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ARYL HYDROCARBON HYDROXYLASE IN RAT BRAIN MICROSOMES

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Introduction Cytochrome P-450 monooxygenases (EC 1.14.14.1) exist in brain where they play important physiological roles such as the aromatization of androgens to estrogens, the 2-hydroxylation of estrogens to yield catechol estrogens, and a participation in prostaglandin metabolism [1-4]. The potential activation of substrates to reactive intermediates is of highest toxicological significance in the brain, as this organ has limited regenerative ability.

Cerebral aryl hydrocarbon hydroxylase (AHH) activity has been studied by several authors, often with indirect metabolite detection [1]. Here we report in preliminary form some results of our investigation of naphthalene oxidation in rat brain preparations using direct GC/MS/MF metabolite quantification.

Material and Methods Male Wistar rats of 150-200 g body weight (purchased from Kleintierfarm AG, Madörin, Switzerland) were sacrificed by cervical dislocation. Whole brains were rapidly removed, rinsed 3 times and homogenized in 6 volumes of ice-cold isotonic KCl solution. Centrifugations were as described by Das et al [5]. The "microsomal pellet" was washed 3 times and resuspended in an isotonic solution of KCl/HEPES (Merck, Darmstadt, FRG) buffer 0.01 M pH 7.6. The protein concentration was measured by the technique of Lowry et al [6] using bovine serum albumin as a standard. NADPH (Serva, Heidelberg, FRG) was used as single cofactor (3 mg/5 ml incubate) after optimization. The substrate in acetonic solution (1 mg/ml) was added to reach a final concentration of 15.6 μM, blank incubates receiving only acetone. Enzymatic activity was stopped by acidification to pH 1, para-chlorophenylethanol added as internal standard, and the metabolites were extracted with diisopropyl ether (freshly distilled, analytical grade, Fluka AG, Buchs, Switzerland), concentrated under nitrogen and silylated with BSA (freshly distilled bis-trimethylsilyl acetamide, Merck) for one hour at room temperature. The extract (2 μl) was injected into a modified Hewlett-Packard 5992A GC/MS with cross-linked silicon phase capillary column, and the main metabolite 1-TMS-naphthol was quantified by mass fragmentometry, monitoring ion 216.2 relative to ion 103.0 from the internal standard.

Results Under 100 % oxygen atmosphere, activity (0.190 ± 0.03 pmol 1-naphthol/mg prot/min) was constant up to 60-70 minutes of incubation, and showed exclusive NADPH dependency without NADH synergism (Fig. 1). Below 2 mg NADPH per incubate (5 ml) containing 5 mg of microsomal proteins, this cofactor was rate-limiting, while above 4 mg some inhibitory effect was observed, the optimal amount being 3 mg. An amount of blood identical to normally used brain weight and treated identically did not show any naphthol formation in all studied subfractions. Addition of blood 105000 g supernatant to brain microsomal incubates caused a slight inhibition rather than the described increase in AHH activity [7].

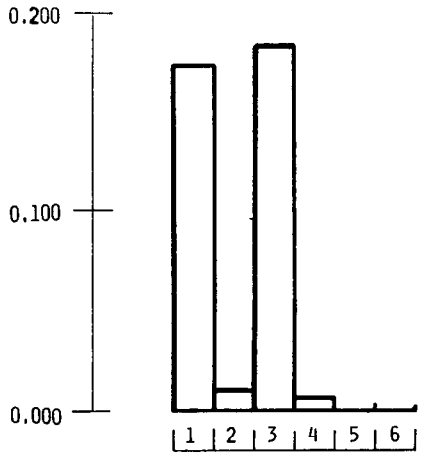

Figure 1 *Rat brain microsomal naphthalene oxidation; activity in pmol/mg prot/min; naphthalene (when present) 15.6 µM; cofactors 3 mg each per incubate of 5 ml; incubation time 60 min. 1: NADPH + NADH; 2: NADH; 3: NADPH; 4: no cofactors; 5: no naphthalene; 6: no microsomes.*

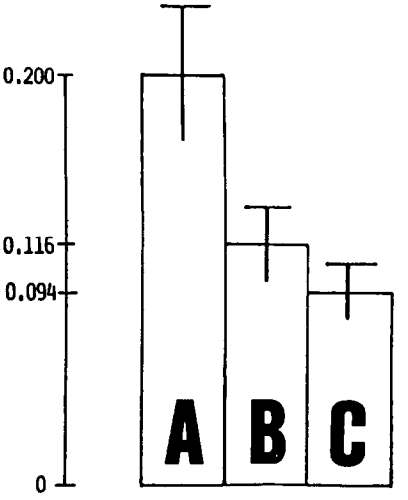

Figure 2 *Activity (pmol/mg prot/min ± SD, n = 4) as a function of atmosphere composition; incubates (5 ml) contained 5 mg of microsomal proteins and 3 mg NADPH; incubation time 60 min. A: O_2 20%/ N_2 80%; B: O_2 20%/N_2 40%/CO 40%; C: O_2 20%/ CO 80%.*

Interestingly, liver microsomes diluted and incubated in conditions optimal for brain preparations yielded in 60 minutes 200 times more 1-naphthol and a fair proportion of 1,2-dihydro-1,2-dihydroxynaphthalene and dihydroxynaphthalenes which would easily have been detected in brain incubates if present in a 1000-fold smaller concentration. In fact, only 1-naphthol and minor amounts of 2-naphthol could be detected in brain incubates. The formation of naphthol was proportional to protein concentration up to 5 mg prot/5 ml incubate, followed by a decrease which above 20 mg prot/ 5 ml, stabilizes around 70 % of maximal 1-naphthol formation.

Using optimal microsomal and cofactor concentrations we observed an apparent Km of 24.1 µM and Vmax of 0.275 pmol 1-naphthol/mg prot/min in a 100 % oxygen atmosphere.

The inhibitory effect of carbon monoxide (Fig. 2) was just below 60 % in an atmosphere of 80 % CO and 20 % O . Metyrapone or SKF-525A when added in identical concentrations as naphthalene (15.6 µM) did not significantly decrease naphthol formation (less than 6 %, NS) after incubation for 60 minutes.

Discussion A sensitive and reproducible method allowing direct determination of aromatic metabolites from non-induced mammalian brain has been developed. The observed oxidation of naphthalene to 1-naphthol by rat cerebral microsomes is clearly due an enzymatic, NADPH-dependent activity of the tissue, and not of remaining blood. The activity is inhibited by CO. Optimal NADPH and protein concentrations were determined. This method is now being used to investigate the behaviour of brain AHH in therapeutically relevant situations.

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