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Rat Brain Flavin-Containing Monooxygenase: Catalytic Activity and Sex-Related Difference

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Abstract

The presence of flavin-containing monooxygenase (FMO) activity was examined in brain microsomes from male and females rats. FMO-mediated oxidation of N, N-dimethylaniline (DMA) and methimazole (MEM) was observed in rat brain microsomes. No sex difference was observed in FMO-mediated metabolism of DMA. The apparent Km for N-oxidation of DMA was 2.83 and 3.02 mM and apparent Vmax was 340 and 360 nmoles of NADPH oxidized/min/mg protein in male and female brain microsomes, respectively. However, a constitutive sex difference was observed in FMO-mediated metabolism of MEM. The apparent Km for the oxidation of MEM was 0.6 and 0.8 mM and apparent Vmax was 32 and 41.5 nmoles of NADPH oxidised/min/mg protein for male and female brain microsomes, respectively. Incubation of male brain microsome at room temperature for 0.5 hr did not have any effect on DMA or MEM oxidation, while 80% of female brain microsomal FMO-mediate oxidation of MEM was lost on similar incubation. Thus a distinct sex-related difference was observed in FMO-mediated oxidation of MEM as indicated by apparent Km and Vmax and sensitivity to temperature.

Key words -

Falvin-containing monooxygenase,
N, N-Dimethylaniline,
Methimazole,
Brain

The major enzyme systems involved in oxidation of xenobiotics are the microsomal cytochrome P-450 (P-450) and associated monooxygenases, and the flavin containing moooxygenase (FMO) while the p-450 and associated monooxygenases catalyse the oxidation at carbon centre. FMO catalyses the N-oxidation of secondary and tertiary amines [1], hydrazine derivatives [2] and S-oxidation of thiols and thiourea [1], [3], [4]. The reactions catalysed by FMO can be differentiated from cytochrome P-450 oxidation by insensitivity of FMO to carbon monoxide and other P-450 inhibitors such as SKF 525-A, metyrapone and n-octylamine and antibody to NADPH cytochrome c (P-450) reductase [5], [6].

Although high concentration of FMO is found in liver, the amount of enzyme present in other tissues varies with species and sex. FMO was first characterized in hog liver [7] and later detected in human liver [8]. In addition to the liver, the pulmonary FMO has been characterized in rabbit [9]. FMO has been purified from rat liver [10], mouse liver [11], rabbit

lung and liver [9], [12], [13] and guinea pig liver [14], [15].

The presence of cytochrome P-450 in brain has been studied in recent years [16], [17] and the xenobiotic metabolizing capability of the brain has been demonstrated. FMO activity was also detected in rat corpus striatum [18]. Studies from our laboratory have indicated high concentration of FMO in male rat brain using N, N-dimethylaniline (DMA), methimazole (MEM) and thiobenzamide as model substrates [19].

Several psychoactive drugs (eg. chlorpromazine, imipramine, etc.) which act on central nervous system are substrates for both liver and rat lung FMO. Both pulmonary and hepatic FMO exhibit tissue specific differences [20]. Since FMO-mediated metabolism is likely to play an important role in pharmacological modulation of drugs acting on the brain, the present study was undertaken to examine the kinetics of FMO-mediated reactions and to determine if sex-related differences existed in brain.

Material and Methods

Male and female wistar rats (4-6 months old) were obtained from Central Animal Research Facility of the Institute. Animals had access to pelleted diet (Lipton India Ltd., Calcutta, India) and water ad libitum. Animals were anaesthetized with ether and perfused transcardially with 20 ml of ice-cold. Tris-HCI buffer (0.1M, pH 7.4), prior to decapitation. The brain and right central lobe of the liver were removed, rinsed in the above buffer and weighed. Both brain and liver were homogenized in 10 volumes of 0.1 M Tris-HCI buffer (pH 7.4) containing 1.15% (w/v) potassium chloride, 0.1mM phenyl methyl sulphonyl fluoride (PMSF), 1mM ethylenediaminetetraacetic acid (EDTA), 10% glycerol (v/v) and 0.1% bovine serum albumin. Microsomes were prepared as described [21]. Microsomes were stored in liquid nitrogen until use. Protein was determined by dye-binding method [22] and by the method of Lowry [23].

FMO activity was measured by following the substrate stimulated rate of NADPH oxidation in presence of n-octylamine. The assay buffer consisted of 0.1 MTris-HCi (pH 8.4), 1mM EDTA, 3mN n-octylamine and 0.1% (v/v) Triton N-101 and was oxygenated before use. Microsomes (30-100μ g) and NADPH (0.1mM) were added to the assay buffer and the mixture was transferred to a spectrophotometric cuvette and warmed at 37° C for 2-3 minutes and the endogenous rates recorded. Reactions were initiated by the addition of appropriate concentration of substrates (DMA or MEM) to the cuvettes, such that the final volume was 1ml. The absorbance changes at 340 mm were recorded. Reaction rates were determined using molar absorptivity of 6220 M^{-1} cm^{-1} for NADPH. Activities are reported as substrate stimulated rates minus the endogenous rates. All assays were performed in duplicate for each batch of microsomes.

Results

FMO activity in brain and liver microsomes from male and female rats is given in Table I. The rate of FMO-mediated metabolism of DMA and MEM by male and female rat brain microsomes was significantly higher than that observed with male rat brain microsomes.

Table I - Flavin-containing monooxygenase activity in male and female rat hepatic and cerebral microsomes

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Activities are mean ± SD (n=4-12 experiments)

The statistical significance is indicate as:

(a) Brain microsomal FMO activity is significantly higher than the corresponding hepatic activity p<=0.05.

(b) Female brain and liver microsomal FMO activities are significantly higher than corresponding male liver microsomal enzyme activities (p<=0.05).

A linear increase in the rat of N-oxidation of DMA was observed with increasing concentration of the microsomal protein (upto 30µ g/ml) from male rat brain. Further increase in protein concentration did not result in increased product formation (Figure 1a). The enzyme activity increased linearly with time (Figure 1b). The relationship between substrate concentration and enzyme activity is depicted in Figure 1c. Apparent Km and Vmax as calculated from Eadie-Hofstee plot (Figure 1d) are 2.83 mM and 340 nmoles of NADPH oxidized min/mg protein respectively.

Figure I: Effect of (a) microsomal protein concentration (b) incubation time (c) substrate concentration - (a) Microsomal protein (30-60µg) was used. The reaction rate was monitored for 5 mins and DMA concentration was 3mM. (b) Microsomal protein concentration was 30µg/ml and DMA (3mM) was added to the reaction mixture and reaction rate monitored for varying period of time. (c) The reaction mixture contained microsomal protein (30µg) and the reaction rate was monitored for 5 mins with varying substrate concentration. 'v' represent velocity expressed as nmoles of NADPH oxidised/min/mg protein. 's' represent DMA concentration in mM. (d) Eadie-Hofstee plot of data from (c). Apparent Km value was 2.8 mM and Vmax 340 nmoles of NADPH oxidized/min/mg protein. Values are mean ±SEM (n=5).

The oxidation of DMA by female brain microsomes was found to increase linearly with protein concentration upto 35µ g/ml (Figure 2a). The reaction rate increased linearly with time (Figure 2b). The relationship between substrate concentration and enzyme activity is shown in Figure 2c. As calculated from the Eadie-Hofstee plot (Figure 2d), the apparent Km and Vmax were 3.02 mM and 360 nmoles of NADPH oxidized/min/mg protein respectively.

Figure II: Effect of (a) microsomal protein concentration (b) incubation time (c) substrate concentration - (a) Microsomal protein (30-60µg) was incubated for 5 minutes and DMA concentration was 3mM. (b) Microsomal protein concentration was 30µg/ml and DMA concentration was (3mM).(c) Incubations were carried out for 5 minutes with 30µg/ml of microsomal protein. 'v' represent velocity expressed as nmoles of NADPH oxidised/min/mg protein. 's' represent DMA concentration in mM. (d) Eadie-Hofstee plot of data from (c). Apparent Km value was 3.02 mM and Vmax 360 nmoles of NADPH oxidized/min/mg protein. Values are mean ±SEM (n=5).

Table II - Effect of temperature on cerebral FMO activity in male and female rats

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Microsomes were preincubated at 4 degree C or room temperature (RT) for 0.5 hr, prior to determination of FMO activity.; Values are mean ± SD of three individual experiments.

Male rat brain microsomal FMO-mediated oxidation of MEM was found to increase linearly with increasing protein concentration upto 50µ g/ml (Figure 3a).The rate of oxidation increased linearly for

60 seconds (Figure 3b). The enzyme activity increased with increasing substrate concentration (Figure 3c). The apparent Km and Vmax were 0.6 mM and 31 nmoles of NADPH oxidized/min/mg protein respectively, as calculated from the Eadie-Hofstee plot (Figure 3d).

- ***Figure III: Effect of (a) microsomal protein concentration (b) incubation time (c) substrate concentratio - (a) Microsomal protein (30-60µg) was incubated for 1 minute and MEM concentration was 1mM. (b) Microsomal protein concentration was 30µg/ml and DMA concentration was 1mM.(c) Incubations were carried out for 1 min with 30µg/ml of microsomal protein. 'v' represent velocity expressed as nmoles of NADPH oxidised/min/mg protein. 's' represent DMA concentration in mM. (d) Eadie-Hofstee plot of data from (c). Apparent Km value was 0.6 mM and Vmax 31 nmoles of NADPH oxidized/min/mg protein. Values are mean ±SEM (n=5).***

Oxidation of MEM by female brain FMO was also found to increase linearly with increasing microsomal protein concentration (Figure 4a) and time (Figure 4b). The relationship between substrate concentration and enzyme activity is shown in Figure 4c. From the Eadie-Hofstee pilot (Figure 4d), the apparent Km and Vmax were calculated to be 0.8 mM and 41.5 nmoles of NADPH oxidized/min/mg protein respectively.

- ***Figure IV: Effect of (a) microsomal protein concentration, (b) incubation time, (c) substrate concentratio - (a) Microsomal protein (100-200µg) was incubated for 1 minute and MEM concentration was 1mM. (b) Microsomal protein concentration was 150µg/ml and MEM concentration was 1mM.(c) Incubations were carried out for 1 min with 150µg/ml of microsomal protein. 'v' represent velocity expressed as nmoles of NADPH oxidised/min/mg protein. 's' represent DMA concentration in mM. (d) Eadie-Hofstee plot of data from (c). Apparent Km value was 0.80 mM and Vmax 41.2 nmoles of NADPH oxidized/min/mg protein. Values are mean ±SEM (n=5).***

The male and female brain microsomes were incubated at room temperature to determine the thermolability of brain FMO. Male brain microsomal FMO-mediated oxidation of both DMA and MEM were unaffected, following the incubation of microsomes for 30 mins at room temperature. However, when female brain microsomes were incubated at room temperature, FMO-mediated oxidation of MEM decreased substantially and was only 20% of the constitutive activity.

Discussion

The specific activity of FMO-mediated oxidation of DMA and MEM by brain microsomes was significantly higher than that observed in liver. This is in concurrence with our earlier observation [19]. The Km for DMA oxidation in brain (2.8 mM) was significantly higher than that reported earlier for hepatic FMO-mediated oxidation of DMA (3.0µ M). The present results indicate that FMO mediated oxidation of DMA in brain has low affinity, but high activity. No sex difference was observed in brain in the rate of DMA oxidation and the Km values did not differ significantly.

The rate of oxidation of MEM in female brain microsomes was significantly higher than that observed in male brain microsomes (Table I). Corresponding results were also observed in the liver. The sex-related difference was also reflected in the Km and Vmax values which differed significantly in the male and the female brain microsomes. The female brain microsomes exhibited higher Vmax and Km values as compared to the male brain.

The present results thus indicate that multiple forms of FMO may exist in the brain. The thermolability

experiments also seem to substantiate the above results. While, male brain FMO-mediated oxidation of DMA and MEM were unaffected by incubation of microsomes at room temperature, the female brain MEM oxidation was selectively and significantly (80%) decreased. In the liver, FMO-mediated oxidation of both DMA and MEM are temperature sensitive. Hepatic FMO activity is totally lost by brief exposure to room temperatures. Thus, the brain FMO seems to be distinct from liver FMO.

The present study demonstrates the sex-related difference in FMO-mediated oxidation of MEM and also points to the possible existence of multiple forms of FMO in brain. This is of significance since several psychoactive drugs are substrates for FMO and are detoxified by FMO mediated reactions.

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