. The amines were common substrates for type A and type B monoamine oxidase.

Monoacetylcadaverine and monopropionylcadaverine were first identified in urine of schizophrenic patients[1]. Recently, Dolezalova et al.[2,3] also identified these acylcadaverines in blood of schizophrenic and normal subjects, and found that the levels of the monoacylcadaverines were higher in blood of schizophrenic patients than in that of the normal subjects. The physiological significance of these amines is not known.

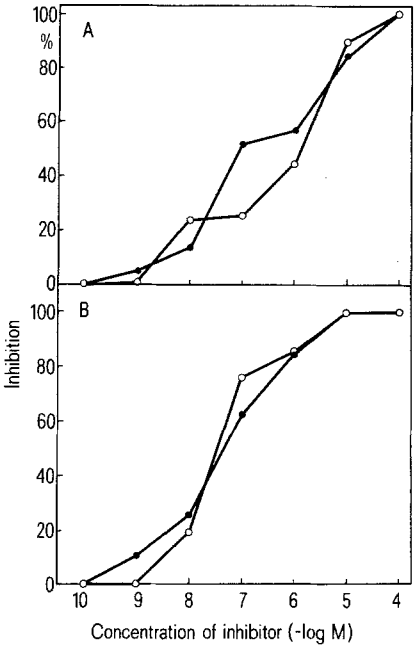
The synthesis and degradation of the monoacylcadaverines have not been studied. Monoacetylcadaverine may be formed from cadaverine via acetylation, because it has been reported that putrescine is preferentially acetylated by rat brain tissue[4]. It is also to be expected that the monoacylcadaverines may be catabolized by monoamine oxidase (EC 1.4.3.4, MAO), since monoacetylputrescine is reported to be a substrate for MAO in the rat[5].

In this paper, we demonstrate that the monoacylcadaverines are substrates for MAO in vitro and further charac-

terize them as substrates for type A and type B MAO. In addition, we have found that these amines are also substrates for hog kidney diamine oxidase (EC 1.4.3.6, DAO).

Materials and methods. Mitochondrial fractions were prepared from the pooled whole brains and livers of 7 male Sprague-Dawley rats weighing 150–200 g as described previously[6]. Hog kidney DAO was purchased from Sigma Chemical Co., St. Louis, Mo. Monoacetylcadaverine and monopropionylcadaverine were synthesized at KOR Isotopes, Cambridge, Ma. Their purities were greater than 99% when checked by nuclear magnetic resonance and mass-spectrometry. Clorgyline, a selective inhibitor of type A MAO[7], was generously supplied by May & Baker Ltd., Dagenham, England. Deprenyl, a selective inhibitor of type B MAO[8], was kindly donated by Prof. J. Knoll, Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary.

MAO and DAO activities were determined fluorometrically by the method of Guilbault et al.[9] and Snyder and Hendley[10]. For each assay (final volume, 0.15 ml), 0.0825–0.165 mg of protein was used. The assays were carried out at pH 7.4 and 37 °C for 60 min. Under the conditions used, the assays were linear during incubation for at least 60 min.



Inhibition of MAO activity in rat liver mitochondria by clorgyline (A) and deprenyl (B) using monoacetylcadaverine (○—○) and monopropionylcadaverine (●—●) as substrates. The substrate concentrations were at their K_m-values, viz. 40.5 and 25.6 mM, respectively. The assay mixtures were preincubated with each inhibitor at 37 °C for 10 min. Each point represents the mean obtained from duplicate determinations upon a single enzyme source prepared from pooled livers of 7 rats.

Protein was measured by a modification[11] of the conventional biuret method.

Results and discussion. Both monoacetylcadaverine and monopropionylcadaverine were found to be substrates for MAO in rat liver mitochondria. The activities in brain mitochondria were also detectable, but represented only 10.3 and 6.2% of those found with the liver enzyme, for monoacetylcadaverine and monopropionylcadaverine, respectively. Although the present MAO assay is comparable in sensitivity to the radiochemical methods[10], the brain MAO was not used to perform determinations for kinetic and inhibition studies due to its low activity. Therefore, all experiments for MAO were carried out with rat liver mitochondria.

Monoacetylcadaverine and monopropionylcadaverine were also found to be substrates for hog kidney DAO. In order to exclude the possibility that the activities found in rat liver mitochondria are due to DAO which may be present in the crude mitochondrial preparation, or that the activities found in hog kidney DAO are due to contamination by MAO, we have checked the effects of pargyline, a selective inhibitor of MAO, and semicarbazide, a selective inhibitor of DAO, on the activities toward both monoacylcadaverines. As can be seen in table 1, the activities with rat liver mitochondria were inhibited almost completely by 1.0 mM pargyline, while semicarbazide in the same concentration induced only a weak inhibition. The inhibition of the activities with hog kidney DAO by pargyline was low, but that by semicarbazide was 100%. These results clearly confirm that both monoacylcadaverines are metabolized by both rat liver mitochondrial MAO and hog kidney DAO.

The Michaelis-Menten kinetic constants for rat liver mitochondrial MAO and hog kidney DAO were determined from Lineweaver-Burk plots as shown in table 2. As can be seen in the table, the K_m-values for both oxidases were very high. The K_m-value for monoacetylcadaverine was higher than that for monopropionylcadaverine when rat liver mitochondrial MAO was used, while it was lower when hog kidney DAO was used. These data suggest that the acyl group of a longer carbon chain may inactivate an amino group of diamines more effectively than a shorter one. The

Table 1. Effects of pargyline and semicarbazide on the oxidation of monoacetylcadaverine and monopropionylcadaverine by rat liver mitochondria and by purified hog kidney DAO*

	Percent inhibition	
	Pargyline	Semicarbazide
Rat liver mitochondria		
Monoacetylcadaverine	96.9	17.4
Monopropionylcadaverine	98.3	12.4
Hog kidney DAO		
Monoacetylcadaverine	10.1	100
Monopropionylcadaverine	6.47	100

* The concentrations of the substrates and the inhibitors were 25.0 mM and 1.0 mM, respectively. Each value is the mean obtained from duplicate determinations.

Table 2. Kinetic constants for MAO in rat liver mitochondria and for hog kidney DAO using monoacetylcadaverine and monopropionylcadaverine as substrates*

Substrate	Rat liver mitochondrial MAO		Hog kidney DAO	
	K_m (mM)	V_{max} (nmoles/mg protein/60 min)	K_m (mM)	V_{max} (nmoles/mg protein/60 min)
Monoacetylcadaverine	40.5	100	7.25	339
Monopropionylcadaverine	25.6	50.0	33.3	149

* Each kinetic constant was determined graphically from Lineweaver-Burk plots using 5–6 substrate concentrations assayed in duplicate.

V_{max}-values for monoacetylcadaverine were higher than those for monopropionylcadaverine for both oxidases.

Since the functional forms of mitochondrial MAO have generally been classified into type A and type B, depending on inhibitor sensitivity and substrate specificity[7], the sensitivity of MAO activities to clorgyline and deprenyl was studied in order to determine the specific type of MAO involved in the catabolism of monoacylcadaverines (figure). In the curves with clorgyline, clear plateaux appeared at 10^{-8}–10^{-7} M and at 10^{-7}–10^{-6} M for monoacetylcadaverine and monopropionylcadaverine, respectively. In the curves with deprenyl, plateaux were not clear. The lack of plateaux in the curves with deprenyl was previously observed, despite the presence of both types of MAO, when enzyme preparations such as rabbit[12] and chick tissues[13], and substrates such as m-octopamine[14] were used. It can be concluded from our results that both monoacylcadaverines are common substrates for type A and type B MAO.

In the present study, although we did demonstrate that monoacetylcadaverine and monopropionylcadaverine are substrates for both rat liver mitochondrial MAO and hog kidney DAO, their K_m-values were extremely high (table 2). Since the levels of the monoacylcadaverines in mammalian tissues and body fluids are low[2,3] (in the order of 10^{-9} M), it seems reasonable to consider that the monoacylcadaverines are not easily metabolized by monoamine oxidase and diamine oxidase in mammalian tissues. It has been suggested that monoacylpolyamines in blood and urine could be useful as biochemical markers for diseases such as schizophrenia[2,3] and leukemias[15]. It seems likely that the monoacylcadaverine levels in blood and urine reflect the level of synthetic processes for these amines, such as the rate of acylation of cadaverine and its availability, rather than the rate at which they are degraded, because monoacylcadaverines may not be further metabolized in the human body.

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2-Halogeno-ethanols as an uncoupler of phosphorylation in rat liver mitochondria

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Summary. 2-Chloroethanol, 2-bromoethanol and 2,2,2-trifluoroethanol at a concentration of 0.79 vol.% stimulated state 4 respiration and released oligomycin inhibition of state 3 respiration. 2-Fluoroethanol and 1-propanol at the same concentration did not affect the respiration.

2-Halogeno-ethanols inhibited α-chymotrypsin (EC 3.4.21.1) effectively[1] by inducing a rapid change in its active site structure[2]. This effect is probably due to the ability of their halogeno groups to cause effective perturbation of the configuration of the hydrophobic groups in a protein[1-3]. Therefore, it seemed of interest to investigate the effects of the halogeno-ethanols on the function of biomembranes. From this point of view, we examined the effect of the halogeno-ethanols and 1-propanol on the succinate oxidation in rat isolated liver mitochondria by measuring the respiration and succinate dehydrogenase activity, and making comparisons between the halogeno groups and the methyl group.

Materials and methods. Male rats (Wistar, 250–300 g) were killed by bleeding. The livers were immediately removed, and a 10% homogenate was prepared in an ice-cold preparation medium containing 210 mM mannitol, 70 mM sucrose, 0.1 mM EDTA and 5 mM Tris-HCl of pH 7.4 in a Potter-Elvehjem homogenizer. Mitochondria were isolated from the homogenate as a fraction between $700 \times g \times 10$ min and $5000 \times g \times 10$ min. After being washed with the same medium, they were resuspended in the same medium to reach a concentration of 3 g of the liver per 2 ml of the medium; the amount of mitochondrial protein was 9.0 to 11.0 mg/ml. Respiration of 0.3 ml of the mitochondrial suspension was measured with an oxygen electrode in a cell of 3.8 ml volume in an air-saturated reaction medium containing 250 mM mannitol, 10 mM KCl, 10 mM K_2HPO_4, 5 mM $MgCl_2$, 0.2 mM EDTA and 10 mM Tris-HCl of pH 7.4 at 25 ± 0.1 °C; the O_2 concentration was 245 μM[4]. Succinate dehydrogenase activity of 0.1 ml of the mitochondrial suspension was anaerobically measured at 25 ± 0.1 °C in a reaction medium containing 37.5 mM sodium succinate, 1.875 mM 2,2′,5,5′-tetraphenyl-3,3′-(p-biphenylene)-ditetrazolium chloride and 25 mM phosphate buffer of pH 7.4. The dehydrogenase activity was determined by the spectrophotometrical estimation of the reduced tetrazolium at 500 nm.

Results and discussion. Figure 1 shows the effects of 2-halogeno-ethanols and 1-propanol at a concentration of