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STUDIES ON THE RATE OF INCORPORATION OF TRYPTOPHAN INTO NICOTINAMIDE–ADENINE DINUCLEOTIDES IN RATS CHRONICALLY EXPOSED TO CARBON DISULPHIDE*

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Abstract—Previous experiments have revealed changes in the metabolism of nicotinic acid and nicotinamide–adenine dinucleotides due to CS_2 intoxication. In this study the incorporation of tryptophan into nicotinamide–adenine dinucleotides in the liver of rats chronically intoxicated with CS_2 has been measured. The concentration and radioactivity of NAD in the liver of rats were determined 5 hr after administration of a loading dose of tryptophan and 2 hr after injection of a trace dose of [^{14}C]tryptophan. It was found that tryptophan was more extensively incorporated into nucleotides in the liver of rats intoxicated with CS_2. This indicates, that tryptophan, rather than nicotinic acid, is the main precursor of NAD synthesis in animals intoxicated with CS_2.

The results from our previous studies have shown chronic exposure to CS_2 of both men [9] and laboratory animals [9, 10, 15] causes an increased urinary excretion of metabolites of nicotinic acid (NAc) and nicotinamide (NAm), but does not produce depletion of nicotinamide–adenine dinucleotides in tissues [11, 12, 14] although the metabolic turnover of the nucleotides is significantly increased [13]. Since, apart from nicotinic acid, tryptophan is the main precursor of NAD, it seemed plausible to suppose that this amino acid could compensate for these requirements in CS_2 intoxicated animals, in which case its utilization for the synthesis of nicotinamide–adenine nucleotides would be significantly increased. Our studies were aimed at verifying this hypothesis.

MATERIALS AND METHODS

The experiments were performed on 3-month-old white rats of the Wistar strain, weighing 170 g at the beginning of the experiment. The animals were exposed to CS_2 vapour at a concentration of 1·7 (1·45–2·05) mg/l of air, for 5 hr daily, 6 days per week over a period of 7–8 months. The control animals were maintained under the same conditions without exposure to CS_2. During the whole experimental period water and diet containing adequate amounts of vitamins and mineral salts were supplied *ad libitum*. Both the control and the exposed animals consumed about 20 g of the diet/day/rat.

The rate of synthesis of nicotinamide–adenine dinucleotides from tryptophan was measured in two experiments, simultaneously in the control and CS_2-intoxicated rats. In the first experiment rats from both groups were injected intraperitoneally with a single dose of 1·2 m-mole per kg body weight of DL-tryptophan. Five hours later the rats were decapitated and the livers excised. The levels of oxidized nicotinamide adenine dinucleotides were determined in the livers before and after the injection of tryptophan according to the method of Sokal *et al.* [7]. In the second experiment intoxicated and control rats were injected intraperitoneally with a single dose of 20 μCi (1 μmole) of DL-tryptophan in physiological solution. DL-Tryptophan [^{14}C] uniformly labeled in the benzene ring, sp. act. 100 mCi/m-mole, was obtained from the Radiochemical Centre, Amersham, England. Two hours later the rats were decapitated and the levels and the specific radioactivity of nicotinamide–adenine dinucleotides in the liver were determined according to the method described in our former paper [13].

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Table 1. The levels of oxidized nicotinamide adenine dinucleotides in the livers of rats exposed to CS_2

	NAD + NADP (m μmole/g)		The rise in NAD + NADP level after tryptophan loading (m μmole/g)
	before tryptophan loading	after tryptophan loading	
Control	652 ± 60 (8)	884 ± 167 (8)	232
CS_2-exposed	666 ± 77 (6)	1036 ± 124* (6)	370

The control rats and rats exposed to CS_2 for 7 months were given intraperitoneally 0·25 m-mole of DL-tryptophan in 0·8 ml of 2·5% $NaHCO_3$. 5 hr later the animals were decapitated and the livers were excised for the determination of the oxidized nicotinamide–adenine dinucleotides, NAD ± NADP.

The results are expressed as mean ± S.D. The numbers of animals in the experimental groups are given in parentheses.

* Significantly different from the control group ($P < 0·05$).

Table 2. Specific radioactivity of NAD + NADP in the livers of control and CS_2-intoxicated rats after administration of [^{14}C]tryptophan

	Number of animals	NAD + NADP (cpm/μmole)
Control	5	6716 ± 1250
CS_2-exposed	5	14600 ± 1983*

Control rats and rats exposed to CS_2 for 7 months were given intraperitoneally 20 μCi (1 μmole) of DL-[^{14}C]tryptophan in 0·5 ml of physiological solution. After 2 hr the animals were decapitated, the livers excised and the specific radioactivity of NAD + NADP determined.

The results are expressed as mean ± S.D.

* Significantly different from the controls ($P < 0·05$).

RESULTS

Rats after 7 months of exposure to CS_2 displayed symptoms of intoxication such as disturbances in equilibrium, muscular weakness and slight paresis of the hind limbs. The mean body weight of the control and exposed animals was 220 and 215 g, respectively. Despite the long period of exposure, concentrations of the oxidized nucleotides in the livers from the control rats and from those exposed to CS_2 before injection of tryptophan were similar (Table 1). Administration of tryptophan in a single dose of 0·25 m-mole per rat resulted in an increased level of oxidized nucleotides in the liver. However, much higher rise in the concentration of the nucleotides was noticed in exposed rats (370 m μmole/g), than in the control animals (230 m μmole/g) (Table 1).

Table results of the second experiment (Table 2) indicate that the incorporation of [^{14}C] tryptophan into liver nucleotides is much faster after exposure to CS_2, since the specific activity of nucleotides is more than 100% higher than that in control animals.

DISCUSSION

Previous studies [6, 9, 10–15] have shown that the increased excretion of metabolites of nicotinic acid and nicotinamide caused by CS_2 intoxication was produced not only by an increased degradation of the nucleotides but was due to the accelerated metabolic turnover of the nicotinamide–adenine dinucleotides. Since, even after long-term exposure to CS_2, the levels of tissue nucleotides are not changed [11], the mechanism of these changes must involve increased delivery of the precursor of nicotinamide dinucleotides to compensate for the higher rate of their metabolic degradation.

Table 3. Effect of chronic CS_2 exposure on the level of tryptophan in rat liver

	Number of animals	Tryptophan (mg/g tissue)
Control	7	3·1 ± 0·2
CS_2-Exposed	7	3·2 ± 0·18

The level of tryptophan was measured in the liver of control rats and experimental animals after 7 months of exposure to CS_2 vapour at a concentration of 1·5–1·7 mg/l of air. The homogenized liver was refluxed with 5N NaOH and tryptophan determined according to method of Spies[8].

The results are expressed as mean ± S.D.

Nicotinic acid and tryptophan are the main precursors of nicotinamide dinucleotides [5]. However, the uptake of nicotinic acid from the diet was shown [13] to be unchanged in rats exposed to CS_2. The results of the present study show an increased rate of utilization of tryptophan for the synthesis of nucleotides in the liver of rats intoxicated with CS_2. This increase was not caused by dietetic factors since the control and intoxicated rats were provided with an adequate and controlled amount of food covering their daily requirements for calories, vitamins and amino acids. The rats of both groups were provided with 22 mg of tryptophan per rat per day, sufficient to cover the daily requirement of the animal for this amino acid [4].

Results, not shown, indicate that the prolonged intoxication with CS_2 does not alter the size of the tryptophan in the liver. The level of endogenous tryptophan in the liver is similar in rats exposed to CS_2 and in control animals (Table 3). Therefore, the difference in specific radioactivity of the nucleotides between control and exposed animals cannot be explained by differences in the specific radioactivity of tissue tryptophan. Any changes in the concentration of liver tryptophan after 7 months of exposure would be reflected in significant changes in the level of nucleotides. Yet the results of a number of our experiments indicated no changes in the level of oxidized nucleotides in the liver of rats intoxicated with CS_2 even after 14 months of exposure [11, 12, 14].

Chronic intoxication with CS_2 induces a significant rise in the activity of tryptophan pyrrolase in the liver of rats [2]. This also supports the conclusion that the rate of conversion of tryptophan to nucleotides is increased.

In conclusion, the results suggest that animals intoxicated with CS_2 use more tryptophan for the synthesis of nucleotides and, in this way, the augmented demands of the organism for the precursor of nicotinamide nucleotides are met.

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