Contribution of Aldehyde Oxidizing Enzymes on the Metabolism of 3,4-Dimethoxy-2-phenylethylamine to 3,4-Dimethoxyphenylacetic Acid by Guinea Pig Liver Slices

Georgios I. Panoutsopoulos

Department of Experimental Pharmacology, Medical School, Athens University

Key Words

Aldehyde oxidase • Aldehyde dehydrogenase • Allopurinol • Disulfiram • Homoveratrylamine • Isovanillin • Liver slices • Monoamine oxidase • 3,4-Dimethoxy-2-phenylethylamine • Xanthine oxidase

Abstract

Background/Aims: 3,4-Dimethoxy-2-phenylethylamine is catalyzed to its aldehyde derivative by monoamine oxidase B, but the subsequent oxidation into the corresponding acid has not yet been studied. Oxidation of aromatic aldehydes is catalyzed mainly by aldehyde dehydrogenase and aldehyde oxidase. **Methods:** The present study examines the metabolism of 3,4-dimethoxy-2-phenylethylamine in vitro and in freshly prepared and cryopreserved guinea pig liver slices and the relative contribution of different aldehyde-oxidizing enzymes was estimated by pharmacological means. **Results:** 3,4-Dimethoxy-2-phenylethylamine was converted into the corresponding aldehyde when incubated with monoamine oxidase and further oxidized into the acid when incubated with both, monoamine oxidase and aldehyde oxidase. In freshly prepared and

cryopreserved liver slices, 3,4-dimethoxyphenylacetic acid was the main metabolite of 3,4-dimethoxy-2-phenylethylamine. 3,4-Dimethoxyphenylacetic acid formation was inhibited by 85% from disulfiram (aldehyde dehydrogenase inhibitor) and by 75-80% from isovanillin (aldehyde oxidase inhibitor), whereas allopurinol (xanthine oxidase inhibitor) inhibited acid formation by only 25-30%. **Conclusions:** 3,4-Dimethoxy-2-phenylethylamine is oxidized mainly to its acid, via 3,4-dimethoxyphenylacetaldehyde, by aldehyde dehydrogenase and aldehyde oxidase with a lower contribution from xanthine oxidase.

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Introduction

Monoamine oxidase (MAO; amine-oxygen oxidoreductase, EC 1.4.3.4) is a flavoenzyme localized in the outer mitochondrial membrane, which catalyzes the oxidative deamination of neuroactive, vasoactive and dietary amines to their corresponding aldehydes [1-3]. This enzyme exists in two different isoenzymatic forms known as MAO A and MAO B, which are differentiated

on the basis of substrate specificity and inhibitor sensitivity [1, 4], and which are encoded by different genes [5].

Aldehydes are highly reactive compounds due to the electrophilic nature of their carbonyl group. Rapid removal of the aldehydes, by oxidation to the carboxylic acid, is important in order to prevent aldehydes from building up within the cells, which may lead to toxicity. The oxidation of aldehydes is generally attributed to aldehyde dehydrogenases [6-8] and the contribution of aldehyde oxidase and xanthine oxidase is usually ignored. However, the importance of aldehyde oxidase in the oxidation of aromatic aldehydes has recently been established [2, 9-12]. Aldehyde dehydrogenase (EC 1.2.1.3; aldehyde-NAD(P)⁺ oxidoreductase) metabolizes irreversibly both aliphatic and aromatic aldehydes to their corresponding acids in a NAD⁺-dependent reaction [8, 13, 14]. In addition, aldehyde oxidase (EC 1.2.3.1; aldehyde-oxygen oxidoreductase) and xanthine oxidase (EC 1.2.3.2; xanthine-oxygen oxidoreductase) also catalyze the oxidation of aldehydes including those arising from endogenous and exogenous amines such as dietary amines and drugs [2, 3, 10, 12]. These enzymes are widely known to metabolize heterocyclic compounds [15, 16]. Nevertheless, aromatic aldehydes are excellent substrates of hepatic aldehyde oxidase with lower activities towards xanthine oxidase [10, 17].

It has been previously shown that 3,4-dimethoxybenzaldehyde [10], a stable analogue of 3,4-dimethoxyphenylacetaldehyde, is a good substrate for guinea pig liver aldehyde oxidase, but only a weak substrate of xanthine oxidase. Therefore, it was thought that these enzymes might also be important in the metabolism of 3,4-dimethoxy-2-phenylethylamine. It is known that 3,4-dimethoxy-2-phenylethylamine is metabolized by MAO B to its aldehyde [18], which can then be converted to 3,4-dimethoxyphenylacetic acid possibly by either aldehyde dehydrogenase, aldehyde oxidase or xanthine oxidase.

In the present study, the metabolism of 3,4-dimethoxy-2-phenylethylamine in freshly prepared and cryopreserved guinea pig liver slices was investigated. Incubations were also performed in the presence of specific inhibitors for each aldehyde oxidizing enzyme. The inhibitors used were isovanillin for aldehyde oxidase [10, 12], allopurinol for xanthine oxidase [19] and disulfiram for aldehyde dehydrogenase [20, 21]. Guinea pig liver was chosen in this study as aldehyde oxidase has a similar specificity to the human liver enzyme [22, 23]. Therefore, the overall contribution of each aldehyde

oxidizing enzyme in guinea pig liver towards the aromatic aldehyde derived from biogenic amines was investigated.

Materials and Methods

Animals

The animals used in this study were female Dunkin-Hartley guinea pigs (450-950 g). They were fed with FD1 pellets supplemented with ascorbic acid and received hay three times weekly. All animals were allowed food and water ad libitum and maintained in a strictly controlled temperature (18±1°C). Humidity was kept at 50% and the lighting cycle was 07.00-19.00 h light and 19.00-07.00 h dark. The animals were handled with humane care according to the National Institutes of Health guidelines for the care and use of laboratory animals [24].

Chemicals

Tetraethylthiuram disulfide (disulfiram), allopurinol, 3-hydroxy-4-methoxybenzaldehyde (isovanillin), diethylamine, 3,4-dimethoxyphenylacetic acid (homoveratric acid), 3,4-dimethoxy-2-phenylethylamine (homoveratrylamine), and 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid) were supplied from Sigma Chemical Company Ltd (Poole, Dorset, United Kingdom). Acetonitrile and tetrahydrofuran (THF) both HPLC grade were supplied from Rathburn Chemicals Ltd (Walkeburn, Scotland), 3,4-dimethoxyphenylethanol was purchased from Acros Organics (Geel, Belgium), and perchloric acid from BDH Chemicals Ltd (Poole, Dorset, United Kingdom). 3,4-Dimethoxyphenylacetaldehyde was prepared enzymatically as a bisulphate complex from homovanillamine with purified human monoamine oxidase B (Sigma-Aldrich Inc, Saint Louis, USA), and subsequently extracted with ether to yield the free aldehyde [7].

Preparation of aldehyde oxidase from guinea pig liver

Centrifugations were carried out using an MSE High Speed 18 centrifuge fitted with an angled rotor at 4°C. Guinea pigs were killed by cervical dislocation, their livers were immediately excised and placed in 3-4 volumes of ice-cold isotonic potassium chloride solution (1.15% KCl w/v). They were homogenized in a Tri-R stir-R Model S63C homogenizer with a Teflon pestle and the partially purified aldehyde oxidase was then prepared as described by Panoutsopoulos and Beedham [10].

Preparation of mitochondrial monoamine oxidase from guinea pig liver

Centrifugations at 100,000×g were carried out at 4°C using a Beckman L265-B preparative ultra-centrifuge fitted with a swing head SW28, and all other lower speed centrifugations were carried out using an MSE High Speed 18 centrifuge.

Guinea pigs were killed by cervical dislocation, their livers were immediately excised and placed in sucrose solution (2.5×10⁻¹ M) to yield a 20% suspension. The mixture was homogenized in a Tri-R stir-R Model S63C homogenizer with a Teflon pestle

and the mitochondrial monoamine oxidase was then prepared as described by Panoutsopoulos et al [2].

Protein content estimation in enzymatic preparations

Protein content in aldehyde oxidase and monoamine oxidase fractions was determined according to the method of Bradford [25] by using Coomassie Blue dye reagent (Pierce, Rockford, Illinois, USA) and bovine serum albumin as the standard, measured at 595 nm.

A) HPLC analysis of 3,4-dimethoxy-2-phenylethylamine and its metabolite. Reverse-phase HPLC was performed using a system supplied from Waters Associates (Northwich, Cheshire, United Kingdom), which consisted of a single piston reciprocating 501 pump, a WISP 710B auto-injector, a Lambda-Max 481 LC spectrophotometer and a Data Module 740. Separation of compounds was achieved using a stainless steel Hypersil ODS column 5 μ m (25 cm $\times$ 4.6 mm internal diameter). A Waters Guard Pak precolumn with a C18 insert was fitted to the HPLC system, in order to avoid contamination of the column with any biological materials. The mobile phase used was 15% acetonitrile/ 1% THF/ 84% of 2.2×10^{-1} M orthophosphate buffer, pH 2.9, containing 1.1×10^{-1} M diethylamine (v/v) at a flow rate of 1.5 ml/min. The mobile phase was filtered and degassed for 10 min under vacuum before use. All solvents were maintained at 4°C in order to obviate any temperature fluctuations in the laboratory. The reactions were monitored at 278 nm and 20 μ l samples were injected for each analysis. 3,4-Dimethoxy-2-phenylethylamine and its possible metabolites were identified by comparison of their HPLC retention times with those of authentic standards.

B) Enzymatic preparation of 3,4-dimethoxyphenylacetaldehyde. In a total volume of 3 ml, 3,4-dimethoxy-2-phenylethylamine (1×10^{-3} M) was incubated with 12.9 mg guinea pig liver monoamine oxidase in oxygenated Krebs-Henseleit buffer, pH 7.4, containing 2.4×10^{-2} M bicarbonate solution at 37°C in a shaking water bath. Aliquots (0.2 ml) were removed at the zero time point and after the indicated time intervals, mixed with 0.1 ml of 3.6% perchloric acid (v/v) and centrifuged for 2.5 min at maximum speed on a Beckman microfuge B. The supernatant was then directly analyzed by HPLC.

C) Production of the acid metabolite from 3,4-dimethoxy-2-phenylethylamine by concurrent incubation with monoamine oxidase and aldehyde oxidase. In a total volume of 3 ml, 3,4-dimethoxy-2-phenylethylamine (1×10^{-3} M) was concurrently incubated with 10.3 mg guinea pig liver monoamine oxidase and 2.67 mg guinea pig liver aldehyde oxidase in oxygenated Krebs-Henseleit buffer, pH 7.4, containing 2.4×10^{-2} M bicarbonate solution at 37°C in a shaking water bath. Aliquots were removed, treated and analyzed as in enzymatic preparation of 3,4-dimethoxyphenylacetaldehyde by monoamine oxidase. Control incubations without the enzymes were also performed in parallel.

Preparation of freshly prepared and cryopreserved guinea pig liver slices

Guinea pigs were killed by cervical dislocation, their livers

were immediately excised and placed in ice-cold Krebs-Henseleit solution pH 7.4 containing 2.4×10^{-2} M bicarbonate, which was continuously purged with 95% O₂/5% CO₂. Freshly prepared liver slices were obtained using a manual slicer as described by Panoutsopoulos et al [26]. The first and last slices from each core were discarded since they contained the liver capsule. The freshly prepared liver slices (liver slice thickness was 0.5 mm) were stored at 4°C in Krebs-Henseleit buffer pH 7.4 containing 2.4×10^{-2} M bicarbonate and saturated with oxygen/carbon dioxide until required. Cryopreserved liver slices were then prepared by direct immersion of the freshly prepared liver slices into liquid nitrogen and kept there until needed.

Metabolism of 3,4-dimethoxy-2-phenylethylamine by freshly prepared and cryopreserved liver slices

3,4-Dimethoxy-2-phenylethylamine (1×10^{-3} M) was incubated with four freshly prepared or cryopreserved liver slices in a total volume of 3 ml Krebs-Henseleit buffer pH 7.4, the addition of the liver slices indicated the beginning of the experiment. The incubations were carried out at 37°C in a shaking water bath and the medium was oxygenated with 95% O₂/5% CO₂ initially and every subsequent hour for 5 min, which was sufficient to maintain optimum metabolite production.

Aliquots were removed, treated and analyzed as in enzymatic preparation of 3,4-dimethoxyphenylacetaldehyde by monoamine oxidase. After analysis, the slices were blotted dry and weighed to determine the total weight of liver used. Control incubations without the addition of liver slices present were also performed in parallel.

3,4-Dimethoxy-2-phenylethylamine incubations with freshly prepared liver slices were also performed in the presence of the inhibitors, isovanillin for aldehyde oxidase [10-12], allopurinol for xanthine oxidase [19] and disulfiram for aldehyde dehydrogenase activity [20, 21], whereas 3,4-dimethoxy-2-phenylethylamine incubations with cryopreserved liver slices were performed in the presence of the inhibitor isovanillin. For comparison reasons, the results on the effect of inhibitors in freshly prepared liver slices have been normalized per 100 mg of liver.

Results

Enzymatic preparation of 3,4-dimethoxyphenylacetaldehyde from 3,4-dimethoxy-2-phenylethylamine by monoamine oxidase

A progressive decrease in 3,4-dimethoxy-2-phenylethylamine concentration (Retention Time, Rt= 4.4 ± 0.2 min, n= 14) was observed with time, which continued until it had almost disappeared within 180 min (Fig. 1). Simultaneously, three metabolites were observed. The major metabolite (Rt= 32 ± 0.8 min, n= 14) corresponded to 3,4-dimethoxyphenylacetaldehyde (Fig. 1), whereas the

Fig. 1. Generation of 3,4-dimethoxyphenylacetaldehyde from 3,4-dimethoxy-2-phenylethylamine catalyzed by guinea pig liver monoamine oxidase. 3,4-Dimethoxy-2-phenylethylamine (1×10^{-3} M) was incubated with 12.9 mg guinea pig liver mitochondrial monoamine oxidase in Krebs-Heinseleit buffer pH 7.4 containing 2.4×10^{-2} M bicarbonate to a total volume of 3 ml at 37°C . Each point represents the mean of five determinations originating from independent experiments. Values are expressed as means $\pm$ SE.

Fig. 2. Production of 3,4-dimethoxyphenylacetic acid from 3,4-dimethoxy-2-phenylethylamine catalyzed by mitochondrial monoamine oxidase and aldehyde oxidase from guinea pig liver. 3,4-Dimethoxy-2-phenylethylamine (1×10^{-3} M) was concurrently incubated with 10.3 mg guinea pig liver mitochondrial monoamine oxidase and 2.67 mg aldehyde oxidase in Krebs-Heinseleit buffer pH 7.4 containing 2.4×10^{-2} M bicarbonate to a total volume of 3 ml at 37°C . Each point represents the mean of five determinations originating from independent experiments. Values are expressed as means $\pm$ SE.

two minor metabolites were identified as 3-methoxy-4-hydroxyphenylacetic acid ($R_t = 10.4 \pm 0.2$ min, $n = 14$) and 3,4-dimethoxyphenylacetic acid ($R_t = 25.3 \pm 0.6$ min, $n = 14$) (Fig. 1).

Production of 3,4-dimethoxyphenylacetic acid from 3,4-dimethoxy-2-phenylethylamine by concurrent incubation with monoamine oxidase and aldehyde oxidase

An approximately 100% reduction in the concentration of 3,4-dimethoxy-2-phenylethylamine was

observed over the 90 min incubation period, which was simultaneously transformed into two metabolites. The major metabolite was 3,4-dimethoxyphenylacetic acid, which accounted for most of the conversion of the amine (Fig. 2). The second metabolite was 3,4-dimethoxy-2-phenylethanol ($R_t = 19.4 \pm 0.3$ min, $n = 14$) which slowly increased with time (Fig. 2).

Metabolism of 3,4-dimethoxy-2-phenylethylamine by freshly prepared guinea pig liver slices

In freshly prepared guinea pig liver slice incubations,

Fig. 3. Metabolism of 3,4-dimethoxy-2-phenylethylamine by freshly prepared guinea pig liver slices. 3,4-Dimethoxy-2-phenylethylamine ($1 \times 10^{-3} \text{M}$) was incubated with four freshly prepared guinea pig liver slices (120 mg) in Krebs-Heinseleit buffer pH 7.4 containing $2.4 \times 10^{-2} \text{M}$ bicarbonate to a total volume of 3 ml at 37°C . Each point represents the mean of seven determinations originating from independent experiments. Values are expressed as means $\pm$ SE.

Fig. 4. Pharmacological characterization of inhibitors on 3,4-dimethoxy-2-phenylethylamine oxidation by freshly prepared guinea pig liver slices. 3,4-Dimethoxy-2-phenylethylamine ($1 \times 10^{-3} \text{M}$) was incubated in the absence of inhibitors and in the presence of (a) disulfiram ($1 \times 10^{-4} \text{M}$), (b) isovanillin ($1 \times 10^{-3} \text{M}$) and (c) allopurinol ($1 \times 10^{-4} \text{M}$), with four guinea pig liver slices in 3 ml of Krebs-Heinseleit buffer pH 7.4 containing $2.4 \times 10^{-2} \text{M}$ bicarbonate to a total volume of 3 ml at 37°C . Each point represents the mean of six determinations originating from independent experiments. Values are expressed as means $\pm$ SE.

3,4-dimethoxy-2-phenylethylamine concentration was steadily decreased with time until it had been completely converted within 180 min (Fig. 3). A single metabolite, 3,4-dimethoxyphenylacetic acid, was formed which accounted for 92% ($t = 180 \text{ min}$) of the 3,4-dimethoxy-2-phenylethylamine conversion (Fig. 3). Thus, 3,4-dimethoxy-2-phenylethylamine is quantitatively converted to 3,4-dimethoxyphenylacetic acid by freshly prepared guinea pig liver slices.

Effect of inhibitors on 3,4-dimethoxy-2-phenylethylamine metabolism by freshly prepared guinea pig liver slices

In the presence of isovanillin ($1 \times 10^{-3} \text{M}$), 3,4-dimethoxy-2-phenylethylamine metabolism by guinea pig liver slices was significantly reduced with complete inhibition of 3,4-dimethoxyphenylacetic acid formation up to 15 min (data not shown) and approximately 82% (Fig. 4), 75% (data not shown) and 70% (data not shown)

Fig. 5. Metabolism of 3,4-dimethoxy-2-phenylethylamine by cryopreserved guinea pig liver slices. 3,4-Dimethoxy-2-phenylethylamine ($1 \times 10^{-3} \text{M}$) was incubated with four cryopreserved guinea pig liver slices (117 mg) in Krebs-Heinseleit buffer pH 7.4 containing $2.4 \times 10^{-2} \text{M}$ bicarbonate to a total volume of 3 ml at 37°C . Each point represents the mean of six determinations originating from independent experiments. Values are expressed as means $\pm$ SE.

Fig. 6. Effect of isovanillin on 3,4-dimethoxy-2-phenylethylamine oxidation by cryopreserved guinea pig liver slices. 3,4-Dimethoxy-2-phenylethylamine ($1 \times 10^{-3} \text{M}$) was incubated with four cryopreserved guinea pig liver slices (124 mg) in the presence of isovanillin ($1 \times 10^{-3} \text{M}$) in Krebs-Heinseleit buffer pH 7.4 containing $2.4 \times 10^{-2} \text{M}$ bicarbonate to a total volume of 3 ml at 37°C . Each point represents the mean of six determinations originating from independent experiments. Values are expressed as means $\pm$ SE.

inhibition at 30, 45 and 60 min, respectively. However, after 60 min most of the isovanillin had been converted into isovanillic acid and therefore, less inhibition in 3,4-dimethoxyphenylacetic acid production was observed after this time (data not shown). Two other minor metabolites, 3-methoxy-4-hydroxyphenylacetic acid and 3,4-dimethoxy-2-phenylethanol, were also present which slowly increased with time. Alcohol metabolites of vanillin, isovanillin and protocatechuic aldehyde were also

produced when these compounds were incubated with liver slices and disulfiram or isovanillin [3, 11, 27].

The addition of disulfiram ($1 \times 10^{-4} \text{M}$) also inhibited the formation of 3,4-dimethoxyphenylacetic acid by 85% (Fig. 4). Two other metabolites, 3-methoxy-4-hydroxyphenylacetic acid and 3,4-dimethoxy-2-phenylethanol, were also observed in the incubations which both increased with time (Fig. 4).

The presence of the inhibitor, allopurinol ($1 \times 10^{-4} \text{M}$), had a lower inhibitory effect on the oxidation of 3,4-dimethoxy-2-phenylethylamine to 3,4-dimethoxyphenylacetic acid in liver slices with the rate of 3,4-dimethoxyphenylacetic acid production been inhibited by about 25-30 % (Fig. 4).

Metabolism of 3,4-dimethoxy-2-phenylethylamine by cryopreserved guinea pig liver slices

Incubations of 3,4-dimethoxy-2-phenylethylamine were also performed with cryopreserved guinea pig liver slices, which contain low aldehyde dehydrogenase activity [8] in order to elucidate the potential role of aldehyde oxidase in the metabolism of its corresponding aldehyde. These gave similar profiles to that with freshly prepared liver slices but with a lower rate of acid production (Fig. 5). There were, however, some minor metabolites produced in cryopreserved liver slice incubations. These were identified as 3,4-dimethoxyphenylacetaldehyde, 3,4-dimethoxy-2-phenylethanol and 3-methoxy-4-hydroxyphenylacetic acid which appeared 10 min, 15 min and 30 min respectively after the initiation of the incubation and gradually increased with time (Fig. 5).

Effect of isovanillin on 3,4-dimethoxy-2-phenylethylamine metabolism by cryopreserved guinea pig liver slices

In the presence of isovanillin ($1 \times 10^{-3} \text{M}$), upon incubating 3,4-dimethoxy-2-phenylethylamine with cryopreserved guinea pig liver slices, the breakdown of 3,4-dimethoxy-2-phenylethylamine was inhibited by about 45-70%. However, the production of its metabolite 3,4-dimethoxyphenylacetic acid was inhibited to a much greater extent by about 75% (Fig. 6). Moreover, there was an increase in the formation of the other metabolites, 3,4-dimethoxyphenylacetaldehyde, 3,4-dimethoxy-2-phenylethanol and 3-methoxy-4-hydroxyphenylacetic acid (Fig. 6).

Discussion

It is known that the oxidation of endogenous and exogenous aldehydes is usually attributed to aldehyde dehydrogenases which catalyze the irreversible pyridine nucleotide-dependent oxidation into their corresponding acids with NAD^+ serving as the hydrogen acceptor [8, 13, 14]. Also aldehyde oxidase and xanthine oxidase catalyze the oxidation of a wide range of aromatic aldehydes, however, in a pyridine nucleotide-independent

manner by utilizing molecular oxygen as the electron acceptor [2, 3, 10-12, 27].

Here it was shown that 3,4-dimethoxy-2-phenylethylamine was converted into its aldehyde derivative in a monoamine oxidase-catalyzed reaction. Houslay and Tipton [18] showed that monoamine oxidase B catalyzes the formation of the intermediate 3,4-dimethoxyphenylacetaldehyde from the parent amine. In addition, they found the K_m value for the reaction of 3,4-dimethoxy-2-phenylethylamine with monoamine oxidase B of rat liver mitochondrial outer membranes ($K_m = 1.4 \times 10^{-3} \text{M}$), suggesting that it is relatively poor substrate of monoamine oxidase. The progressive conversion of 3,4-dimethoxy-2-phenylethylamine reported in the present studies using the isolated enzyme, but also in guinea pig liver slices, is in accordance with the kinetic results found by Houslay and Tipton.

When both monoamine oxidase and aldehyde oxidase were included in the incubations, the results showed that 3,4-dimethoxy-2-phenylethylamine is gradually oxidized to its corresponding acid derivative, via 3,4-dimethoxy-2-phenylacetaldehyde, by aldehyde oxidase. This indicates that the 3,4-dimethoxyphenylacetaldehyde, like 3,4-dimethoxybenzaldehyde [10], is a good substrate of aldehyde oxidase which can play a role in the metabolism of the aldehyde, in addition to other aldehyde oxidizing enzymes which are present in vivo. However, the contribution of each enzyme on the metabolism of 3,4-dimethoxyphenylacetaldehyde will depend on its relative oxidation rate, the concentration of the enzyme within the tissue and the affinity of the aldehyde for each enzyme.

Precision-cut liver slices have been shown to be a powerful in vitro system for investigating drug metabolism, xenobiotic-induced hepatotoxicity, or induction properties of drugs [3, 9, 11, 28, 29]. Since no proteolytic treatment is required for their preparation, the normal tissue architecture, the cell heterogeneity, and cell-to-cell contacts are maintained in slices [29-31]. They resemble the organ which they are derived from, and all cell types are present as in the whole organ in vivo [28-30]. Consequently, the oxidation of 3,4-dimethoxyphenylacetaldehyde has been investigated using freshly prepared liver slices, where a high degree of hepatic lobular architecture of the tissue and intercellular communication are maintained, giving similar conditions to those in vivo.

The metabolism of 3,4-dimethoxy-2-phenylethylamine in guinea pig liver is shown in Fig. 7. It is known [18] that monoamine oxidase B catalyzes the

Fig. 7. Proposed metabolic pathways of 3,4-dimethoxy-2-phenylethylamine by guinea pig liver slices.

formation of the intermediate aldehyde. From the results in the present investigation, this form of monoamine oxidase seems to be active in liver slices. 3,4-Dimethoxy-2-phenylethylamine was converted into its acid metabolite, via the intermediate 3,4-dimethoxyphenylacetaldehyde, by freshly prepared guinea pig liver slices. However, in the presence of the inhibitors three other minor metabolites, 3,4-dimethoxyphenylacetaldehyde, 3,4-dimethoxy-2-phenylethanol and 3-methoxy-4-hydroxyphenylacetic acid, were observed in addition to its acid derivative (Fig. 7).

Isovanillin, a specific inhibitor of aldehyde oxidase [10-12], inhibited 3,4-dimethoxyphenylacetic acid production by about 70-80%. This indicates that aldehyde oxidase activity may be a significant factor in the oxidation of 3,4-dimethoxyphenylacetaldehyde. This assumption is confirmed with cryopreserved liver slices where aldehyde dehydrogenase activity is lower by 70% [8], whereas molybdenum hydroxylase activity is not affected. Under these conditions, 3,4-dimethoxyphenylacetic acid production was about half the concentration of that

produced in freshly prepared liver slices, which was significantly inhibited (75% inhibition) in the presence of isovanillin throughout the 180 min incubation period. This is a further indication of the involvement of aldehyde oxidase in the oxidation of 3,4-dimethoxyphenylacetaldehyde in guinea pig liver. Additionally, the spectrophotometric results [10] showed that 3,4-dimethoxybenzaldehyde, an analogue of 3,4-dimethoxyphenylacetaldehyde, is a good substrate of aldehyde oxidase but only a poor substrate of xanthine oxidase. This supports the contention that the equivalent phenylacetaldehyde compound might also be a good substrate of aldehyde oxidase, which thus contributes to its metabolism in guinea pig liver slices.

However, when disulfiram was present in the incubation mixture with freshly prepared liver slices, the formation of 3,4-dimethoxyphenylacetic acid was greatly inhibited (85% inhibition). Since disulfiram is a relatively specific inhibitor of aldehyde dehydrogenase [20, 21], it is suggested that aldehyde dehydrogenase also plays an

important role in the 3,4-dimethoxy-2-phenylethylamine metabolism in liver slices. This indicates that aldehyde dehydrogenase also contributes to the formation of 3,4-dimethoxyphenylacetic acid in guinea pig liver slices. This is also suggested by the cryopreserved liver slice incubations as formation of 3,4-dimethoxyphenylacetic acid was approximately half of that formed in fresh liver slices due to its low aldehyde dehydrogenase activity [8].

In contrast, allopurinol caused a much lower inhibition in acid production (25-30% inhibition) indicating that xanthine oxidase also catalyzes this reaction, but plays a less important role in the metabolism of 3,4-dimethoxyphenylacetaldehyde. This is further supported by its analogue 3,4-dimethoxybenzaldehyde, which was found to be only a poor substrate of xanthine oxidase [10]. In addition, previous studies have been shown that the contribution of xanthine oxidase to aromatic aldehydes in liver slices is minimal, due to the low concentrations of

the enzyme being present in guinea pig liver [3, 12, 27]. However, the present investigation has shown that xanthine oxidase plays a role, although to a much lower extent, in the oxidation of 3,4-dimethoxyphenylacetaldehyde.

In conclusion, the results of this study have shown that 3,4-dimethoxy-2-phenylethylamine is metabolized to its intermediate 3,4-dimethoxyphenylacetaldehyde by monoamine oxidase, which is further oxidized mainly by both aldehyde oxidase and aldehyde dehydrogenase with a much lower contribution by xanthine oxidase. It would therefore appear that both aldehyde dehydrogenase and aldehyde oxidase not only contribute to the metabolism of xenobiotic aldehydes derived from drugs, xenobiotics, foods and flavourings, but also to the metabolism of the biogenic aldehydes derived from biogenic amines, such as 3,4-dimethoxy-2-phenylethylamine.

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