Metabolism of Homovanillamine to Homovanillic Acid in Guinea Pig Liver Slices

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Key Words

Aldehyde oxidase • Aldehyde dehydrogenase • Allopurinol • Disulfiram • Isovannillin • Liver slices • Monoamine oxidase • Homovanillamine • Homovanillic acid • Xanthine oxidase

Abstract

Background/Aims: Homovanillamine is a biogenic amine that it is catalyzed to homovanillyl aldehyde by monoamine oxidase A and B, but the oxidation of its aldehyde to the acid derivative is usually ascribed to aldehyde dehydrogenase and a potential contribution of aldehyde oxidase and xanthine oxidase is usually ignored. **Methods:** The present investigation examines the metabolism of homovanillamine to its acid derivative by concurrent incubation with monoamine oxidase and aldehyde oxidase. In addition, the metabolism of homovanillamine in freshly prepared and cryopreserved liver slices is examined and the relative contribution of aldehyde oxidase, xanthine oxidase and aldehyde dehydrogenase activity by using specific inhibitors of each oxidizing enzyme is compared. **Results:** Homovanillamine was rapidly converted mainly to homovanillic acid when incubated with both monoamine oxidase and aldehyde oxidase. Homovanillic acid was also the main metabolite in the

incubations of homovanillamine with freshly prepared or cryopreserved liver slices, via the intermediate homovanillyl aldehyde. The acid formation was 70-75 % inhibited by disulfiram (specific inhibitor of aldehyde dehydrogenase), whereas isovanillin (specific inhibitor of aldehyde oxidase) inhibited acid formation to a lesser extent (50-55 %) and allopurinol (specific inhibitor of xanthine oxidase) had almost no effect. **Conclusions:** Homovanillamine is rapidly oxidized to its acid, via homovanillyl aldehyde, by aldehyde dehydrogenase and aldehyde oxidase with little or no contribution from xanthine oxidase.

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Introduction

Monoamine oxidase (MAO; EC 1.4.3.4) is a flavin-adenine dinucleotide-containing enzyme that is located in the outer mitochondrial membrane. Although monoamine oxidase activity is distributed in several mammalian tissues, its highest levels are found in the liver [1]. The enzyme exists in two different forms in mammalian tissues, MAO A and MAO B, which differ in their substrate and inhibitor specificities [2] as well as in tissue and cell

distribution [3]. Monoamine oxidase catalyzes the oxidative deamination of a variety of exogenous and endogenous amines, such as dietary amines and neurotransmitters, to their corresponding aldehydes [2].

Sequential oxidation of aldehydes is generally attributed to the cytosolic or mitochondrial aldehyde dehydrogenases [4, 5] and the contribution of other aldehyde oxidizing enzymes such as aldehyde oxidase and xanthine oxidase is unknown. However, the importance of aldehyde oxidase in the oxidation of aromatic aldehydes has recently been established [6-10].

Aldehyde dehydrogenase (EC 1.2.1.3; aldehyde-NAD(P)⁺ oxidoreductase) catalyzes the irreversible oxidation of a broad range of aldehydes to their corresponding acids in an NAD⁺-dependent reaction [11-13]. Although aldehyde dehydrogenase activity is distributed in all mammalian tissues, its maximum activity is found in the liver [14] with different isozymes present in mitochondria [15], cytosol [16] and microsomes [17].

In addition, aldehyde oxidation is also catalyzed by aldehyde oxidase (EC 1.2.3.1; aldehyde-oxygen oxidoreductase) and xanthine oxidase (EC 1.2.3.2; xanthine-oxygen oxidoreductase) [6-8]. These enzymes are more widely known for their role in the metabolism of heterocyclic compounds [18, 19]. Nevertheless, aromatic aldehydes are excellent substrates of hepatic aldehyde oxidase with lower activities towards xanthine oxidase [6, 20]. Vanillin (4-hydroxy-3-methoxybenzaldehyde) is rapidly oxidized by guinea pig liver aldehyde oxidase whereas its structural isomer, isovanillin (3-hydroxy-4-methoxybenzaldehyde), is a potent competitive inhibitor [6, 7].

It has been shown that vanillin [6-8], a stable analogue of 4-hydroxy-3-methoxyphenylacetaldehyde (homovanillyl aldehyde), is an excellent substrate for guinea pig liver aldehyde oxidase, and was thought that this enzyme might also be important in the metabolism of homovanillamine.

Homovanillamine is metabolized by both MAO A and B to homovanillyl aldehyde [21], which can then be converted to homovanillic acid by either aldehyde dehydrogenase or possibly by aldehyde oxidase and xanthine oxidase.

Since homovanillyl aldehyde is not commercially available, it can be prepared enzymatically from homovanillamine as a bisulphate complex using rat liver monoamine oxidase [5]. Subsequent extraction with ether yields the free aldehyde. However, this aldehyde is relatively unstable and it was found that the bisulphite has an inhibitory effect on aldehyde oxidase.

Consequently, homovanillyl aldehyde has been generated in situ from homovanillamine with guinea pig liver monoamine oxidase in the presence of aldehyde oxidase.

In this investigation the metabolism of homovanillamine in guinea pig liver was investigated. Guinea pig liver was chosen in this study as aldehyde oxidase has similar specificity to the human liver enzyme [22, 23] and therefore guinea pig is a good model for in vivo and in vitro screening of human aldehyde oxidase activity. The amine was concurrently incubated with guinea pig liver monoamine oxidase and aldehyde oxidase. Homovanillamine incubations were also performed with freshly prepared or cryopreserved guinea pig liver slices both in the absence and in the presence of specific inhibitors for each aldehyde oxidizing enzyme. The inhibitors used were isovanillin for aldehyde oxidase, allopurinol for xanthine oxidase and disulfiram for aldehyde dehydrogenase. Therefore, the overall contribution of each aldehyde oxidizing enzyme in guinea pig liver towards aromatic aldehydes derived from biogenic amines was investigated and the role of aldehyde oxidase towards the oxidation of biogenic derived aldehydes was elucidated.

Materials and Methods

Animals

Dunkin-Hartley female guinea pigs, weighing 450-950 g, were used in this study. They were fed with FD1 pellets supplemented with ascorbic acid and received hay three times weekly. All animals had free access to food and tap water and housed in a strictly controlled temperature (18±1°C) with 50 % humidity and a 12h light/12h dark cycle. The animals were handled with humane care in accordance with the National Institutes of Health guidelines.

Chemicals

Tetraethylthiuram disulfide (disulfiram), allopurinol, 4-hydroxy-3-methoxy-2-phenylethanol (homovanillyl alcohol), 3-hydroxy-4-methoxybenzaldehyde (isovanillin), diethylamine, 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid), 4-hydroxy-3-methoxy-2-phenylethylamine (homovanillamine), 3-methoxy-4-hydroxy-2-phenyl-2-hydroxyethylamine (normetanephine), 3,4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxybenzyl alcohol (vanillyl alcohol) were supplied from Sigma Chemical Company Ltd. Acetonitrile and tetrahydrofuran (THF) both HPLC grade were supplied from Rathburn Chemicals Ltd and perchloric acid was purchased from BDH Chemicals Ltd.

Preparation of partially purified 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 placed in 3-4 volumes of ice-cold isotonic potassium chloride solution (1.15 % KCl w/v) and they were homogenized in a Tri-R stir-R Model S63C homogenizer with a Teflon pestle. The partially purified aldehyde oxidase was then prepared according to the method of Panoutsopoulos and Beedham [6].

Preparation of mitochondrial monoamine oxidase from guinea pig liver

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

Guinea pigs were killed by cervical dislocation and their livers were placed in sucrose solution (2.5×10^{-1} M) to yield a 20 % suspension. The mixture was then homogenized in a Tri-R stir-R Model S63C homogenizer with a Teflon pestle. Mitochondrial monoamine oxidase was then prepared according to the method of Panoutsopoulos et al [10].

Protein content in aldehyde oxidase and monoamine oxidase fractions was determined with Coomassie Blue dye reagent (Pierce, Rockford, Illinois, USA) and bovine serum albumin as the standard, measured at 595 nm, according to the method of Bradford [24].

HPLC analysis of homovanillamine and its metabolites

Reverse-phase HPLC was carried out using a system 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. Chromatographic separation of compounds was achieved using a stainless steel Hypersil ODS column 5 μ m (25 cm×4.6 mm internal diameter) with a Waters μ Bondapak C18 Guard-Pak insert and 8.5 % acetonitrile/ 1 % tetrahydrofuran/ 90.5 % 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. The reactions were monitored at 280 nm and 20 μ l samples were injected for each analysis. Homovanillamine and its possible metabolites were identified by comparison of their HPLC retention times with those of authentic standards.

Concurrent incubation of homovanillamine with monoamine oxidase and aldehyde oxidase for the production of the acid metabolites

In a total volume of 3 ml, homovanillamine (1×10^{-3} M) was concurrently incubated with 19.56 mg guinea pig liver monoamine oxidase and 2.67 mg 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 (0.2 ml) were removed at 0 min and at various time intervals, mixed with 0.1 ml 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. Standard solutions of homovanillamine and its

metabolites were also analyzed by HPLC. Control incubations omitting 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 according to the method of Panoutsopoulos et al [25]. The first and last slices from each core were discarded since they contained the liver capsule. The freshly prepared liver slices (each liver slice had a thickness of 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.

Incubation conditions with freshly prepared and cryopreserved liver slices

Homovanillamine (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 to facilitate mixing 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 (0.2 ml) were removed at various time intervals, treated with 0.1 ml of 3.6 % perchloric acid and centrifuged for 2.5 min at maximum speed on a Beckman microfuge B and the supernatant was then analyzed by HPLC. Control incubations without the addition of liver slices present were also performed in parallel. Standard solutions of the amine and its possible metabolites were also analyzed by HPLC. After analysis, the slices were blotted dry and weighed to determine the total weight of liver used.

Homovanillamine incubations with freshly prepared liver slices were also performed in the presence of the inhibitors, isovanillin for aldehyde oxidase [6, 7], allopurinol for xanthine oxidase [26] and disulfiram for aldehyde dehydrogenase activity [27, 28]. For comparison reasons, the results on the effect of inhibitors in freshly prepared liver slices have been normalized per 100 mg of liver. Finally, homovanillamine incubations with cryopreserved liver slices in the presence of the inhibitor isovanillin were performed.

Results

Concurrent incubation of homovanillamine with guinea pig liver monoamine oxidase and aldehyde oxidase

When homovanillamine (Retention time, $R_t = 3.7 \pm 0.1$ min, $n = 8$) was incubated in the presence of both

guinea pig liver monoamine oxidase and aldehyde oxidase, a 100 % reduction in the amine concentration was observed over the 120 min incubation period, which was simultaneously transformed into three metabolites. The major metabolite was homovanillic acid ($R_t = 22.4 \pm 1.0$ min, $n = 8$), which accounted for almost quantitative conversion of the amine (Fig. 1). The second metabolite was homovanillyl alcohol ($R_t = 15.0 \pm 0.4$ min, $n = 8$) which gradually increased to its maximum concentration at 30 min and thereafter gradually disappeared (Fig. 1). Finally, the third metabolite with retention time of 2.8 ± 0.1 min ($n = 6$) was slowly increased with time (data not shown) and was thought to be 3-methoxy-4-hydroxy-2-phenyl-2-hydroxyethylamine (normetanephrine), which is formed via the enzyme dopamine β -hydroxylase. Thus the results show that, like vanillin [6, 7], homovanillyl aldehyde is a good substrate for aldehyde oxidase.

Metabolism of homovanillamine with freshly prepared guinea pig liver slices

When homovanillamine was incubated with freshly prepared guinea pig liver slices, a rapid decrease in homovanillamine concentration occurred (Fig. 2) and one major metabolite and two minor metabolites were produced. The major metabolite was homovanillic acid, which accounted for most of the parent amine (Fig. 2). The minor metabolites were slowly formed and were identified as homovanillyl alcohol (Fig. 2) and 3,4-dihydroxyphenylacetic acid ($R_t = 9.4 \pm 0.2$ min, $n = 6$) (data not shown).

Effect of inhibitors on homovanillamine metabolism by freshly prepared guinea pig liver slices

The presence of isovanillin (1×10^{-3} M) in the incubation of homovanillamine in freshly prepared guinea pig liver slices gave a 50-55 % inhibition in the amounts of homovanillic acid produced at 30 and 45 min. After this time, less inhibition was observed (Fig. 3(i), (ii), (iii)) as after 45 min most of the isovanillin had been metabolized into isovanillic acid. Higher concentrations of two other metabolites were noted in the presence of isovanillin. Homovanillyl alcohol, showed a moderate increase (Fig. 3(i), (ii), (iii)), whereas 3,4-dihydroxyphenylacetic acid was significantly increased (data not shown).

In the presence of disulfiram (1×10^{-4} M), the production of homovanillic acid was greatly inhibited by 70-75 %, whereas there was a 7 to 10-fold increase in the formation of homovanillyl alcohol (Fig 3(i), (ii) and (iii)).

Fig. 1. Concurrent incubation of homovanillamine with mitochondrial monoamine oxidase and aldehyde oxidase from guinea pig liver. Homovanillamine (1×10^{-3} M) was incubated with 19.56 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 is the mean of six determinations. Values are expressed as means $\pm$ SE.

Fig. 2. Metabolism of homovanillamine with freshly prepared guinea pig liver slices. Homovanillamine (1×10^{-3} M) was incubated with four freshly prepared guinea pig liver slices (120 mg) 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 is the mean of seven determinations. Values are expressed as means $\pm$ SE.

Incubations of homovanillamine with freshly prepared guinea pig liver slices in the presence of allopurinol (1×10^{-4} M) had similar profiles to those seen without any inhibitors (Fig. 3(i), (ii), (iii)). However, homovanillic acid concentrations were slightly higher than those in the not inhibited incubations.

Metabolism of homovanillamine with cryopreserved guinea pig liver slices

When homovanillamine was incubated with cryopreserved guinea pig liver slices, its concentration rapidly decreased and homovanillic acid was produced (Fig. 4). Breakdown of the amine seemed to be no different to that observed in freshly prepared guinea pig liver slices. However, the production of homovanillic acid only corresponded to approximately 70 % of that seen in freshly prepared liver slice incubations.

Other metabolites observed in the chromatograms were normetanephrine (data not shown) which increased with time and was thought to be formed via dopamine β -hydroxylase and 3,4-dihydroxyphenylacetic acid which decreased with time (data not shown). In addition, two other minor metabolites (Fig. 4) also appeared to be formed in reasonable amounts which co-eluted with homovanillyl alcohol and 4-hydroxy-3-methoxybenzyl alcohol ($R_t = 7.2 \pm 0.1$ min, $n = 6$), respectively.

Effect of isovanillin on homovanillamine metabolism by cryopreserved guinea pig liver slices

The presence of isovanillin (1×10^{-3} M), in cryopreserved liver slice incubations, resulted in a slower breakdown of homovanillamine (Fig. 5) than that observed in absence of any inhibitor. Not surprisingly, isovanillin significantly inhibited the production of homovanillic acid (approximately 75 %) and an increase in homovanillyl alcohol formation was observed. 4-Hydroxy-3-methoxybenzyl alcohol concentrations also increased during the 180 min incubation period (Fig. 5). One other minor metabolite was 3,4-dihydroxyphenylacetic acid which was produced in similar amounts to that observed without isovanillin being present (data not shown).

Discussion

The oxidation of endogenous and exogenous aldehydes is usually attributed to aldehyde dehydrogenases in a NAD^+ -dependent reaction [11-13]. However, both aldehyde oxidase and xanthine oxidase also catalyze the oxidation of a wide range of aromatic aldehydes [6-8], but their potential contribution has largely been ignored. Consequently, the relative importance of these enzymes, compared to that of aldehyde dehydrogenase, in the metabolism of these compounds has only recently been established [6-10].

Fig. 3. Effect of inhibitors on homovanillamine oxidation by freshly prepared guinea pig liver slices. Homovanillamine (1×10^{-3} M) was incubated in the absence of inhibitors and in the presence of (a) isovanillin (1×10^{-3} M), (b) disulfiram (1×10^{-4} M) and (c) allopurinol (1×10^{-4} M), with four guinea pig liver slices in 3 ml of 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 is the mean of six determinations. Values are expressed as means $\pm$ SE.

The results obtained from the incubations where both monoamine oxidase and aldehyde oxidase were included, have shown that homovanillamine is rapidly oxidized to its corresponding acid derivative, via homovanillyl aldehyde, by aldehyde oxidase. This shows that

Fig. 4. Metabolism of homovanillamine with cryopreserved guinea pig liver slices. Homovanillamine ($1 \times 10^{-3} \text{M}$) 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 is the mean of six determinations. Values are expressed as means $\pm$ SE.

Fig. 5. Effect of the inhibitor isovanillin on homovanillamine oxidation by cryopreserved guinea pig liver slices. Homovanillamine ($1 \times 10^{-3} \text{M}$) was incubated with four cryopreserved guinea pig liver slices (124 mg) in the presence of isovanillin ($1 \times 10^{-3} \text{M}$) with four liver slices 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 is the mean of six determinations. Values are expressed as means $\pm$ SE.

homovanillyl aldehyde is a good substrate of aldehyde oxidase. The ability of aldehyde oxidase to catalyze homovanillyl aldehyde oxidation does not necessarily imply that this enzyme will play a major role in vivo when other aldehyde oxidizing enzymes are also present. Therefore, the contribution of these enzymes will depend on the comparative affinity of homovanillyl aldehyde for each enzyme, their relative oxidation rates and the concentration of the enzymes within the tissue under study.

Consequently, the oxidation of homovanillyl aldehyde has been studied 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, and the activities of endobiotic and xenobiotic-metabolizing enzymes are maintained for many hours [29, 30].

The metabolism of homovanillamine in freshly prepared guinea pig liver slices is shown in Fig. 6. It is known that both rat liver monoamine oxidase A and B catalyze the formation of the intermediate homovanillyl aldehyde from the amine [23] and therefore one or both of these isozymes are active in guinea pig liver slices.

From the results, it was seen that homovanillamine was converted to its acid metabolite, via its intermediate homovanillyl aldehyde, by freshly prepared guinea pig liver slices. In addition, small amounts of homovanillyl alcohol and 3,4-dihydroxyphenylacetic acid were also produced. In the presence of allopurinol, acid production was not affected and to the contrary a slight enhancement of the acid metabolite was produced which was similar to that seen in vanillin incubations with freshly prepared guinea pig liver slices [8]. Therefore, xanthine oxidase is not important in the metabolism of homovanillamine. Isovanillin, a specific inhibitor of aldehyde oxidase [6-8], inhibited homovanillic acid production by about 50 % indicating that aldehyde oxidase activity may be a significant factor in the oxidation of homovanillyl aldehyde which is generated from homovanillamine. However, when disulfiram was present in the incubation mixture, the formation of homovanillic acid was greatly inhibited. Since disulfiram is a relatively specific inhibitor of aldehyde dehydrogenase [27, 28], it is suggested that aldehyde dehydrogenase also plays an important role in the homovanillamine metabolism in liver slices. This is also suggested by the cryopreserved liver slice incubations as formation of homovanillic acid was approximately half of that formed in fresh liver slices due to low aldehyde dehydrogenase activity [13]. The presence of isovanillin inhibited homovanillamine oxidation to a greater extent in cryopreserved liver slices than in freshly prepared liver slices. This is probably due to the low activity of aldehyde dehydrogenase (about 70% lower) found after freezing the liver slices in liquid nitrogen [13], whereas molybdenum hydroxylase activity is not affected. This is a further indication of the involvement of aldehyde oxidase in the oxidation of homovanillyl aldehyde in guinea pig liver. In view to the similarity between guinea pig and human liver aldehyde oxidase [21, 22] it is likely that human hepatic aldehyde oxidase may also be important in biogenic amine

Fig. 6. Proposed metabolic pathways of homovanillamine by freshly prepared guinea pig liver slices.

metabolism. Although central dopaminergic activity can be measured from peripheral plasma levels of homovanillic acid (which is a metabolite of dopamine), it has been shown that homovanillic acid is also produced locally from circulating DOPA [31]. Aldehyde oxidase may be the enzyme responsible for the peripheral formation of homovanillic acid which occurs during dopamine metabolism.

Incubation of vanillin with guinea pig liver mitochondrial aldehyde dehydrogenase [7] showed that it is a poor substrate of mitochondrial aldehyde dehydrogenase. This suggests that the equivalent phenylacetaldehyde compound might also be a poor substrate of this form of aldehyde dehydrogenase. However, the results here indicate that an aldehyde dehydrogenase isozyme may be involved in the metabolism of homovanillamine. This could be cytosolic aldehyde dehydrogenase [32], which is more susceptible to disulfiram inhibition than the mitochondrial isozyme.

Incubation of vanillin with aldehyde oxidase [7] and the kinetic data [6] revealed that it is a good substrate of aldehyde oxidase and only a poor substrate of xanthine oxidase [6, 7]. This suggests that homovanillyl aldehyde might also be metabolized by aldehyde oxidase in the integrated system of guinea pig liver slices.

Since there was some acid formed in the incubation of homovanillamine when isovanillin was present and also in incubations when disulfiram was included, it is evident that the oxidation of homovanillyl aldehyde is metabolized by both aldehyde oxidase and aldehyde dehydrogenase.

Catecholamines such as dopamine, noradrenaline and adrenaline and their acid metabolites play an important role in the central and peripheral nervous systems. A number of diseases are related to catecholamines dysfunction such as Parkinson's disease, hypertension, and certain neural tumours such as pheochromocytoma and neuroblastoma. These diseases may be characterized by the release of abnormally high quantities of amines and their metabolites, such as VMA (vanillylmandelic acid) and HVA

(homovanillic acid) in the urine [33, 34]. Therefore, the determination of various catecholamines and their metabolites is very useful in etiological investigations, diagnosis, and the follow-up of catecholamine-related diseases. Quantitative measurement of urinary VMA and HVA levels is a useful indication for diagnosis of neuroblastoma [35]. Furthermore, the ratio VMA/HVA is important for the prognosis after surgical removal of the tumour [36].

In conclusion, the results of this study have shown that homovanillamine is metabolized to its intermediate homovanillyl aldehyde by monoamine oxidase, which is further oxidized by both aldehyde oxidase and aldehyde dehydrogenase in guinea pig liver slices whereas xanthine oxidase did not appear to contribute to its metabolism. Therefore, it would appear that aldehyde oxidase not only contributes to the metabolism of aldehydes derived from drugs, xenobiotics, foods and flavorings, but also to the metabolism of the intermediate aldehyde derivatives from biogenic amines, such as homovanillamine.

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