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INTERFERENCE OF ALDEHYDE METABOLIZING ENZYMES WITH DIAMINE OXIDASE/HISTAMINASE/ACTIVITY AS DETERMINED BY ^{14}C PUTRESCINE METHOD

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Abstract—The Δ^1 pyrroline formation, as an indicator of diamine oxidase activity according to Okuyama and Kobayashi ^{14}C putrescine test [1], has been investigated in several tissue homogenates. When guinea pig liver homogenate was used as a source of enzyme in the presence of aldehyde dehydrogenase inhibitors chloral hydrate and acetaldehyde the level of formation Δ^1 pyrroline was strongly increased in a dose-dependent manner. Also inhibition of aldehyde reductase by phenobarbital enhanced Δ^1 pyrroline formation, but to a lesser degree. In other tissues, with very high initial diamine oxidase activity (rat intestine, dog kidney) or with very low diamine oxidase activity (guinea pig skin, dog liver) the influence of these inhibitors was only slight. Pyrazole, an inhibitor of alcohol dehydrogenase exerted only a small effect on Δ^1 pyrroline formation. All aldehyde-metabolizing enzymes inhibitors, except pyrazole, were without effect on purified pea seedling and hog kidney diamine oxidases. The use of aldehyde-metabolizing enzymes inhibitors may help to reveal the real values of diamine oxidase activity, when tissues homogenates are used as a source of enzyme.

Diamine oxidase (DAO, EC 1.4.3.6) activity is often estimated with ^{14}C putrescine as a substrate by a measurement of the amount of ^{14}C Δ^1pyrroline and its polymers formed during reaction [1]. Oxidation of putrescine by DAO leads to γ-aminobutyraldehyde formation, which cyclizes into an internal aldimine ring, Δ^1pyrroline. This cyclic compound may undergo a variety of further spontaneous changes, i.e. polymerization, interaction with o-aminobenzaldehyde or extensive cleavage [1–4]. Unlike putrescine, the aldimine and its polymers are soluble in organic solvents, e.g. toluene, and may be thus easily separated.

Degradative pathways of putrescine are not fully understood, but there is evidence of its transformation to γ-aminobutyric acid GABA via γ-aminobutyraldehyde [5], this latter compound may also undergo other metabolic transformations. The formation of the alcohol product from γ-aminobutyraldehyde 4-amino-1-butanol in tissues is also suspected. The formation of 4-amino-1-butanol has been reported when putrescine was used as a substrate for purified pig kidney diamine oxidase and liver or yeast alcohol dehydrogenases [6].

The possible pathways of γ-aminobutyraldehyde metabolism are shown on Fig. 1.

The enzymes which metabolize the aldehydes (4-imidazolylacetaldehyde, γ-aminobutyraldehyde) resulting from an oxidation of histamine or putrescine, respectively, by DAO have been not investigated until now, mainly because of inadequate methods. These aldehydes are very unstable and undergo rapid transformations. A supposed product of histamine reaction with DAO (4-imidazolylacetaldehyde) is unstable. Kapeller–Adler and Fletcher [8] described its separation as a corresponding dinitrophenylhydrazone, but Kivits and Hora [9] recently demonstrated that it was an artefact and acetone dinitrophenylhydrazone was regarded as imidazolylacetaldehyde derivative. The use of γ-aminobutyraldehyde as a substrate is also impossible because of its immediate, spontaneous cyclization to Δ^1pyrroline. However a new approach, with the use of the inhibitors of aldehyde metabolizing enzymes, might partially fill this gap.

Recently several inhibitors of aldehyde metabolizing enzymes have been described: chloral hydrate [10, 11], acetaldehyde [10–12] and disulfiram [11, 13] inhibit aldehyde dehydrogenase, barbiturates inhibit an aldehyde reductase [11, 14] and pyrazole inhibits an alcohol dehydrogenase [15].

We now present data concerning influence of these inhibitors on the enzymes metabolizing γ-aminobutyraldehyde with a special reference to ^{14}C putrescine assay for DAO. Monoamine oxidase inhibitors [16] have been also applied to check whether γ-aminobutyraldehyde formed during reaction may be transformed by MAO into dialdehyde.

Abbreviations—ADH—alcohol dehydrogenase, AldDH—aldehyde dehydrogenase, AldR—aldehyde reductase, GABA—γ-aminobutyric acid, DAO—diamine oxidase, MAO—monoamine oxidase.

MATERIALS AND METHODS

Chemicals. 1,4-^{14}C Putrescine dihydrochloride/65mCi/m-mole/was purchased from Radiochemical Center, Amersham; putrescine dihydrochloride and acetaldehyde from Fluka, perchloric acid from Boehringer and hog kidney DAO from Sigma. Pea seedling DAO was a gift from Dr Jürgen Kusche

(Abt. Exp. Chirurgie, University of Marburg Fedn. Rep. Germany) and iproniazid, tranylcypromine and pargyline were a gift from Dr Richard W. Schayer (Rockland Res. Inst. U.S.A.). All other chemicals were of the highest analytical grade available and were obtained from POCh, Poland.

Enzyme preparation. Tissue samples, guinea pig liver and skin, intestine of Wistar rats and liver, and kidney of mongrel dogs, were homogenized with three volumes of sodium-potassium phosphate buffer (0.15 M, pH 7.4), centrifuged for 20 min at 25,000 *g* and the supernatants were used as a source of enzyme. Purified DAO from pea seedlings and hog kidney DAO were used as reference enzymes.

Determination of ^{14}C Δ^1pyrroline. The method of isotopic assay was essentially that of Okuyama and Kobayashi as described by Kusche *et al.* [17].

The complete reaction mixture contained 0.15 M phosphate buffer (pH 7.4), enzyme preparation, 10^{-4}M [^{14}C]putrescine (0.05 μCi) and in corresponding samples, an inhibitor. The total volume of the medium was 0.75 ml. After the definite incubation periods the reactions were terminated by the addition of 0.2 ml of perchloric acid (0.4 M) then treated with 1 ml of strong alkaline buffer (saturated $NaHCO_3$ solution and 2N NaOH, pH about 12) to obtain pH 9.9 exactly, followed by the addition of 5 ml of toluene—0.35% PPO. These conditions allow for the optimal ^{14}C Δ^1pyrroline and its polymers extraction [17]. After vigorous shaking for 4 min samples were centrifuged for 2 min at 2000 *g* and placed into dry ice—methanol bath (−20°–40°) to freeze aqueous phase. A liquid, organic layer containing labelled Δ^1pyrroline was transferred into scintillation vials with 5 ml of toluene—0.35% PPO. Radioactivity was measured in LKB Wallac 81000 scintillation counter.

RESULTS

Influence of aldehyde dehydrogenase (AldDH) and aldehyde reductase (AldR) inhibitors on Δ^1pyrroline formation in guinea pig liver homogenates. The influence of acetaldehyde, chloral hydrate and disulfiram, inhibitors of AldDH, and barbital and phenobarbital, inhibitors of AldR on Δ^1pyrroline formation in guinea pig liver homogenates is presented in Table 1.

Chloral hydrate and acetaldehyde produced a dose dependent increase in Δ^1pyrroline formation up to 5 and 10 times respectively. Disulfiram, another AldDH inhibitor was ineffective even at 10^{-3}M. Phenobarbital, an inhibitor of AldR, increased Δ^1pyrroline formation only in concentration of 10^{-3}M, while in lower concentrations it was without effect. Barbital, another AldR inhibitor, had no effect even at 10^{-3}M.

Influence of aldehyde metabolizing enzymes inhibitors on Δ^1pyrroline formation in various rat, dog and guinea pig tissue homogenates (Table 2). AldDH and AldR inhibitors (Table 2) exert a very feeble effect, if any, on Δ^1pyrroline formation in other tissues tested i.e. rat intestine, dog kidney and liver, and guinea pig skin.

Influence of pyrazole, an inhibitor of alcohol dehydrogenase (ADH) on Δ^1pyrroline formation. Pyrazole 10^{-3}M, an inhibitor of ADH inhibited Δ^1pyrroline formation by pea seedling DAO up to 82 ± 3 per cent of initial value ($P < 0.02$), while its effect on

Table 1. The influence of aldehyde dehydrogenase (AldDH) and aldehyde reductase (AldR) inhibitors on Δ^1pyrroline formation by guinea pig liver homogenates

Percentage of initial* Δ^1pyrroline level after inhibition of:				
Aldehyde dehydrogenase			Aldehyde reductase	
Acetaldehyde	Chloral hydrate	Disulfiram	Barbital	Phenobarbital
5×10^{-4} M no effect	5×10^{-4} M no effect	10^{-3} M	10^{-5} M	10^{-5} M
10^{-3} M 152 ± 35	10^{-3} M 138 ± 31	no effect	no effect	no effect
$n = 5$	$n = 2$			
5×10^{-3} M 332 ± 81	5×10^{-3} M 153 ± 16		10^{-3}	10^{-3}
$n = 5$	$n = 3$		no effect	253 ± 58
10^{-2} M 1070 ± 262	2×10^{-2} M 552 ± 166			$n = 14$
$n = 3$	$n = 12$			

* Initial value of DAO (36 ± 6 dpm, $n = 24$) is expressed as Δ^1pyrroline formed per 1 min of incubation.

Table 2. The influence of aldehyde dehydrogenase and aldehyde reductase inhibitors on Δ^1pyrroline formation in tissues with high and low diamine oxidase activities

Tissue homogenates	Initial value dpm/min	Percentage of initial Δ^1pyrroline level after inhibition of: Ald. DH Acetaldehyde 10^{-2}M	Ald. DH Chloral hydrate 2×10^{-2}M	Ald. R Phenobarbital 10^{-2}M
Rat	1336 ± 259	108 ± 12	117 ± 9	106 ± 10
intestine	$n = 14$	$n = 5$	$n = 14$	$n = 6$
Dog	2447 ± 267	115 ± 18	109 ± 3	100 ± 2
kidney	$n = 3$	$n = 3$	$n = 3$	$n = 3$
Guinea pig	13 ± 1	—	no effect	114 ± 13
skin	$n = 3$		$n = 3$	$n = 3$
Dog liver	~ 3	—	no effect	no effect
	$n = 3$		$n = 3$	$n = 3$

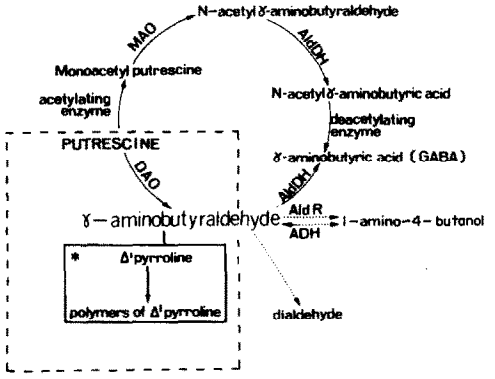

Fig. 1. The possible pathways of γ-aminobutyraldehyde metabolism. Putrescine acetylation leading to GABA is taken from N. Seiler [7]. *Described as an indicator of DAO activity [1].

guinea pig liver, dog kidney and rat intestine homogenates was small, if any statistically not significant.

Influence of monoamine oxidase (MAO) inhibitors on Δ^1pyrroline formation. MAO inhibitors, iproniazid 5×10^{-4}M, pargyline 1×10^{-7}M and tranylcypromine 1×10^{-6}M did not influence Δ^1pyrroline formation in guinea pig liver homogenates ($n = 3$).

The formation of Δ^1pyrroline in the presence of purified pea seedling and dog kidney diamine oxidases was not changed even by the highest concentrations of all examined aldehyde metabolizing enzymes inhibitors, except pyrazole.

DISCUSSION

We have shown that the inhibitors of aldehyde metabolizing enzymes enhanced the level of Δ^1pyrroline formation. Δ^1pyrroline is a product of a spontaneous cyclization of γ-aminobutyraldehyde, the latter being the direct product of oxidative deamination of putrescine. Thus Δ^1pyrroline level has been treated as a direct reflection of putrescine oxidation process, and was commonly used as the indicator of DAO activity. However, γ-aminobutyraldehyde will only be nearly entirely transformed into Δ^1pyrroline when the millieu—a supposed source of DAO, is completely devoid of any aldehyde metabolizing enzymes. The situation becomes different when millieu e.g. tissue homogenate contains enzymes, which are able to utilize γ-aminobutyraldehyde as a substrate. It could be oxidized by AldDH to GABA [19] or reduced by AldR or/and ADH to 4-amino-1-butanol [6]. Reduction of aldehyde group to an alcoholic hydroxyl group has been found to prevent cyclization (Dupré). The same may be valid as an oxidation of the aldehyde group to acidic carboxyl group is concerned. In these cases Δ^1pyrroline level decreases and this final effect might be and was commonly interpreted as a low DAO activity. It became quite obvious, that the applicability of the method of Okuyama and Kobayashi for an estimation of DAO activity should either be restricted to purified enzyme preparations, or, if tissue homogenates must be assayed, the validity of the assay must be checked out by the use of inhibitors of aldehyde metabolizing enzymes or/and measurement of final products of aminoaldehyde transformation i.e. GABA and aminohydroxybutane.

The use of inhibitors has revealed the existence at least in a guinea pig liver of aldehyde metabolizing enzymes, which interfere with DAO assay. In this respect our studies confirm and extend the earlier results of Schmutzler *et al.* [18] with chloral hydrate.

Therefore the use of inhibitors may give better approximation to the real value of DAO activity in tissue homogenates. However, it is not very obvious, that this approach could reveal the full DAO activity in tissues. First of all, the inhibitors used in this work were described previously only as inhibitors of enzymes metabolizing aldehydes deriving from monoamines [10–15], but not for diamines. Thus they should be not necessarily the most specific for diamine aldehydes. It is even reasonable to suppose, that the aldehyde metabolizing enzymes of tested tissues have somewhat different properties towards inhibitors from the other known aldehyde metabolizing enzymes (mainly bovine and rats' brain and liver). Thus disulfiram, reported to be specific inhibitor of rat's liver and brain aldehyde dehydrogenases [11, 13] showed no effect on the guinea pig liver enzyme, even at 10^{-3}M, while two other inhibitors, acetaldehyde and chloral hydrate exerted strong, dose-dependent effects. Barbital described as an inhibitor of AldR in bovine brain [14] was ineffective as an inhibitor of AldR in guinea pig liver. This enzyme was inhibited only by phenobarbital at high 10^{-3}M concentration (Table 1). Nevertheless, the results concerning the inhibition of AldR by barbiturate suggest a possibility of transformation of aminoaldehyde into its alcohol derivative. The existence of this route of putrescine catabolism has not been reported in tissue homogenates until now.

The weak inhibitory effect of pyrazole on Δ^1pyrroline formation in various tissue homogenates, and its stronger inhibitory action on purified pea seedling DAO, may suggest an existence of a low alcohol dehydrogenase activity in all examined tissues. Monoamine oxidase activity against γ-aminobutyraldehyde does not appear likely, because none of MAO inhibitors used in our experiments was able to change Δ^1pyrroline level. One cannot exclude however, that MAO activity might appear, if other metabolic pathways of γ-aminobutyraldehyde were blocked.

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