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Acetaldehyde—an inhibitor of the enzymatic oxidation of 5-hydroxyindoleacetaldehyde*†

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ETHANOL has been reported to reduce the amount of 5-hydroxyindoleacetic acid (5-HIAA)[1, 2] and 3-methoxy-4-hydroxymandelic acid (VMA)[3] excreted in urine by animals. This alteration was attributed either to an inhibition of monoamine oxidase (MAO)[1] or to a depletion of NAD,[2] the cofactor necessary for aldehyde dehydrogenase activity. Studies using labeled serotonin[4] and norepinephrine[5] in man further demonstrated the decrease in excreted 5-HIAA and VMA and also demonstrated that this decrease occurred concomitantly with an increase in the excretion of 5-hydroxy-tryptophol (5-HTOH)[4] and 3-methoxy-4-hydroxyphenylglycol(MHPG).[5] These results are supported in a previous publication[6] in which we demonstrated that *in vitro* acetaldehyde did have an effect on serotonin catabolism and that this effect was at the aldehyde dehydrogenase locus and not MAO. The net result was a decrease in the formation of 5-HIAA and an increase in the "neutrals" (5-hydroxyindoleacetaldehyde and 5-HTOH).

In this communication we have examined more closely the inhibition of aldehyde dehydrogenase by acetaldehyde.

Preparation and measurement of 5-hydroxyindoleacetaldehyde (*5-HIAA*ld). 5-HIAAld was prepared according to the method of Renson *et al.*[7] by incubating serotonin with a dialyzed preparation of MAO.[8] The aldehyde was measured enzymatically by using a partially purified preparation of AldDH prepared according to Racker.[9] The increase in absorption at 340 mμ, due to formed NADH, was used to calculate the amount of 5-HIAAld metabolized. A molar extinction coefficient of $6{\cdot}2 \times 10^3$ was used in the calculations.[10] The validity of this method was checked by measuring the 5-HIAA formed.[11, 12] The results obtained by the two methods were in close agreement.

Isolation of brain mitochondria. Rat brain mitochondria were prepared by homogenizing a rat brain in 20 parts of 0·4 M sucrose containing 0·04 mM EDTA at pH 7·4. The homogenate was centrifuged at 1000 *g* for 10 min; the supernatant was decanted and centrifuged at 11,500 *g* for 15 min. The supernatant was discarded and the pellet was resuspended in one-half the volume of the homogenizing solution and centrifuged at 11,500 *g* for 15 min. The tubes were then swirled to remove a white fluffy layer on top of the mitochondria, which would correspond to gross nerve ending particles.[13] The mitochondria were resuspended in 3·0 ml of 0·05 M phosphate buffer at pH 7·4 containing 0·04 mM EDTA.

To determine the type of inhibition, if any, and the K_i of acetaldehyde and the K_m for 5-HIAAld, the data were treated by the method of Dixon[14] by plotting $1/v$ vs. I, where v is the velocity of the reaction and I is the inhibitor concentration. In each of 5 separate experiments at least two substrate concentrations were used, ranging from $2{\cdot}8 \times 10^{-6}$ to $11{\cdot}4 \times 10^{-6}$ M. Acetaldehyde was used at four different concentrations ranging from $1{\cdot}0 \times 10^{-6}$ to $7{\cdot}5 \times 10^{-6}$ M. Controls were also used which did not contain acetaldehyde. Blanks were prepared by incubating the enzyme and other components and adding the substrate after the reaction had been terminated. 5-HIAA was extracted and assayed as described above and at least three standards were prepared for a standard curve. All standards and samples were corrected by subtracting blank values.

The incubation mixtures consisted of: substrate at various concentrations; redistilled acetaldehyde, where designated; $1{\cdot}25 \times 10^{-3}$ M neutralized NAD; and 0·3 ml mitochondria in 0·05 M phosphate at pH 7·4, in a total volume of 3·0 ml. Incubation was for 12 min at 37° in glass-stoppered Erlenmeyer flasks. The reaction was terminated by the addition of 1·0 ml of 0·1 N HCl. 5-HIAA was extracted and assayed as described above. Conditions were predetermined to limit the maximum amount of substrate metabolized to less than 20 per cent of that initially present. The reaction rate was linear for at least 20 min.

A typical plot of the data is shown in Fig. 1. The apparent K_i (± S.D.) from 5 determinations was found to be $2{\cdot}62 (\pm 1{\cdot}00) \times 10^{-6}$ M and is in the neighborhood of the K_m for acetaldehyde deter-

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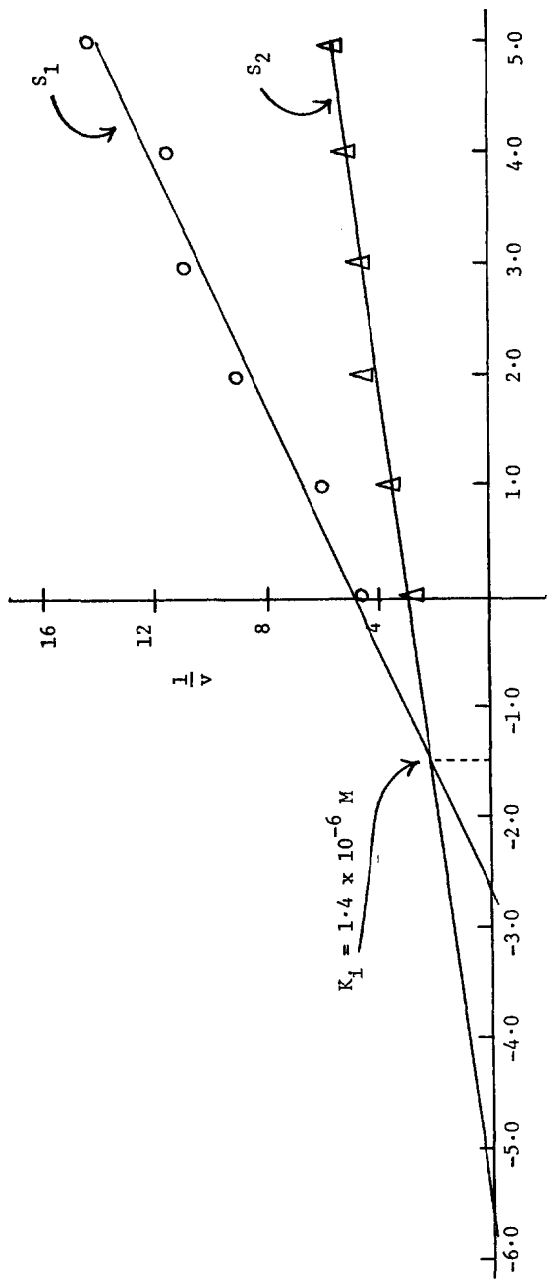

FIG. 1. Brain aldehyde dehydrogenase, showing competitive inhibition of 5-HIAAld oxidation by 10^{-6} M acetaldehyde. Ordinate, reciprocal of micrograms of 5-HIAA formed per incubation mixture; abscissa, acetaldehyde $\times 10^{-6}$ M. $S_1 = 2{\cdot}8 \times 10^{-6}$ M 5-HIAAld; $S_2 = 11{\cdot}4 \times 10^{-6}$ M 5-HIAAld. Incubation mixture contained NAD at $1{\cdot}25 \times 10^{-3}$ M, 0·3 ml mitochondria in 0·5 M phosphate at pH 7·4, acetaldehyde and 5-HIAAld. Total volume of the mixture was 3·0 ml. Incubation was for 12 min at 37° in glass-stoppered flasks.

mined with bovine aldehyde dehydrogenase.[15] It can also be observed that the inhibition is of the competitive type, as has been suggested by others.[10] The apparent K_m for 5-HIAAld was also determined from the Dixon plots.[14] The 5 experiments gave 10 determinations of the K_m for 5-HIAAld. The K_m ($\pm$ S.D.) was found to be $5{\cdot}44$ ($\pm$ $2{\cdot}4$) $\times$ 10^{-6} M, which is in agreement with that reported by Erwin and Deitrich[15] using bovine brain AldDH.

NAD was shown not to be a limiting factor in these results, since doubling the amount of NAD present in the incubation mixture, where acetaldehyde and substrate were at their maximum concentrations, did not alter the amount of product formed or the amount of inhibition.

It was reported in a previous publication[6] that in rat liver homogenate acetaldehyde had essentially no effect on MAO activity. However, it was found that the production of 5-HIAA by AldDH was inhibited by acetaldehyde at concentrations approaching those found physiologically. The inhibition was shown not to be due to a depletion of NAD, since excess cofactor had no effect on the rate of metabolism or the amount of inhibition. The net result of this inhibition was an accumulation of the neutrals, 5-HTOH and 5-HIAAld, and a corresponding decrease in 5-HIAA.[6]

A more detailed kinetic study of the inhibition of AldDH by acetaldehyde was carried out in this publication by using rat brain mitochondrial AldDH. A Dixon plot[14] of $1/v$ vs. I indicates that the observed inhibition is competitive (Fig. 1). Studies with bovine aldehyde dehydrogenase indicated that this should be the case.[10] The K_m for 5-HIAAld was $5{\cdot}4 \times 10^{-6}$ M and the K_i for acetaldehyde was $2{\cdot}6 \times 10^{-6}$ M. These values are in close agreement with those published for bovine brain aldehyde dehydrogenase.[15] Since the above inhibition is a competitive one, the K_i for acetaldehyde is equivalent to its K_m; therefore the above correlation of K_m values could be made.

If the concentrations of 5-HIAAld and acetaldehyde present in the brain were known, one could calculate the theoretical percentage inhibition of 5-HIAAld oxidation in the brain by using the constants K_m and K_i and their values determined above. The following formula[16] permits such a calculation.

$$\frac{V_i}{V_o} = \frac{K_m + s}{K_p + s}$$

where V_i is the velocity in the presence of inhibitor; V_o is the velocity in absence of inhibitor; s is the substrate concentration in brain; and K_p is calculated as $[i + K_i]/[K_i] \times K_m$, i being the inhibitor concentration in the brain.

Since 5-HIAAld has not been found in the brain, possibly due to its rapid metabolism, this type of calculation is not possible without making certain assumptions. If one considered s, the substrate concentration in the brain, to be less than 10^{-7} M and therefore, for all practical purposes, zero, the above equation reduces to- $V_i/V_o = K_m/K_p$. By using the K_m and K_i determined in this study and an acetaldehyde concentration of $7{\cdot}5 \times 10^{-5}$ M, which has been found in the rat brain after ethanol administration,[17] a K_p of $1{\cdot}61 \times 10^{-4}$ is calculated. Then $V_i/V_o = 0{\cdot}034$, which gives a theoretical inhibition of 96·6 per cent for 5-HIAAld oxidation by AldDH in the brain. It would also be predicted that the aldehyde derived from norepinephrine (DHMald) would be inhibited to the same extent, since the kinetic constants for the two aldehydes and the amounts found in the brain are comparable.[15]

It thus seems plausible that the observed[1-3] alteration in biogenic amine metabolism is due to an inhibition of aldehyde dehydrogenase and not necessarily to changes in NADH.

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The time course of covalent binding of [^{14}C]-4-*N*,*N*-di-(2′-chloroethyl) aniline (aniline mustard to mouse liver and kidney nucleic acids and proteins *in vivo*

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4-*N*,*N*-di-(2′-chloroethyl) aniline (aniline mustard, ¦CB1074) inhibits completely the growth of the transplanted ADJ/PC5 myeloma in mice. It causes complete regression of established tumours weighing up to 3g at a dose of 40 mg/kg. Using the tritiated (ring labelled) compound at this dose level the maximum levels of binding to myeloma DNA, RNA and cytoplasmic proteins were 0·25 μm/g, 0·23 μm/g and 0·19 μm/g respectively.[1]

In this communication the level of binding of this alkylating agent to the DNA, ribosomal RNA, and proteins of mouse liver and kidneys at various times after a similar single intraperitoneal injection are reported.

MATERIALS AND METHODS

^{14}C-4-*N*,*N*-di-(2′-chloroethyl) aniline was prepared from ^{14}C-aniline hydrogen sulphate (3 mc) (obtained from the Radiochemical Centre, Buckinghamshire, England), essentially as described before.[2] The specific radioactivity of the product was 2·2 mc/mM.

Groups of five male Bal c$^-$ mice weighing about 25 g were used to determine each point on the curves. The animals were fed rat cake and water *ad libitum*.

The labelled compound was administered intraperitoneally in arachis oil (40 mg/kg; 0·2 ml oil/mouse).

Groups of animals were killed at 2 hr, 5·5 hr, 1 hr, 48 hr, 1 week, 2 weeks and 3 weeks. DNA, ribosomal RNA, nuclear and cytoplasmic proteins were extracted from the pooled organs and purified,