Biochemical Pharmacology, Vol. 30, No. 21, pp. 2893–2900, 1981.
Printed in Great Britain.

0006-2952/81/212893-08 $02.00/0

MONOAMINE OXIDASE IN HUMAN PLATELETS
KINETICS AND METHODOLOGICAL ASPECTS

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(*Received* 22 *December* 1980; *accepted* 7 *April* 1981)

Abstract—The kinetic properties of human platelet monoamine oxidase (MAO) were examined in 20 apparently healthy controls. The mean value (±S.D.) of the maximum velocity (V) was found to be 5.36 ± 1.97 pmoles of product formed/10⁸ platelets/min and the Michaelis–Menten constants were for phenethylamine (K_m^{PEA}) 14.6 ± 8.20 μM and for oxygen ($K_m^{O_2}$)254 ± 125 μM, when assayed in 0.1 M phosphate buffer, pH 7.4. The relation between the value of the corresponding apparent constants was studied. Inhibition of the enzyme activity was seen at 20 μM of PEA and 180 μM of oxygen. The enzyme kinetics were also studied at different pH. Two pK values were found, pK_1 = 6.65 and pK_2 = 6.95. The influence of homogenization on the MAO activity was compared with the activity in the undisrupted platelet. At PEA concentrations below 10 μM higher MAO activities were found in the intact cell. A 15 per cent loss of activity was detected in platelet samples after storing at −20° for three and a half years.

Monoamine oxidase (MAO; amine:oxygen oxidoreductase/deaminating/flavine containing; EC 1.4.3.4) is involved in the metabolism of the biogenic amines both in the peripheral and central nervous system. Due to its availability, platelet MAO has been extensively studied during recent years in order to examine if there exists any association between the enzyme activity and various psychiatric disorders, e.g. schizophrenia [1–3]. In a previous interlaboratory study on platelet MAO in a pedigree with schizophrenia [2] some methodological problems in assaying the enzyme activity were identified. In order to follow up this report we have in the present study, paid attention to some of these problems, e.g. disruption of cells, linearity with dilution of enzyme and stability of enzyme activity during storage.

Monoamine oxidase from various tissues has been shown to follow a double-displacement (ping-pong) reaction pathway [4–8] involving both amine and oxygen as substrate. Some reports on platelet MAO have dealt with the role of oxygen as one of the substrates for the enzyme [9–11]. We now report on the kinetic properties of human platelet MAO with regard to both the substrates, phenethylamine and oxygen. In addition we also report on the influence of pH on these enzyme properties.

MATERIALS AND METHODS

Preparation of blood platelets. Venous blood (approximately 9 ml) was collected into Vacutainer tubes containing 1 ml of 3.8 per cent sodium citrate as anticoagulant. After 30 min in room temperature the tubes were centrifuged at 70 *g* for 10 min to remove the erythrocytes. The number of platelets in the obtained platelet rich plasma (PRP) was determined in an automatic cell counter (Thrombocounter). The platelets were spun down by centrifugation at 1300 *g* for 10 min and washed once with cold 0.1 M phosphate buffer, pH 7.4. The pelleted platelets were either used directly in the assay or immediately frozen and stored at −20° until use. Prior to the assay the fresh or thawed pellet was disrupted in buffer by sonication at 300 W for 15 sec (Braun, Labsonic 1510 with a microprobe). The amount of protein in the sonicated solution was assayed according to Lowry *et al.* [12].

Monoamine oxidase assay. The enzyme activity was assayed radiochemically using radioactively labelled PEA as monoamine substrate. The purity of the amine was checked by thin layer chromatography (TLC) in a system of methanol:acetic acid:water (70:15:15) and yielded one single spot as detected both by fluorescence and liquid scintillation. An aliquot of 0.4 ml of sonicated platelets was prewarmed for 5 min at 37° in a 10 ml borosilicate glass tube in which the whole assay procedure, including extraction, was performed. The reaction was started by the addition of 0.6 ml prewarmed (37°) 0.1 M phosphate buffer, pH 7.4 containing various concentrations of PEA, 2.4–20 μM (sp.act. 7 mCi/mmol), and oxygen, 65–180 μM (final concentrations).

After 5 min of incubation the reaction was terminated by adding 1.0 ml of 0.1 M HCl. The acidified solution was extracted with 6.0 ml of ethyl acetate by vigorous shaking on a Vortex mixer for 10 sec. The two phases were separated by centrifugation at 1000 *g* for 5 min and 4.0 ml of the organic phase were transferred to a scintillation counting vial containing 10 ml of scintillation liquid. The radioactivity

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was measured in a liquid scintillation counter (Packard 3380 with an additional absolute activity analyzer).

Oxygenated buffer was prepared by bubbling oxygen through the warmed buffer (37°) for 30 min to obtain saturation. After adjusting the pH to 7.4 or to the pH used in the particular experiment, the desired concentration of oxygen was obtained by mixing the saturated buffer with non-oxygenated buffer in adequate proportions. The concentration of oxygen in the buffer and the reaction mixture was determined by measuring the partial pressure of oxygen and by using a Clark oxygen electrode calibrated as described by Robinson and Cooper [13].

Evaluation of MAO activities. Unless otherwise specified all assays were performed in duplicates. At least three concentrations of PEA and a minimum of two concentrations of oxygen were used in each determination. The incubation periods, unless otherwise stated, were short enough to give the initial velocity (v). The reaction has been assumed to proceed through a series of binary complexes, with an intermediate form of the enzyme free from either of the substrates [14]. In a general form the system may be illustrated as two successive reaction sequences

$$E + A \rightleftharpoons EA \rightleftharpoons E'C \rightleftharpoons E' + C,$$
$$E' + B \rightleftharpoons E'B \rightleftharpoons ED \rightleftharpoons E + D, \quad (1)$$

where E represents the enzyme with the flavine residue in its oxidized state and E' the enzyme with a reduced flavine group. A and B represent in this case amine and oxygen substrates respectively and C and D illustrate the products formed in the reaction.

Under initial rate measurements when the relative concentrations of the products formed are zero, the velocity of the enzyme reaction can be shown to obey the equation,

$$v = \frac{V}{1 + (K_m^A/[A]) + (K_m^B/[B])}, \quad (2)$$

where K_m^A and K_m^B are the Michaelis constants for the reactants A and B respectively and V is the velocity of the enzyme reaction when saturated with substrates (maximal velocity).

In reciprocal form equation (2) becomes

$$\frac{1}{v} = \frac{K_m^A}{V}\frac{1}{[A]} + \left(1 + \frac{K_m^B}{[B]}\right)\frac{1}{V} \quad (3)$$

Thus, reciprocal plots with respect to one of the substrates (e.g. A) will result in families of parallel lines at a series of fixed concentrations of the other substrate (B) (e.g. see Fig. 3). The lines intersect the v^{-1} axis at $(1 + K_m^B/[B])$ (V^{-1}) and the $[A]^{-1}$ axis at $-(1 + K_m^B/[B])K_m^{A^{-1}}$ giving apparent values on the kinetic constants for each concentration of the alternative substrates. Therefore accurate values for the constants can not be obtained from this primary plot. However, the kinetic constants K_m and V may be determined from secondary plots where the intercepts on the v^{-1} axis in the primary plot is plotted vs the concentration of the second substrate B. This line intersects the 'intercept' axis at V^{-1} and $[B]^{-1}$ axis at $-(K_m^B)^{-1}$ [14].

The values of v and V were expressed as pmoles of product formed/10^6 platelets/min or nmoles of product formed/mg protein/min. The K_m values were expressed as μM of PEA and μM of oxygen.

All values were calculated using linear regression analysis.

Materials. Radioactively labelled phenethylamine-(ethyl-1-^{14}C) hydrochloride was obtained from New England Nuclear, Boston, MA, U.S.A. All other reagents were standard laboratory reagents of analytical grade wherever possible. Disposable glass culture tubes were purchased from Corning Glass Wares, Corning, NY, U.S.A.

RESULTS

Stability of enzyme activity

Platelets from seven individuals were sampled and prepared and the pellets were frozen at −20°. An aliquot of each preparation was assayed within a month after sampling according to the method described by Ask *et al.* [2]. Three and a half years later, 1979, another aliquot of the samples was assayed under atmospherical condition of oxygen as described in the present study. The values of the enzyme activity obtained at the two occasions of assay were compared at one concentration of PEA, 10 μM. There was a high correlation between the results from the assays, $r = 0.90$. The mean value ±S.D. was 0.93 ± 0.21 nmol/mg/min in 1976 whereas corresponding values 1979 were 0.79 ± 0.16 nmol/mg/min, indicating a decrease of 15 per cent over the period of three and a half years.

Disruption of platelets

Platelet rich plasma from one individual was divided into three equal parts. From two of these, platelet pellets were prepared. These pellets were suspended in the same volume of phosphate buffer as the volume of PRP from which they originated. One of these platelet suspensions was homogenized by sonication, the other one by homogenization in a tight fitting Potter–Elvejhem glass homogenizer. These homogenates and the untreated PRP were used in a comparative assay. The activity of MAO was determined at one concentration of oxygen (atmospheric) and six different concentrations of PEA, 1.7, 2.5, 5.0, 10.0, 13.4 and 20.0 μM. The results of the experiment are illustrated in a double-reciprocal plot, (Fig. 1). There was little or no difference between the two homogenized preparations in regard to the enzyme activity and the apparent kinetic constants, V and K_m. However, the PRP exhibited an increased MAO activity at lower concentrations of PEA whereas the activity was the same or slightly decreased at concentrations above 10 μM of PEA. Although the apparent K_m value was decreased in PRP the apparent V value was almost the same as compared to the value obtained in the homogenized platelets.

Linearity of activity with dilution of the enzyme

Sonicated human platelets were diluted 1, 2, 4, 8 and 16 times with buffer. The same volume, 0.4 ml, of the dilutions were used in the assay. There was

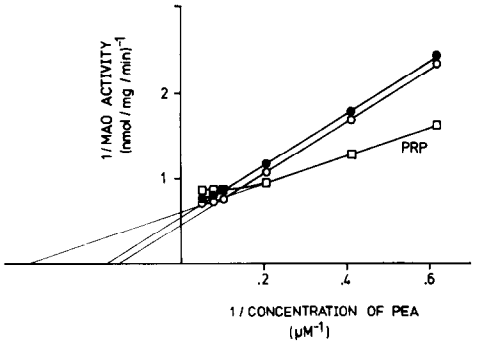

Fig. 1. The influence of disruption on the MAO activity studied in three aliquots from the same platelet preparation. The activities in intact platelets (□), after homogenization in a Potter–Elvejhelm glass homogenizer (●) and after sonication (○), are presented as the reciprocal values at each reciprocal concentration. The standard error of any given mean value ($\bar{y}$) did not exceed $0.05 \times \bar{y}$.

a high correlation ($r = 0.96$) between the extracted radioactivity and the degree of dilution, when assayed under atmospheric conditions and at 10 μM of PEA. At three different concentrations of enzyme (undiluted, 1:4 and 1:8) the assay was performed at three different concentrations of PEA, 2.5, 5.0 and 10.0 μM. There was no difference in apparent K_m (K_m^{app}) and apparent V (V^{app}) values at the three dilutions (K_m^{app}); 5.1, 5.4, 4.8 μM and V^{app}; 1.30, 1.44, 1.34 (pmoles of product formed/10^6 platelets/min).

Relation between number of platelets and the content of protein in platelet pellets

The activity of MAO was assayed in platelets from nine individuals. The number of platelets was determined in PRP and the protein concentration was determined in the phosphate buffer homogenate. The rate of reaction was very similar when related

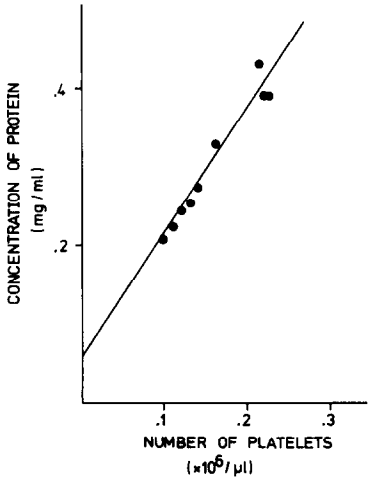

Fig. 2. The relation between the number of platelets in PRP and the protein content of the platelet pellet. The concentration of protein was determined in the pellet homogenized in buffer. The standard error of the intercept on the y-axis was 0.022.

to the number of platelets or content of protein, which was in agreement with the findings by others [15, 16]. There was also a high correlation ($r = 0.98$) between the number of platelets in PRP and the concentration of protein in the homogenate, (Fig. 2). The plotted line intersects the protein axis away from the origin, indicating a non-platelet protein content in the washed pellet of approximately 60 μg/ml PRP.

Linearity with period of incubation

The amount of product formed increased in a linear fashion with increasing periods of incubation up to approximately 7 min. After 10 min of incubation the enzyme activity decreased to about 80–85 per cent of the value obtained after 5 min.

Kinetics

Human platelet MAO from one apparently healthy individual was assayed using four different concentrations of PEA (2.5, 5.0, 10.0 and 20.0 μM) and four different concentrations of oxygen (65, 101, 138 and 180 μM). The activity increased with increasing concentrations of both PEA and oxygen. However, at the highest concentrations used (PEA; 20.0 μM and O_2; 180 μM) the increase was no longer proportional to the increase in concentrations of the substrates. This was displayed in both the primary and secondary double-reciprocal plots [Figs. 3(a and b)]. The calculated values on K_m for PEA and oxygen were 30.7 and 659 μM respectively. The maximum velocity, V, of the reaction was calculated to 14.8 pmoles of product formed/10^6 platelets/min.

Correlation between kinetic properties

The activity of MAO was assayed in platelet samples from 20 apparently healthy individuals. The assay was performed at three concentrations of PEA (2.5, 5.0 and 10.0 μM) and at two different concentrations of oxygen (65 and 122 μM). Significant correlations were found between both v and the apparent V, v and V as well as apparent V and V, and between apparent K_m and V (Fig. 4 and Table 1). However, no significant correlations were found between the kinetic parameters when compared with either of K_m^{PEA} or $K_m^{O_2}$, (Table 1). Table 1 also gives the mean values of the kinetic parameters.

The effect of pH

The effect of pH on the MAO activity was studied in the range pH 6.0–8.0. Optimum enzyme activity (v) was seen between pH 7.0–8.0, at both concentrations of oxygen studied and at one concentration of PEA (10 μM), [Fig. 5(a)]. The concentrations of oxygen were estimated to be 65 and 122 μM. The apparent K_m value for PEA exhibited a drastic decrease (90–95 per cent) over the whole pH range examined. However, the apparent V value decreased only about 25 per cent in the pH range. The course of the curves for the apparent K_m and V values was almost parallel for both concentrations of oxygen in the studied range of pH [Fig. 5(a)].

The real values for V, $K_m^{O_2}$ and K_m^{PEA} were calculated from the secondary double reciprocal plots, [Fig. 5(b)]. The value of V was doubled when increasing the pH from 6.0 to 8.0. The Michaelis constant

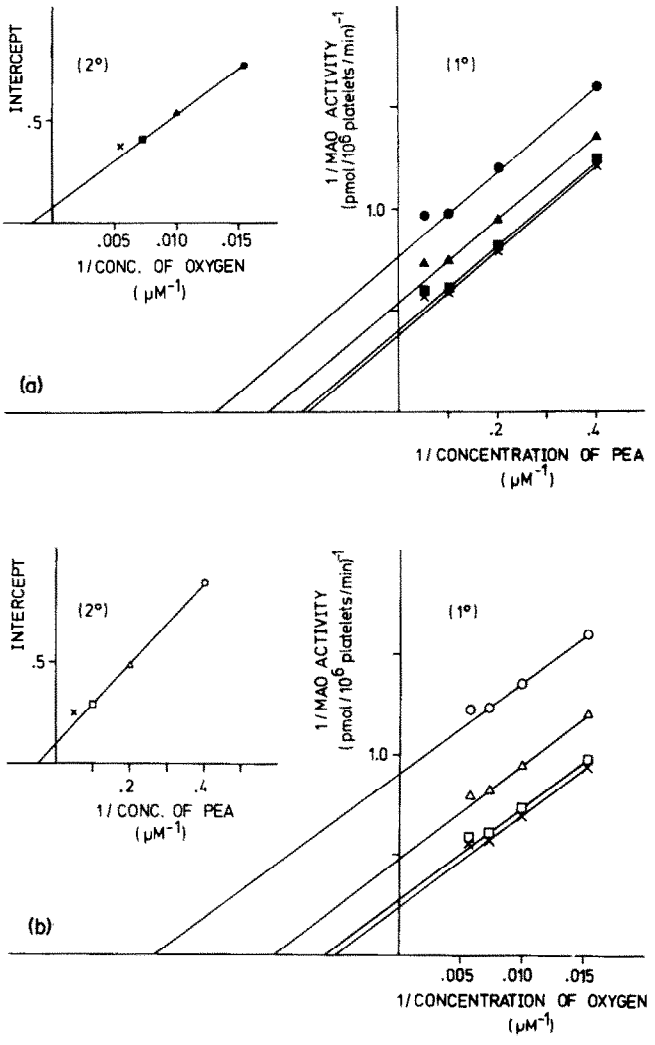

Fig. 3. Determination of the kinetic parameters V, $K_m^{O_2}$ and K_m^{PEA} of MAO in a platelet sample from one apparently healthy individual. Each point represents the mean ($\bar{y}$) of two determinations. The standard error of any given value did not exceed $0.05 \times \bar{y}$. (a) Primary plot (1°); the influence of the concentration of PEA on platelet MAO activity at four different concentrations of oxygen, 65, 101, 138 and 180 μM. Secondary plot (2°); the intercepts, from the primary plot, plotted against the reciprocal concentrations of oxygen. The V and $K_m^{O_2}$ values calculated from the secondary plot were 14.8 pmoles of product formed/10^6 platelets/min and 659 μM respectively. (b) Primary plot (1°); the influence of the oxygen concentration on platelet MAO activity at four different concentrations of PEA, 2.5, 5.0, 10.0 and 20.0 μM. Secondary plot (2°); the intercepts, from the primary plot, plotted against the reciprocal concentrations of PEA. The V and K_m^{PEA} values, calculated from the secondary plot were 14.8 pmoles of product formed/10^6 platelets/min and 30.7 μM respectively.

for oxygen increased from 50 to 350 μM in the same pH range. However, the K_m value for PEA decreased 80–85 per cent between pH 6.0–7.0 and was then almost stable up to pH 8.0, [Fig. 5(b)]. The influence of pH on the enzyme became more easily interpreted when the three quantities were plotted together as logarithmic values as proposed by Dixon [17], [Fig. 5(c)]. The pK value at pH 6.95 indicated by the sharp bend of the pK_m^{PEA} curve corresponds well with the pK values (6.90–6.95) obtained from the lg v curves. From the p$K_m^{O_2}$ curve a pK value is observed at pH 6.60. This pK was not seen in either the lg v or the pK_m^{PEA} curves.

DISCUSSION

The diversity of the reports on human platelet monoamine oxidase kinetics in various diseases have necessitated more extensive studies on the methodological problems in determining the activity of the enzyme [2, 18–21]. The degree of aggregation and the density of the platelets have been shown to vary [22], probably with the age of the cells [23]. It was also recently described that the MAO activity in human platelets of different density were unequally susceptible to inhibition by various psychotropic drugs [24]. Since different individuals may have pla-

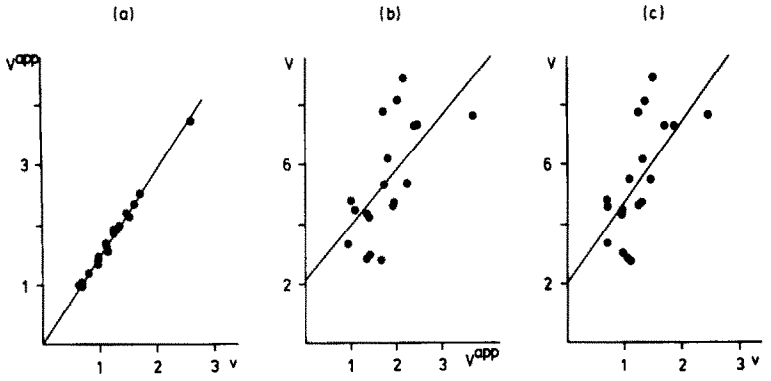

Fig. 4. The relation between the activity and maximum activity of human platelet MAO in 20 apparently healthy subjects. (a) The initial velocity at 10 μM PEA and 122 μM oxygen versus the apparent V value, obtained from primary double-reciprocal plots. (b) The apparent V value versus V obtained from secondary double-reciprocal plots. (c) The initial velocity at 10 μM PEA and 122 μM oxygen versus V obtained from secondary double-reciprocal plots. All values were expressed as pmoles of product formed/10^6 platelets/min. For correlation coefficients see Table 1.

Table 1. Correlation coefficients between kinetic properties of human platelet MAO in 20 apparently healthy subjects

	V^{app}	V	K_m^{app}	$K_m^{O_2}$	K_m^{PEA}	$\bar{x} \pm$ S.D.	
v	0.995	0.638	0.164	0.234	0.222	1.22	0.46
V^{app}		0.611	0.374	0.249	0.259	1.78	0.67
V			0.695	0.375	0.040	5.36	1.97
K_m^{app}				0.176	0.454	4.61	0.59
$K_m^{O_2}$					0.149	254	125
K_m^{PEA}						14.6	8.20

Mean values ±S.D. are expressed in μM for all K_m and in pmoles of product formed/10^6 platelets/min for all velocities.

The v value given is the activity assayed with 10 μM PEA and 122 μM = O_2.

For symbols see text

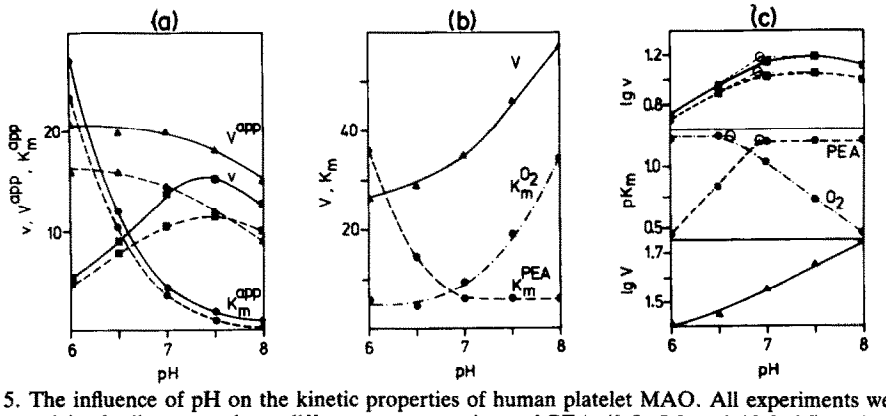

Fig. 5. The influence of pH on the kinetic properties of human platelet MAO. All experiments were performed in duplicates at three different concentrations of PEA (2.5, 5.0 and 10.0 μM) and two different concentrations of oxygen (65 and 122 μM). Each value represents the mean ($\bar{y}$) of at least two determinations. The standard error of any given value did not exceed $0.05 \times \bar{y}$. All velocities are expressed as pmoles of product formed/10^6 platelets/min. The apparent K_m and K_m^{PEA} are expressed as μM and $K_m^{O_2}$ as $\mu M \times 10^{-1}$. (a) The dependency of the initial velocity (10 μM PEA) on pH is illustrated by the filled squares and solid line (122 μM oxygen) and by the filled squares and broken line (65 μM oxygen). The influence of pH on the apparent V is represented by (▲——▲) (122 μM oxygen) and by (▲----▲) (65 μM oxygen). The influence of pH on the apparent K_m value for PEA is represented by (●——●) (122 μM oxygen) and by (●----●) (65 μM oxygen). (b) The influence of pH on V is illustrated by (▲——▲). The influence of pH on $K_m^{O_2}$ is illustrated by (●-·-·-●) and on K_m^{PEA} by (●----●). (c) The influence of pH on the kinetic properties illustrated in logarithmic scale. The symbols used are the same as in (a) and (b). The big open circles indicates pK values of the enzyme.

telet populations with different distribution of age this may give rise to platelet preparations of various composition. An altered composition of the platelet population could be expected to be found, not only in different diseases or after drug treatment, but also under physiologically altered conditions such as the menstrual cycle [25]. A difference in MAO activity between men and women in a population of apparently healthy subjects was recently described [26]. It was however not shown whether the higher value of enzyme activity in platelets from women was due to a difference in the platelet population or to some other endogenous factor. Since the density of the platelets varies the preparation of the platelet rich plasma which includes one or several steps of centrifugation, may be critical. This was recently studied extensively and described by others [23].

The activity of human platelet MAO appears to be fairly stable when stored at −20°. Over a period of three and a half years at the most 15 per cent of the activity was lost. As it appears this loss in activity was due to a decrease in V, rather than an increase in K_m for PEA or O_2. However, if the platelet samples were repeatedly frozen and thawed there was a change in both apparent K_m and V as well as in enzyme activity (v).

The kinetics follow, in the absence of products, a simple kinetic equation (2). The apparent values of V and K_m obtained from primary double reciprocal plots [see equation (3)], may vary many times with only a small change in the concentration of either one of the substrates. This is most obvious when the concentration of a substrate is of the same magnitude or lower than its K_m value. The high K_m values for oxygen (254 ± 125 μM, mean ±S.D.), as determined in the 20 apparently healthy subjects were all close to or higher than the solubility of oxygen in air-saturated water. Thus, it appears that the MAO will not be saturated with oxygen under normal conditions. The intracellular enzyme activity may, therefore, be affected by the competition from other mitochondrial enzymes, e.g. the respiratory chain, for the dissolved oxygen. However, as previously pointed out by Tipton [27], a consequence of the ping-pong mechanism is that the activity of the enzyme is quite insensitive to fluctuations in the available oxygen concentration providing the concentration of the amine is relatively low. If though, the concentration of amine is increased to the magnitude of its K_m value (14.6 ± 8.2 μM), which is the case in most assays used, the enzyme activity now becomes sensitive to changes in the available oxygen. It is unlikely that the intracellular concentration of amines under physiological conditions will reach this value. The concentration of PEA has been shown in the human post mortem brain to be approx. 10^{-9} g/g brain (wet wt). This low concentration is probably due to the high efficiency of MAO since the administration of MAO inhibitors significantly increases the concentration of unmetabolized amines [28].

The products ammonia, hydrogen peroxide and aldehyde formed in the reaction may act as inhibitors of the MAO activity [4]. Since the systems metabolizing peroxide are extremely efficient and the K_i for ammonia relatively high it is unlikely that either of these two products will have any inhibitory effect on MAO *in vivo*. On the other hand, the aldehyde exhibits a low K_i and has thus been suggested to have a significant regulatory effect on the enzyme activity [29]. At the highest concentrations of either one substrate (O_2; 180 μM, PEA; 20 μM) a slight inhibition of the enzyme activity was observed in the present investigation. However, it could not from the present results be distinguished whether this decrease in activity was due to inhibition by the substrates or by the products.

In order to avoid inhibition all further kinetic experiments were performed at lower, non-saturating conditions. In a previous study [26] a high correlation coefficient (0.99) between the MAO activity at 10 μM of PEA and the calculated apparent V value was reported. This high correlation, obtained in a population of 32 apparently healthy subjects, indicated that the activity (v) well reflected the apparent V value for most individuals. On the basis of the results in the present investigation, the v value at one concentration did also seem to reflect the value of V in the studied population of 20 apparently healthy controls. This confirms the finding by Fowler and Wiberg [11] that the activity of MAO was positively correlated to the number of enzyme molecules. Since it is not possible to assay the enzyme under saturating conditions it must however be emphasized that the obtained v value only may serve as a very rough estimate of the corresponding V value in different individuals. No correlation between v and K_m values was observed. Neither was there any correlation between the two K_m values. It is worth noting, however, that a low correlation coefficient does not in itself rule out the possibility of a dependency between the two variables [30].

The relation between the discussed kinetic parameters was also studied at different pH in platelets from one individual. So far there is to our knowledge no report on the influence of pH on K_m and V for human platelet MAO. The relation between the kinetic properties was completely changed if the true values of K_m and V were calculated instead of the apparent values. When using apparent K_m and V values it appeared as if the increase in MAO activity with higher pH was only due to a decrease in K_m for the amine substrate. The following decrease in activity seemed to be caused by a decrease in V. Besides, there were no concordance between the apparent pK values when the parameters were plotted in a logarithmic scale (data not shown). However, the calculation of the more accurate V and K_m values made the relation easier to interpret. The increase in enzyme activity observed between pH 6.0 and 7.0 was caused by a decrease in K_m for PEA. Above this pH K_m remained at a constant level up to pH 8.0. The value of $K_m^{O_2}$, on the other hand, being at a low level from pH 6.0 to 7.0 increased rapidly between pH 7.0 and 8.0. This increase in $K_m^{O_2}$ was at least one of the reasons for the plateau and the decrease in enzyme activity which was observed within the same pH range. The value of V did not reach a maximum but increased with increasing pH, suggesting that there were no ionizing groups in the enzyme–substrate (ES) complexes with a pK value within the studied range of pH [14]. The logarithmic plots of

the K_m values support this suggestion. A pK of a group situated in the ES complex produces an upward bend, i.e. a bend with the concave side upwards. No such bend could be detected. A pK of a group in the free enzyme or in the free substrate produces a downward bend. Two distinct downward bends were found, one at pH 6.6 in the $K_m^{O_2}$ curve and one at pH 6.9 in the K_m^{PEA} curve. No one of these could be caused by PEA since its pK value has been determined to be 9.88 [31]. The ionizing groups must therefore, be situated in the free enzyme. The lower pK value was caused by a group in the reduced state of the enzyme and the higher by a group in the oxidized state of the enzyme. As the only part of the enzyme being different in the two states is the flavine residue, the ionizing groups can be assumed to be located to either the flavine molecule itself or in its close vicinity. The possibility of the two pK values to originate from the protolysis of the same hydrogen is also conceivable. The presence or absence of the 'reactive' flavine hydrogen may affect the protolysis of some other closely associated hydrogen. The observed pK value at 6.9 corresponds well with the value (7.00–7.05) reported by Oi *et al.* [32] in a study on beef liver MAO.

There was a difference in enzyme activity when assaying MAO in PRP and disrupted platelets. The activity was significantly higher in intact platelets although the apparent V values were almost identical. Consequently there was a decrease in the apparent K_m value in the intact cells. The activity was in these experiments determined only at one concentration of oxygen. Therefore, it was not possible to calculate the more accurate values of V or K_m. It is, however, possible to discuss the cause of the difference in enzyme activity in the intact and disrupted platelets. From the intercept, $v^{-1} = V^{-1}(1 + K_m^{O_2}/[O_2])$, it can be assumed that the transport of oxygen across the membrane was not a regulating factor. A change in the oxygen concentration or a change in $K_m^{O_2}$ should give the same change in the apparent V as in the apparent K_m value. The marked decrease in apparent K_m must therefore be caused by some other factor. A decrease in the apparent K_m value for PEA could be the result of a decrease in the concentration of the available amine, i.e. the expected total concentration in the incubate is not reached inside the plasma membrane. This could occur if the transport of PEA into the cell was a slower process than the metabolization to aldehyde by MAO or if PEA was pumped out from the cell towards its gradient. Naturally, this change in concentration would also be exhibited in a decreased enzyme activity (v), resulting in a parallel upward shift of the whole curve. Since the apparent V value did not change a decreased intracellular concentration is not likely to have occurred. In the same way, an accumulation of PEA inside the platelet can be ruled out. It is therefore plausible that the increase in MAO activity in the intact human platelet as compared to the activity in disrupted platelets, is due to an increase in the affinity for PEA. This should result in a decrease in the apparent K_m value, since $-[PEA]^{-1} = K_m^{PEA^{-1}}(1 + K_m^{O_2}/[O_2])$, indicating a dependency of the enzyme activity on the environmental structure and molecular organization, i.e. the enzyme is working in a more effective manner when situated in the undisrupted platelet. However, the influence of other factors such as a MAO activator in plasma [33] or a different pH inside the platelet could not from the present results be excluded.

Acknowledgements—This work was supported by a grant from the Swedish Medical Research Council (project No. 3371). We thank Elsa Rylander for typing the manuscript.

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