In vitro Metabolism of Ethionine, N-Acetyl-ethionine and N-Acetylmethionine by Rat Liver Microsomes

It is not unusual for a foreign compound to undergo more than one metabolic conversion before it is excreted[1]. The formation of sulfoxides and N-acetyl derivatives of alkylated thio amino acids is a prevalent pathway for the metabolism of these compounds in the rat[2]. S-*n*-Propyl-L-cysteine was converted to its sulfoxide by microsomal preparations from rat liver which required either an NADPH generating system or NADH and CN^-[3]. Rats injected with either ethionine or ethionine sulfoxide excreted N-acetylethionine sulfoxide in the urine[4,5]. The major pathway for the metabolism of ethionine by the rat has been suggested to be: ethionine→ethionine sulfoxide→N-acetylethionine sulfoxide. The present study was conducted to determine whether the reactions of ethionine could be demonstrated in vitro.

Materials and methods. Adult male Charles River rats (150–200 g) were allowed free access to Purina laboratory chow and water. Animals were sacrificed by decapitation and hepatic microsomes were prepared[6]. Microsomal protein was estimated as described by LOWRY et al.[7]. Metabolism of substrates was accomplished in a 3-ml incubation mixture containing 5 m*M* $MgCl_2$, 12 m*M* glucose-6-phosphate, 1 enzyme unit of glucose-6-phosphate dehydrogenase, 0.33 m*M* NADP, 50 m*M* *Tris* buffer (pH 7.4), 5–10 mg of microsomal protein, and various amounts of substrates. The mixtures were incubated with shaking for various periods of time. The substrates were either [ethyl-1-^{14}C] ethionine, [ethyl-1-^{14}C] N-acetylethionine, [ethyl-1-^{14}C] N-acetylethionine sulfoxide, [methyl-^{14}C] N-acetylmethionine, or [methyl-^{14}C] N-acetylmethionine sulfoxide. They were synthesized from ethionine or methionine by methods described previously[2,5]. [Ethyl-1-^{14}C] L-ethionine (1.34 μCi per μmole) was purchased from ICN, Irvine, California. At the end of the incubation period, the reaction was stopped by placing the incubation flask in boiling water for 5 min and the precipitated protein was collected by centrifugation for 10 min at 2000 × *g* in an IEC model K centrifuge. The supernatant was decanted and 0.2 ml was spotted on Whatman 3 MM filter paper and chromatographed in a solvent system containing tert-butanol-methylethylketone-water-diethylamine (10:10:4:1 by volume)[8]. All metabolites were identified by cochromatography with known standard compounds.

Ethionine, N-acetylethionine, ethionine sulfoxide, N-acetylmethionine, N-acetylmethionine sulfoxide, and N-acetylethionine sulfoxide were detected by spraying the chromatograms with a platinic iodide solution[9]. Methionine, ethionine, methionine sulfoxide and ethionine sulfoxide were quantitatively determined by developing chromatograms with 0.5% ninhydrin in acetone[10]. The radioactivity on the chromatograms was detected on radioautograms prepared with Kodak No-screen X-ray film. The radioactive spots on the chromatograms were eluted overnight with water and an aliquot counted.

Results and discussion. In vitro experiments were carried out to determine whether a microsomal enzyme system from liver could metabolize ethionine or N-acetylethionine to the corresponding sulfoxides. When [ethyl-1-^{14}C] ethionine (0.2 μCi, 400 μg) was incubated for 1 h, about 32% of the radioactivity was metabolized. About 45% of it was converted to ethionine sulfoxide; the other products were not identified.

When N-acetylethionine was incubated under the same conditions to determine if microsomes could metabolize N-acetylethionine to N-acetylethionine sulfoxide, it was found that instead of the compounds being oxidized, they were deacetylated. About half of the N-acetylethionine was deacetylated to ethionine. A time study of the deacetylation of N-acetylethionine incubated with liver microsomes is given in Figure 1. The deacetylation was fairly rapid with approximately half of the N-acetylethionine converted to ethionine at 120 min of incubation.

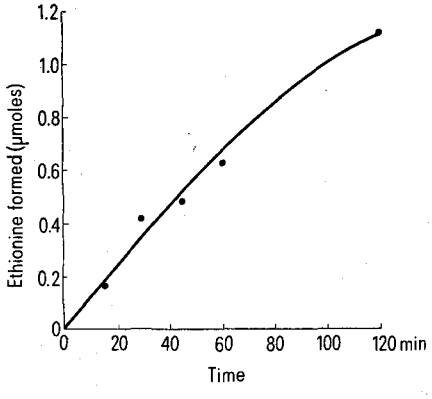

Fig. 1. The deacetylation of N-acetylethionine as a function of time. N-Acetylethionine (2.1 μmoles) was dissolved in 1 ml of *Tris*-HCl buffer (pH 7.4) and incubated for 2 h with 1 ml of NADPH generating system and 1 ml of microsomal suspension (5 mg/ml).

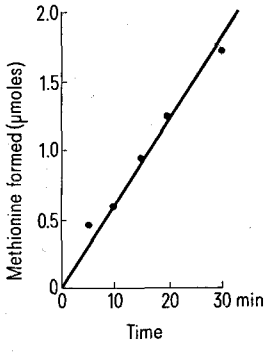

Fig. 2. The deacetylation of N-acetylmethionine as a function of time. N-Acetylmethionine (2.1 μmoles) was dissolved in 1 ml of *Tris*-HCl buffer (pH 7.4) and incubated for 30 min with 1 ml of NADPH generating system and 1 ml of microsomal suspension (5 mg/ml).

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The deacetylation of N-acetylmethionine occurred more rapidly than that of N-acetylethionine. Deacetylation of N-acetylmethionine was followed by assaying for the formation of methionine (Figure 2). A linear relationship resulted with 1.8 μmoles of methionine being formed by 30 min. These results show that N-acetylmethionine and N-acetylethionine are deacetylated by liver microsomes, but are not converted to the sulfoxide derivative. N-acetylethionine sulfoxide and N-acetylmethionine sulfoxide incubated under the same conditions with liver microsomes were not deacetylated. Carcinogenic arylacetamides have also been reported to be deacetylated by microsomes from dog liver[11].

BENNEDETTI et al.[12] reported that the growth rate of rats fed a methionine-deficient diet (12% casein or enzymatic casein hydrolysate) supplemented with N-acetylmethionine was as good as if the diet was supplemented with methionine. They suggested that the hydrolysis of the acetyl group of N-acetylmethionine took place gradually so that the methionine released mixed with the amino acids released by the hydrolysis of casein. The present study suggests that the rat handles N-acetylethionine in a similar manner.

Summary. Rat liver microsomes deacetylated N-acetylethionine and N-acetyl-methionine to ethionine and methionine. The deacetylation of N-acetylmethionine was more rapid than the deacetylation of N-acetylethionine. Ethionine was slowly converted to ethionine sulfoxide by the microsomal preparations. N-Acetylethionine and N-acetylmethionine were not oxidized by the microsomes.

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Monoamine Oxidase Activity of the Hypothalamus and Pituitary: Alterations after Pinealectomy, Changes in Photoperiod, or Additions of Melatonin in vitro[1]

The pineal gland appears to be involved in the control of gonadal function, possibly acting through the hypothalamic-hypophyseal axis, by altering the synthesis and/or release of gonadotropins[2,3]. The search for an antigonadal agent from the pineal gland has resulted in the isolation of melatonin as well as other compounds[2]. Melatonin fulfills many of the requirements of an antigonadal agent[4]: it reduces ovarian[5–7] and testicular size[8–11]; affects androgen synthesis in vitro[12,13]; and inhibits the release of LH[14,15] and FSH[16,17]. It has been suggested that this inhibition is mediated in part through the hypothalamus[15].

Injections of melatonin increase brain serotonin (5–HT) concentrations[18] as well as other brain biogenic amines[19]. Moreover, 5–HT has been reported to suppress the release of both LH[15] and FSH[17] and can decrease testicular size and alter testicular morphology[19]. Because of the similarities of action between melatonin and 5–HT and the increase in tissue levels of 5–HT caused by melatonin, we decided to ascertain whether melatonin could alter monoamine oxidase activity (MAO, monoamine: O_2 oxidoreductase [deaminating] E.C.1.4.3.4. – The enzyme that metabolizes 5–HT to 5-hydroxyindole acetaldehyde) of neurogenic tissues both in vivo and in vitro.

Materials and methods. The effects of photoperiod on MAO activity were determined by exposing 24-day-old rats (Sprague-Dawley derived strain, 7 animals per group) to 3 different lighting schedules for 10 days prior to sacrifice: the 1st group was placed in constant light (LL); the 2nd group received 12 h light followed by 12 h dark (LD); the 3rd group was placed in constant darkness (DD). At the time of sacrifice, organs were removed, weighed and frozen for later assay.

Another group of seven 24-day-old rats (Sprague-Dawley strain) was pinealectomized, while the control group was sham-operated. Both groups were sacrificed 9 days after sugery. Hypothalami and pituitary glands were assayed for MAO activity as previously described[20]. Statistical comparisons were accomplished by use of the students *t*-test.

In vitro effects of melatonin on MAO activity were determined by using rats from our small animal colony (Holtzman strain). Aliquots (2 mg) of pituitary, or hypothalami were weighed, homogenized, and assayed for MAO activity[20]. Various concentrations of melatonin (dissolved in 95% ethanol) were added to homogenates in

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