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METABOLISM OF BIOGENIC ALDEHYDES IN ISOLATED HUMAN BLOOD CELLS, PLATELETS AND IN PLASMA

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Abstract—The metabolism of biogenic aldehydes was measured in different human blood fractions. Isolated erythrocytes, leukocytes, platelets and plasma were incubated with 3,4-dihydroxyphenylacetaldehyde (DOPAL) or 5-hydroxyindole-3-acetaldehyde (5-HIAL), the aldehydes derived from dopamine and 5-hydroxytryptamine, respectively. The disappearance of the aldehydes and the formation of acid and alcohol metabolites were analysed using high-performance liquid chromatography with electrochemical detection. The aldehydes were unstable in phosphate-buffered saline, but this non-enzymatic oxidation was prevented in the presence of EDTA, pyrophosphate or blood tissue. When DOPAL or 5-HIAL were incubated with erythrocytes, only acid metabolites were formed, whereas both acid and alcohol metabolites were formed in incubations with leukocytes or platelets. The amount of the acid metabolite exceeded that of the alcohol metabolite, both with leukocytes and platelets. No metabolites were formed when the aldehydes were incubated in plasma. The oxidation of the aldehydes in incubations with erythrocytes or platelets was totally inhibited in the presence of 50 μM of the aldehyde dehydrogenase inhibitor disulfiram. However, disulfiram did not inhibit the metabolism of DOPAL and 5-HIAL in incubations with leukocytes, suggesting that different isozymes of aldehyde dehydrogenase are present in leukocytes as compared to erythrocytes and platelets.

Biogenic aldehydes are formed by oxidative deamination of biogenic amines by the action of monoamine oxidase (MAO, EC 1.4.3.4). The aldehydes are either oxidized to the corresponding acids by aldehyde dehydrogenase (ALDH, EC 1.2.1.3), or reduced to the corresponding alcohols by aldehyde reductase (ALR, EC 1.1.1.2) or alcohol dehydrogenase (ADH, EC 1.1.1.1). In both brain and peripheral systems of humans, the aldehydes derived from dopamine (DA) and 5-hydroxytryptamine (5-HT, serotonin) are predominantly metabolized via the oxidative pathway, whereas the aldehydes of epinephrine (E) and norepinephrine (NE) are preferentially reduced in the brain but oxidized in peripheral systems [1, 2].

Biogenic amines are normally present in the blood. The catecholamines (DA, E, NE) are released into the circulation from the adrenal medulla and peripheral nerve endings [3, 4], whereas 5-HT originates from the enterochromaffin cells of the gastrointestinal tract [5, 6]. 5-HT, and to some extent also the catecholamines, is actively taken up by platelets and stored within dense granules [7]. During platelet aggregation, 5-HT is released and causes vasoconstriction of vascular smooth muscles and also induces further platelet aggregation [8]. The biogenic amines are removed from the circulation either by uptake into presynaptic nerve terminals, or by uptake into extraneuronal tissues, e.g. vascular smooth muscle [1]. The reuptake into nerve terminals is followed either by accumulation within vesicles or by enzymatic degradation, whereas the extraneuronal uptake normally is followed by enzymatic degradation [1].

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Oxidative deamination of biogenic amines probably occurs in the blood, since MAO activity is present in human platelets [9] and plasma [10]. Recently, Kodaira *et al.* [11] observed the formation of the aldehyde metabolites when DA or 3-methoxytyramine were incubated with rabbit platelets. The further metabolism of the aldehydes, either formed in the blood or possibly released into the circulation from surrounding tissues, is, however, likely to take place within the blood cells or the platelets. ALDH activity has been observed both in human erythrocytes and leukocytes [12], and ALR activity has been detected in erythrocytes [13] and platelets [14]. No aldehyde metabolizing activity has been observed in human plasma.

This study was carried out to examine the metabolism of 3,4-dihydroxyphenylacetaldehyde (DOPAL) and 5-hydroxyindole-3-acetaldehyde (5-HIAL), the aldehydes derived from DA and 5-HT, respectively, in isolated human erythrocytes, leukocytes and platelets as well as in plasma.

MATERIALS AND METHODS

Chemicals. The bisulphite forms of DOPAL and 5-HIAL were prepared enzymatically by the use of rat liver MAO [15]. The free aldehydes were obtained from the bisulphite forms by ether extraction [16]. Dopamine hydrochloride (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine creatinine sulphate complex (5-HT, serotonin), 5-hydroxyindole-3-acetic acid (5-HIAA), 5-hydroxytryptophol (5-HTOH) and 2-propylpentanoic acid (valproate) were obtained from Sigma Chemical Co. (St. Louis, MO). Human serum albumin was obtained from AB Kabi (Stockholm, Sweden). Disulfiram (Fluka AG, Buchs, Switzer-

land) was recrystallized twice from 99.5% ethanol. 3,4-Dihydroxyphenylethanol (DOPET) was obtained from Serva AG (Heidelberg, F.R.G.), 4-methylpyrazole from Labkemi AB (Stockholm, Sweden), EDTA and sodium octylsulphate from Merck AG (Darmstadt, F.R.G.) and Percoll from Pharmacia AB (Uppsala, Sweden).

Fractionation of blood. Fresh human citrate-treated blood was obtained from the University Hospital of Uppsala, Sweden. The blood was centrifuged at 400 *g* for 10 min and erythrocytes and leukocytes were isolated from the blood cell pellet. The supernatant was separated into platelet-rich and platelet-poor plasma. The blood cells were diluted 1:1 with phosphate-buffered saline (PBS, 10 mM NaH_2PO_4 and 10 mM Na_2HPO_4 in 0.15 M NaCl), pH 7.4, and layered on a Percoll separation medium (density 1.092 g/ml) and centrifuged at 800 *g* for 10 min. The erythrocyte pellet obtained was resuspended in Percoll of the same density, and centrifuged at 800 *g* for 10 min. The pellet was resuspended in PBS and centrifuged at 800 *g* for 10 min and this procedure was repeated twice. After the final centrifugation, the cells were resuspended in PBS. The erythrocyte fraction contained less than 0.001% leukocytes, as compared to about 0.15% in whole blood.

Leukocytes were isolated from the buffy coat, diluted 1:1 with PBS, which was layered on Percoll (1.085 g/ml) and centrifuged at 800 *g* for 10 min. The leukocytes, obtained from the top of the separation medium, were resuspended in PBS and layered on Percoll of the same density and centrifuged as above. The leukocytes were then layered on Percoll (1.050 g/ml) to remove platelets (see below). The cells were washed as described for the erythrocytes, and after the final centrifugation the cells were resuspended in PBS. The leukocyte fraction contained less than 3% erythrocytes.

Platelets were isolated from platelet-rich plasma (PRP) obtained as described above. PRP was layered on Percoll (1.050 g/ml) and centrifuged at 400 *g* for 5 min. The platelets were isolated from the top of the separation medium. The platelets were suspended in PBS and centrifuged at 400 *g* for 10 min. This procedure was repeated twice and after the final centrifugation the platelets were resuspended in PBS. The platelet fraction contained less than 0.1% erythrocytes or leukocytes.

Cell-free plasma was prepared by centrifugation at 5000 *g* for 10 min.

Erythrocytes, leukocytes and platelets were counted on a Hycel cell counter HC 333 (Clinicon, Mannheim, F.R.G.) equipped with a multichannel analyser.

Assay conditions. Assays were performed by following the disappearance of the aldehydes and the formation of the acid and alcohol metabolites, when DOPAL or 5-HIAL were incubated with the different blood fractions. Erythrocytes, leukocytes or platelets were suspended in 5 ml of PBS, pH 7.4, containing 50 μM EDTA, and were incubated under gentle shaking in polyethylene flasks at 37° in a water bath. The assay mixture was preincubated for 5 min before addition of the substrate. In assays with enzyme inhibitors, these were added at the start of the preincubation. In assays with human plasma, the aldehydes were incubated in 5 ml of plasma.

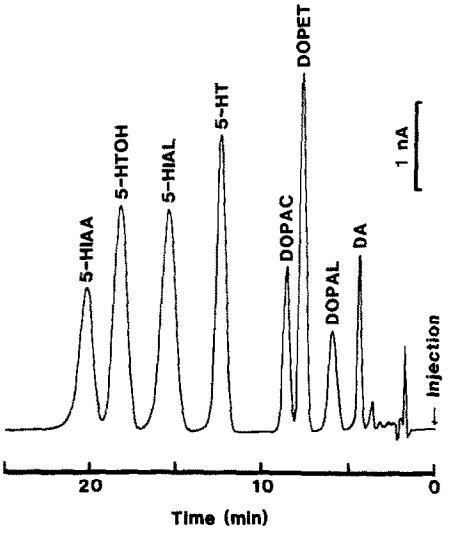

Fig. 1. Separation of DA and 5-HT metabolites by reversed phase HPLC with electrochemical detection. The standard solution contained 1 μM of each compound in 3.4% (w/v) PCA. The sensitivity was set at 5 nA/V. The chromatographic conditions are given in the Materials and Methods.

The assays were started by addition of the aldehyde. Aliquots of 0.5 ml were taken at different times during 90 min of incubation and were immediately deproteinized by mixing with 0.125 ml ice-cold 16.8% (w/v) perchloric acid (PCA). The samples were centrifuged at 20,000 *g* for 10 min and the supernatants were immediately analysed or stored at −80° until analysis. The metabolites were stable for at least one week in samples stored at −80°.

Analyses of metabolites with HPLC. The aldehydes and their acid and alcohol metabolites were analysed using high-performance liquid chromatography (HPLC) with electrochemical detection. The chromatographic system was composed of a Milton Roy Minipump, Mod. 396 (Laboratory Data Control, Riviera Beach, FL) and a Rheodyne injector, Mod. 7125, with a 20 μl sample loop (Rheodyne Inc., Cotati, CA). A Model LC-3 electrochemical detector from Bioanalytical Systems Inc. (West Lafayette, IN) with a glassy carbon working electrode was used, and the potential was set to +0.75 V versus an Ag/AgCl reference electrode. The analytical column was a Nucleosil C_{18} reversed phase column (250 × 4.0 mm i.d., particle size 5 μm) from Macherey-Nagel (Düren, F.R.G.).

The mobile phase was pumped at a flow rate of 1 ml/min and consisted of 105 mM citrate, 2.5% (v/v) methanol, 0.002% (w/v) EDTA and 5 mg/l of sodium octylsulphate, pH 2.2. The mobile phase was filtered under vacuum through a 0.45 μm Millipore HA filter (Millipore Corp., Bedford, MA) and deaerated before use. Chromatography was carried out at room temperature.

The HPLC system made it possible to simultaneously determine DOPAL and 5-HIAL, the acid (DOPAC and 5-HIAA) and alcohol (DOPET and 5-HTOH) metabolites, and also the parent amines, DA and 5-HT respectively (Fig. 1). Standard solu-

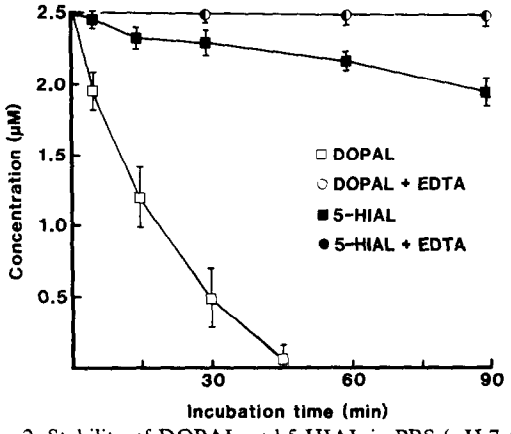

Fig. 2. Stability of DOPAL and 5-HIAL in PBS (pH 7.4, 37°) in the absence and presence of EDTA. Aliquots were taken at different times for HPLC analysis of the aldehydes. Values are the means ± SD (N = 5).

tions (1.0–2.5 μM) were prepared in 3.4% (w/v) PCA and the amounts of the metabolites were calculated from the peak-heights in the chromatograms. Good linearity between peak-heights and amounts of the metabolites was observed in the concentration range used.

RESULTS

Stability of DOPAL and 5-HIAL at different incubation conditions

When 2.5 μM of DOPAL was incubated in PBS at 37°, pH 7.4, no aldehyde could be detected after 60 min of incubation, whereas when 2.5 μM of 5-HIAL was incubated at the same conditions, about 80% remained after 90 min of incubation (Fig. 2). Similar results were obtained in experiments with the parent amines (DA and 5-HT). DOPAL was, however, more stable in the presence of sodium pyrophosphate, and about 90% was recovered after 90 min of incubation in PBS containing 1 mM of sodium pyrophosphate. The recovery of 5-HIAL was unaffected by sodium pyrophosphate. DOPAL and 5-HIAL were more stable at 25° than at 37° and both aldehydes were more stable at pH 7.4 than pH 8.8, and even more stable at pH 6.0. The aldehydes were also less susceptible to destruction in incubation buffers saturated with nitrogen gas. When 50 μM of EDTA was added to the PBS, almost 100% of both DOPAL and 5-HIAL could be detected after 90 min of incubation (Fig. 2).

The initial recovery of DOPAL or 5-HIAL, when 2.5 μM of the aldehydes were added to plasma followed by deproteinization within 15 sec, was about 80% with DOPAL, whereas less than 50% of the 5-HIAL added could be detected. Some 60% of the recovered aldehydes disappeared during the following incubation period, but no acid or alcohol metabolites were formed. However, in experiments with DA and 5-HT, the initial recovery was close to 100% and no disappearance was observed during the incubation. When DOPAL or 5-HIAL were incubated in PBS supplemented with 45 mg/ml of human serum albumin, which corresponds to the amount in human plasma, the initial recovery was almost 100%, both with DOPAL and 5-HIAL. The disappearance of the aldehydes during the incubation did, however, resemble that observed in plasma.

Metabolism of DOPAL and 5-HIAL in human blood fractions

When DOPAL or 5-HIAL were incubated with isolated erythrocytes ($5 \cdot 10^7$ cells/ml), only the acid metabolites, DOPAC and 5-HIAA, respectively, were formed (Fig. 3a and b). Even when the oxidative pathway was totally inhibited by disulfiram, no formation of the reduced metabolite occurred. The recovery of the aldehydes as acid metabolites after 90 min of incubation was about 85–90%. This metabolism was completely inhibited in the presence of 50 μM of the ALDH-inhibitor disulfiram. The aldehydes were very stable in PBS in the presence of intact cells even without EDTA and about 95% of DOPAL and 5-HIAL were recovered after 90 min of incubation with erythrocytes in the presence of disulfiram. This stabilizing effect in the presence of tissue was also observed when the aldehydes were incubated in the presence of isolated blood cell membranes. However, when DOPAL or 5-HIAL were incubated with sonicated erythrocytes, the initial recovery of DOPAL was only about 20% and no 5-HIAL at all could be detected. Furthermore, no formation of DOPAC occurred, unless NAD was added to the assay mixture, but even then the recovery of the acid metabolite was only about 30%, as compared with intact erythrocytes. No formation of 5-HIAA was observed in the presence of NAD. The metabolism of the aldehydes by intact erythrocytes was not limited by the intracellular NAD-concentration, since the formation of the acid metabolites proceeded linearly for 90 min when higher concentrations of the aldehydes were used (10 μM). The aldehyde metabolizing activity of human erythrocytes with DOPAL or 5-HIAL as the substrates was about 50 nmol of DOPAC and 40 nmol of 5-HIAA formed $\cdot hr^{-1} \cdot 10^9$ cells^{-1}, as calculated from the initial velocities in Fig. 3a and b.

When the aldehydes were incubated with intact leukocytes ($5 \cdot 10^7$ cells/ml), both the acid and alcohol metabolites were formed (Fig. 4a and b). The ratio between the acid and alcohol metabolites was about 2:1 with 5-HIAL as the substrate and the recovery of the aldehyde as acid and alcohol metabolites was 85–90% after 90 min of incubation. The ratio between the acid and alcohol metabolites with DOPAL as the substrate was about 15–20:1. The recovery of the DOPAL metabolites was about 80% after 15 min of incubation and the recovery subsequently decreased to about 50% after 90 min of incubation (Fig. 4a). The metabolism of the aldehydes was not limited by the intracellular NAD- or NADPH-concentrations, since the formation of the acid and alcohol metabolites proceeded linearly for at least 30 min when higher concentrations of the aldehydes were used (10 μM). The metabolism, measured after 90 min of incubation, was unaffected in the presence of 50 μM disulfiram. The aldehyde metabolizing activity of human leukocytes with DOPAL as the substrate was about 420 nmol of DOPAC and 25 nmol of DOPET formed $\cdot hr^{-1} \cdot 10^9$ cells^{-1}, and with 5-HIAL as the substrate 125 nmol

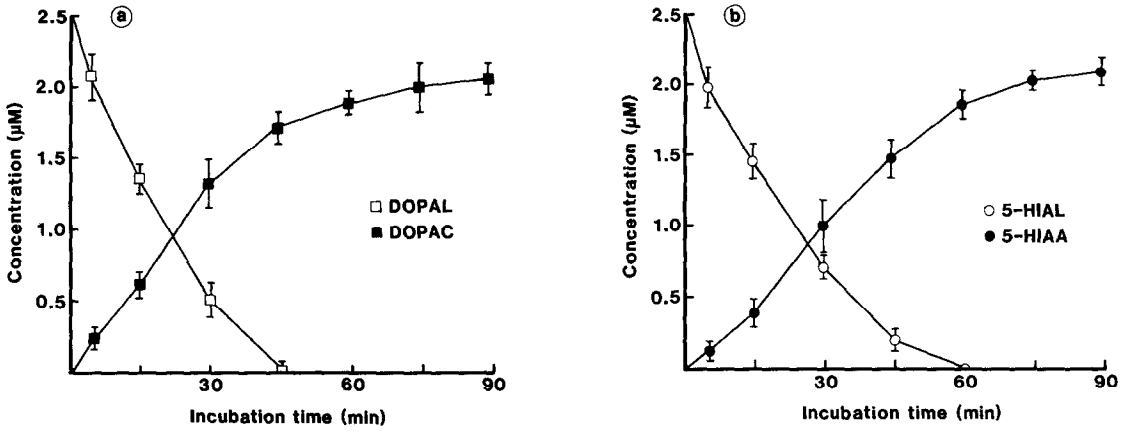

Fig. 3. Metabolism of DOPAL (a) and 5-HIAL (b) in isolated human erythrocytes. The erythrocytes were suspended in PBS, pH 7.4, ($5 \cdot 10^7$ cells/ml) and incubated at 37° with 2.5 μM DOPAL or 5-HIAL. Aliquots were taken at different times for HPLC analysis of metabolites. Values are the means ± SD from duplicate determinations on five different erythrocyte preparations.

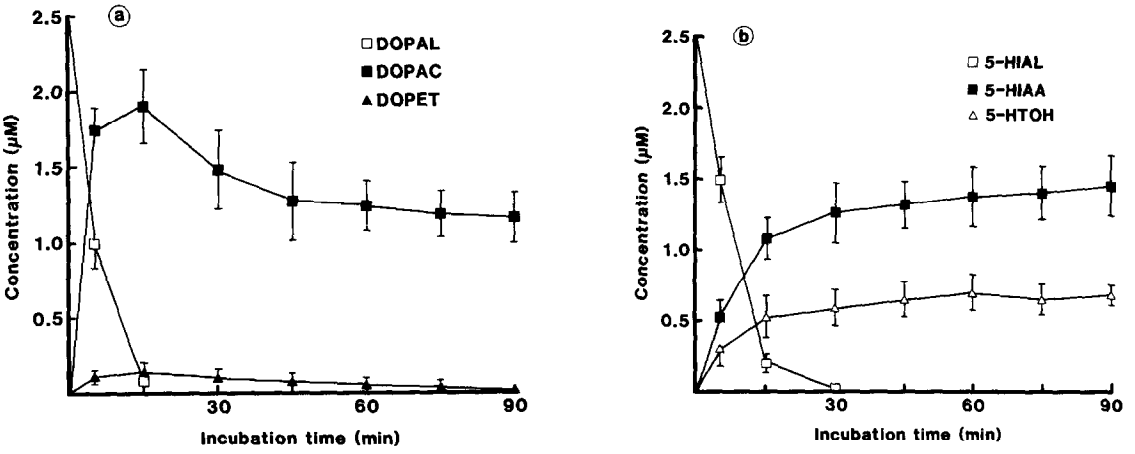

Fig. 4. Metabolism of DOPAL (a) and 5-HIAL (b) in isolated human leukocytes. The leukocytes were suspended in PBS, pH 7.4, ($5 \cdot 10^7$ cells/ml) and incubated at 37° with 2.5 μM DOPAL or 5-HIAL. Aliquots were taken at different times for HPLC analysis of metabolites. Values are the means ± SD from duplicate determinations on five different leukocyte preparations.

of 5-HIAA and 75 nmol of 5-HTOH formed·$hr^{-1} \cdot 10^9$ $cells^{-1}$, as calculated from the initial velocities in Fig. 4a and b.

Incubation of DOPAL or 5-HIAL with isolated platelets ($5 \cdot 10^8$ platelets/ml) also resulted in the formation of both acid and alcohol metabolites (Fig. 5a and b). The ratio between the acid and the alcohol metabolites was about 4:1 and 2:1 with DOPAL and 5-HIAL, respectively. About 85% of the metabolized DOPAL and 5-HIAL were recovered as acid and alcohol metabolites after 90 min of incubation. When platelets were incubated in the presence of 50 μM disulfiram, both the formation of the acid and alcohol metabolites were inhibited. Incubation of intact platelets in the presence of 50 μM of valproate or 4-methylpyrazole, inhibitors of ALR and ADH, respectively, did not affect the metabolism of the aldehydes. The aldehyde metabolizing activity of platelets with DOPAL as the substrate was about 3.0 nmol of DOPAC and 0.7 nmol of DOPET formed·$hr^{-1} \cdot 10^9$ $platelets^{-1}$, and with 5-HIAL as the substrate 2.0 nmol of 5-HIAA and 1.0 nmol of 5-HTOH formed·$hr^{-1} \cdot 10^9$ $platelets^{-1}$, as calculated from the initial velocities in Fig. 5a and b.

As mentioned above, no acid or alcohol metabolites were formed when the aldehydes were incubated in human plasma. When plasma assays were performed in the presence of 50 μM NAD and NADPH, only small amounts of the acid and alcohol metabolites were formed.

DISCUSSION

The biogenic aldehydes were previously considered as inactive degradation intermediates in the metabolism of biogenic amines, since they lacked the biological activities of the parent amines. Renson *et al.* [17] studied the effects of the aldehydes derived from 5-HT and E on smooth muscle preparations from the rat. The aldehydes were found to be taken up and metabolized by the smooth muscle preparations, but they possessed none of the effects of the parent amines, even at concentrations several

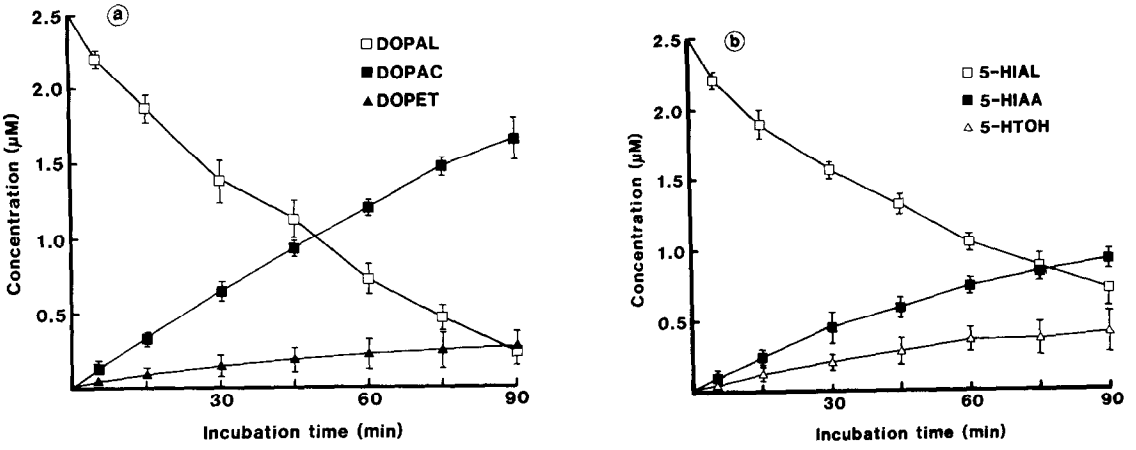

Fig. 5. Metabolism of DOPAL (a) and 5-HIAL (b) in isolated human platelets. The platelets were suspended in PBS, pH 7.4, ($5 \cdot 10^8$ platelets/ml) and incubated at 37° with 2.5 μM DOPAL or 5-HIAL. Aliquots were taken at different times for HPLC analysis of metabolites. Values are the means ± SD from duplicate determinations on five different platelet preparations.

hundred times higher than of the amines. However, it has been suggested that the biogenic aldehydes possess distinct physiological properties of their own [2, 18]. They may be involved in the regulation of sleep [19] and also in the pharmacological actions of ethanol [2, 20]. It has recently been shown that the aldehyde of 5-HT causes depression of firing rates of single neurons in the cerebellum and neocortex of rats [21] and that the aldehydes derived from DA and 3-methoxytyramine inhibit platelet aggregation [11]. Furthermore, biogenic aldehydes are very reactive and bind to neuronal membranes [22] and proteins [23].

Experiments with biogenic aldehydes are complicated due to their reactivity and instability. Therefore, before conducting the metabolic experiments, the stability of the aldehydes under different incubation conditions was tested. Especially DOPAL was found to be very unstable when incubated in PBS. The parent amines showed similar stability properties and since electrochemical analysis was used in the assays of both the aldehydes and the amines, these results indicate that the disappearance was caused by oxidation of the hydroxyl groups. The amount of aldehyde that disappeared was also measured enzymatically using rat liver ALDH. Similar results to those observed in the HPLC-assays were obtained (unpublished results), suggesting that the aldehyde function of DOPAL and 5-HIAL was lost, or that the oxidation products, if retaining their aldehyde groups, did not react with ALDH. However, this instability of the aldehydes disappeared when EDTA was added to the mixture. Since EDTA forms stable complexes with metal ions, these results indicated that the stability of the aldehydes was also affected by trace amounts of metal ions in the incubation mixture. Metal ions catalyze the oxidation of catecholamines to orthoquinones [24] and EDTA has previously been shown to stabilize catecholamines in physiological buffers [25]. Furthermore, DOPAL showed a good stability in the presence of sodium pyrophosphate. This may be due to the pyrophosphate ion forming complexes with metal ions, or by the formation of complexes with the catechol ring, thereby protecting the hydroxyl groups from oxidation.

The aldehydes were protected from destruction in the presence of intact blood cells or blood cell membranes. Since biogenic aldehydes are known to bind to membranes, these results indicate that membrane binding protects the aldehydes from destruction. However, when the aldehydes were incubated with sonicated erythrocytes, there was a considerable initial binding of the aldehydes. This may be due to binding to free hemoglobin and might explain the low initial recovery of the aldehyde and the low recovery of the acid metabolite obtained, as compared to incubations with intact erythrocytes.

No formation of the acid and alcohol metabolites was observed when the aldehydes were incubated in plasma unless NAD and NADPH was added, but even then only small amounts of the metabolites were formed. The low activity observed can probably be explained by leakage from the blood cells. However, a considerable initial binding and disappearance during the incubation was observed when 5-HIAL and DOPAL were incubated in human plasma, whereas no such binding of the parent amines occurred. These results indicate that the aldehyde group is responsible for this binding. Since the initial binding was not observed in incubations with human serum albumin, some other plasma component must be involved.

Only formation of the acid metabolites was observed when DOPAL or 5-HIAL were incubated with erythrocytes, which indicated that these aldehydes were oxidized by ALDH in human erythrocytes. It has previously been shown that human erythrocytes contain ALDH activity with high affinity for the biogenic aldehydes DOPAL and indole-3-acetaldehyde [12]. It has also been shown that erythrocytes contain ALR activity [13]. However, our results suggest that this reducing capacity is of minor importance for the metabolism of DOPAL and 5-HIAL in human erythrocytes.

Both acid and alcohol metabolites were formed, when the aldehydes were incubated with leukocytes or platelets. The amount of the acid metabolite

exceeded that of the alcohol metabolite, both in incubations with leukocytes and platelets. These results suggest that human leukocytes and platelets possess ALDH and ALR activity. Human leukocytes have previously been shown to contain ALDH activity [12], and ALR activity has been observed in human platelets [14]. Pletscher *et al.* [26] observed, however, the formation of both acid and alcohol metabolites when 5-HT or DA were incubated with isolated rabbit platelets. When platelets were supplemented with erythrocytes, the acid metabolite was mainly produced [26]. Those results indicate that the oxidative pathway is of major importance in the erythrocytes, and are in agreement with the data reported here for human erythrocytes.

The metabolism of DOPAL and 5-HIAL in leukocytes was not affected by disulfiram. These results suggest that different isozymes of ALDH are present in erythrocytes and platelets as compared to leukocytes. The human liver cytosolic ALDH II, which is similar to the erythrocyte ALDH, is strongly inhibited by disulfiram whereas the mitochondrial ALDH I is less sensitive [27], suggesting that the ALDH activity observed in leukocytes was mainly caused by an isozyme similar to ALDH I.

The present experiments were performed with intact cells metabolizing exogenously added aldehydes, whereas under *in vivo* conditions, these aldehydes are formed inside the cells at the outer mitochondrial membrane. Therefore, no conclusions can be drawn from the present findings about the kinetics or the intracellular location of the metabolism of biogenic aldehydes *in vivo*. However, the results show that human erythrocytes, leukocytes and platelets have the ability to metabolize biogenic aldehydes. Since these aldehydes are very reactive and bind to tissue components, ALDH and ALR probably fulfil important roles in keeping the aldehyde level low.

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