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.

1 T.L. Perry, S. Hansen and L. MacDougall, Nature *214*, 484 (1967).
2 H. Dolezalova, M. Stepita-Klauco, J. Kucera, H. Uchimura and M. Hirano, J. Chromat. *146*, 67 (1978).
3 H. Dolezalova, M. Stepita-Klauco, C. van der Velde and V. Cassone, Biol. Psychiat. *14*, 27 (1979).
4 N. Seiler and M.J. Al-Therib, Biochim. biophys. Acta *354*, 206 (1974).
5 N. Seiler and M.J. Al-Therib, Biochem. J. *144*, 29 (1974).
6 O. Suzuki, Y. Katsumata, M. Oya, V.M. Chari, R. Klapfenberger, H. Wagner and K. Hostettmann, Biochem. Pharmac. *27*, 2075 (1978).
7 J.P. Johnston, Biochem. Pharmac. *17*, 1285 (1968).
8 J. Knoll and K. Magyar, in: Advances in Biochemical Psychopharmacology, vol.5, p.393. Ed. E. Costa and M. Sandler. Raven Press, New York 1972.
9 G.G. Guilbault, P.J. Brignac, Jr and M. Juneau, Analyt. Chem. *40*, 1256 (1968).
10 S.H. Snyder and E.D. Hendley, J. Pharmac. exp. Ther. *163*, 386 (1968).
11 O. Suzuki, E. Noguchi and K. Yagi, Brain Res. *135*, 305 (1977).
12 R.F. Squires, in: Advances in Biochemical Psychopharmacology, vol.5, p.355. Ed. E. Costa and M. Sandler. Raven Press, New York 1972.
13 O. Suzuki, M. Oya, Y. Katsumata and M. Asano, Experientia *35*, 167 (1979).
14 O. Suzuki, H. Hattori, Y. Katsumata and M. Oya, Life Sci. *25*, 1231 (1979).
15 M. Tsuji, T. Nakajima and I. Sano, Clinica chim. Acta *59*, 161 (1975).

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

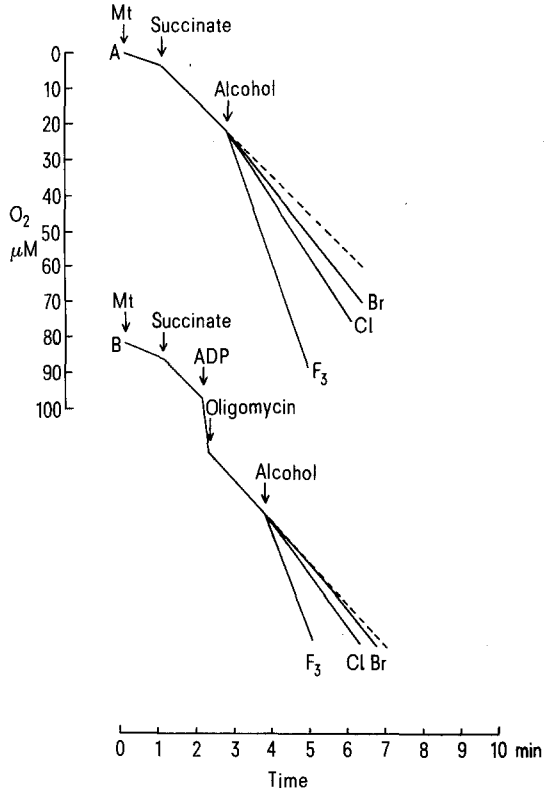

Fig. 1. Effects of 2-halogeno-ethanols at a concentration of 0.79 vol.% on isolated rat liver mitochondria respiration with 2.4 mM succinate as a substrate at 25 °C: A, state 4; B, state 3 (with 118 μM ADP) in the presence of 1.5 μg/ml oligomycin. The amount of mitochondrial protein was about 3.0 mg in an oxygen electrode apparatus of 3.8 ml volume. ——, respiration with: Cl, 2-chloroethanol (0.12 M); Br, 2-bromoethanol (0.11 M) and F_3, 2,2,2-trifluoroethanol (0.11 M). - - - - -, respiration without any alcohol; the lines were the same as those with 2-fluoroethanol or 1-propanol at the same concentration.

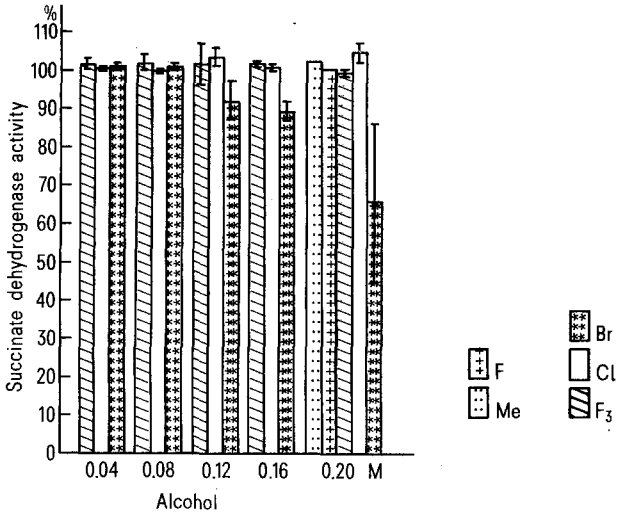

Fig. 2. Effects of 2-halogeno-ethanols and 1-propanol on succinate dehydrogenase activity in isolated rat liver mitochondria at 25 °C. The activity of about 1.0 mg mitochondrial protein was anaerobically determined in a reaction medium containing 37.5 mM succinate, 1.875 mM tetrazolium and 25 mM phosphate buffer, and is given as relative to that without any alcohol. Each short vertical bar shows the mean value ± SE (n = 3). F, 2-fluoroethanol; Me, 1-propanol; Br, Cl and F_3 as for figure 1.

0.79 vol.% (about 0.14 M for 2-fluoroethanol, 0.12 M for 2-chloroethanol, and 0.11 M for 2-bromoethanol, 2,2,2-trifluoroethanol and 1-propanol) on the respiration in state 4[5](A) and in state 3 in the presence of oligomycin[6](B) with 2.4 mM succinate as a substrate. 2-Chloroethanol, 2-bromoethanol and 2,2,2-trifluoroethanol of the alcohols stimulated state 4 respiration and released the inhibition of state 3 respiration by oligomycin. The same stimulation and release are produced by uncouplers of phosphorylation such as dinitrophenol[7]. 2-Fluoroethanol and 1-propanol at the same concentration, however, had no effect on the respiration. The effect of the alcohols up to 0.20 M on the succinate dehydrogenase activity was examined and is given in Fig. 2. 2-Bromoethanol above 0.12 M inhibited but the others, within 0.20 M, had no effect on the activity. All of the alcohols within 0.20 M did not activate the enzyme. Therefore, the succinate dehydrogenase was neither involved in the stimulation of the state 4 respiration, nor in the release of the oligomycin inhibition of the state 3 respiration by the alcohols. Figure 3 shows the effects of 2,2,2-trifluoroethanol at 0.018 M, 0.055 M and 0.110 M on the respiration in order to examine the alcohol-concentration dependence of the effects, and the effects of dinitrophenol at 0.155 mM on the respiration in the presence of 2,2,2-trifluoroethanol at 0.018 M, in order to compare the effects of dinitrophenol with those of 2,2,2-trifluoroethanol. Both the stimulation and the release were equally pronounced with increase in the alcohol concentration. Dinitrophenol increased the stimulation and the release. Therefore, 2-chloroethanol, 2-bromoethanol and 2,2,2-trifluoroethanol affected the succinate oxidation as uncouplers of phosphorylation. Most uncouplers act as protonophores across the inner membrane of mitochondria[8,9]. However, these alcohols cannot directly transport a proton. The uncoupling power of 2,2,2-trifluoroethanol was only

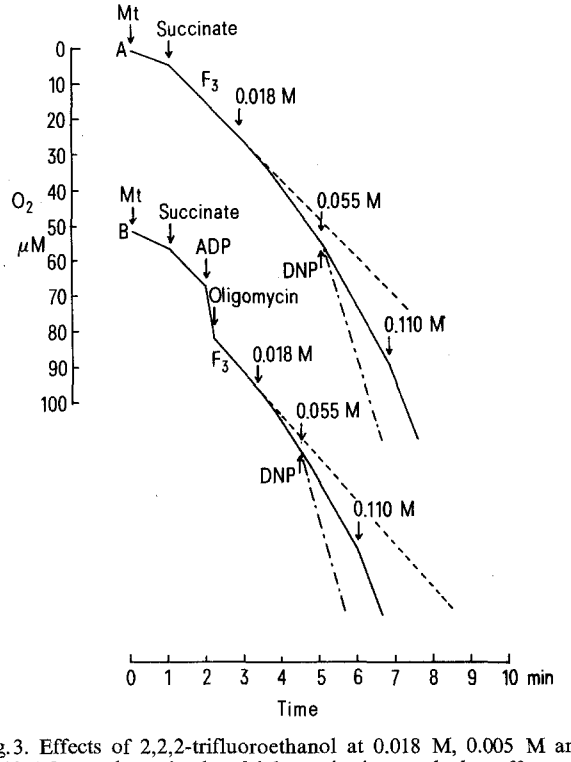

Fig. 3. Effects of 2,2,2-trifluoroethanol at 0.018 M, 0.005 M and 0.110 M on the mitochondrial respiration and the effects of dinitrophenol (DNP) at 0.16 mM on the respiration in the presence of 2,2,2-trifluoroethanol at 0.018 M. Conditions as for figure 1 except that the ADP concentration was 354 μM instead of 118 μM.

about $^1/_{500}$ of that of dinitrophenol, which is now classified as a classical uncoupler because of its relatively weak uncoupling power in comparison with that of a powerful uncoupler[10,11]. As described in the introductory statement the halogeno-ethanols have a significant ability to cause perturbation of the configuration of the hydrophobic groups in a protein, but 1-propanol, on the other hand, has a poor ability to cause such perturbation. The difference in their ability is probably due to the following; the nature of the hydrophobicity of the protein hydrophobic groups is different from that of the halogeno groups but identical with that of the nonpolar group of 1-propanol[3]. This leads us to a hypothesis interpreting the mode of uncoupling by these halogeno-ethanols; 2-chloroethanol, 2-bromoethanol and 2,2,2-trifluoroethanol perturb the structure of the inner membrane to cause leakage of protons from the outside to the inside. The ineffectiveness of 2-fluoroethanol in figure 1 may be due to the poor hydrophobicity of its fluoroethyl group - insufficient to cause the above uncoupling effect. It is now recognized that the enhancement of the uncoupling power the introduction of halogeno groups into a protonophore-type uncoupler results from both an increase in its hydrophobicity and a decrease in the pK_a[12-14]. This study, however, indicates that the introduction also gives the uncoupler the ability to cause an effective change in the structure of the inner membrane.

1 S. Ebina, T. Kobayashi and Y. Nagai, Experientia *35*, 1011 (1979).
2 S. Ebina and Y. Nagai, unpublished data.
3 S. Ebina and H. Uedaira, Bull. chem. Soc. Japan *50*, 1305 (1977).
4 B. Hagihara, Biochim. biophys. Acta *46*, 134 (1961).
5 B. Chance, J. biol. Chem. *217*, 409 (1955).
6 H.A. Lardy, D. Johnson and W.C. McMurray, Archs biochem. Biophys. *78*, 587 (1958).
7 W.F. Loomis and F. Lipmann, J. biol. Chem. *173*, 807 (1948).
8 P. Mitchell, Nature *191*, 144 (1961).
9 A. Finkelstein, Biochim. biophys. Acta *205*, 1 (1970).
10 R.L. Williamson and R.L. Metcalf, Science *158*, 1694 (1967).
11 S. Muraoka and H. Terada, Biochim. biophys. Acta *275*, 271 (1972).
12 H.C. Hemker, Biochim. biophys. Acta *63*, 46 (1962).
13 M.W. Whitehouse, Biochem. Pharmac. *13*, 319 (1964).
14 E. Weinbach and J. Garbus, J. biol. Chem. *240*, 1811 (1965).

Adenosine-3, 5-phosphate levels in brain structures of rats submitted to four different behavioral procedures

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Summary. Shuttle avoidance training decreased the cAMP content of rat brain (excluding hippocampus and caudate nucleus), amygdala and hypothalamus. Stimulation by tones alone had a similar effect, but only in the brain fraction. Pseudoconditioning or footshocks alone had no effect.

Brief aversive learning experiences in rats or mice are accompanied by increased rates of phosphorylation of brain chromosomal[2-4] and synaptosomal[5] proteins. This effect probably depends on the activation of protein kinases by adenosine-3′, 5′-phosphate (cAMP)[2,4]. Therefore, regional changes in brain cAMP levels might be expected during the initial stages of aversive conditioning. Hambley and Rose[6,7] reported an early fall, followed by a late increase, of cAMP levels in the brain of chickens submitted to another form of learning, namely, visual imprinting. The present note reports on the effect of 5- or 25-min of shuttle avoidance training, pseudoconditioning, footshocks, and tones, on the cAMP content of various brain regions of the rat.

Material and methods. Adult male Wistar rats from our own breeding stock were used (age 59–70 days; weight 135–195 g). Behavioral procedures were carried out in 50 × 25 × 25 cm noncompartmentalized shuttleboxes made of acrylic[4,8]. The floor of these boxes was a grid of 2 mm bronze bars spaced 7 mm apart. A flat, 0.5-cm-wide, piece of acrylic at the midline was the only mark between the right and left sides of the floor. A loudspeaker was attached to the rear wall 15 cm above floor level, at the midline. Animals were divided into 5 groups, each submitted to a different behavioral treatment, as follows:

1. Pseudoconditioning. 5 sec, 1 kHz, ≅ 70 db tones were presented at randomly variable intervals of 10–50 sec, at a rate of 10 tones every 5 min. Footshocks (60 Hz, 1 mA, 2 sec) were interspersed among the tones at randomly variable tone-shock intervals of 5–45 sec, as follows: 8 shocks among the first 10 tones, 7 among the following 10 tones, 5 among the next 10 tones, 3 among the next 10 tones, and 2 among the last 10 tones. This approximately matches the distribution of footshocks over time obtained in the following behavioral procedure[4,8].
2. Shuttle avoidance. Tones were presented as above, but each tone was immediately followed by a footshock (contiguity) unless the animals performed a shuttle response to the tone (avoidance contingency)[8].
3. Tones alone. Tones were presented every 10–50 sec; no shocks.
4. Footshocks alone. Footshocks delivered every 10–50 sec; no tones.
5. Intact controls. Animals taken out from their home cages and sacrificed right away.

In the 4 former groups, the behavioral treatments were carried out during either 5 or 25 min. In all cases, interstimulus intervals were programmed in such a way, that the animals submitted to 5 min sessions received 10 tones (or trials, or footshocks), and those submitted to the 25-min sessions received 50 tones (or trials, or footshocks).

Animals were sacrificed by decapitation within 30 sec from termination of each of the above described behavioral procedures. The brain was immediately taken out, put on ice and dissected into either hippocampus, caudate nucleus and rest of the brain (excluding cerebellum but including amygdala and hypothalamus), or amygdala and hypothalamus. Tissues were homogenized at less than 4 °C in 20 times their volume of 6% trichloroacetic acid, and subsequently processed for cAMP determination by radioimmuncassay[9] using the New England Nuclear Corporation kit NEX-132. Protein content of each tissue was measured in aliquots of the homogenates by the Folin phenol reagent method[10]. Statistical comparisons were by a randomized-group analysis of variance followed by a Duncan multiple range test[11].