Biochemical Pharmacology, Vol. 34, No. 3, pp. 445–446, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

BIOTRANSFORMATION OF SYMMETRIC DIALKYLNITROSAMINES IN THE RAT INTESTINE

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The importance of small intestinal mucosa in the metabolism of foreign compounds during absorption has been well established. It has been proposed that especially at low concentrations the mucosal barrier may represent an important site of defence against orally ingested carcinogens (1). Although nitrosamines are strongly carcinogenic and widespread in our environment very little is known about their fate during intestinal absorption. Using at first an in vitro technique (2) we studied the capacity of rat jejunal and ileal segments to metabolize symmetric dialkylnitrosamines with 2 to 5 carbon atoms per side chain.

MATERIALS AND METHODS

Chemicals All nitrosamines used in this investigation were 1-^{14}C-labeled and synthetized by one of us (M.W.) except for NDEA which was purchased from NEN (Dreieich, F.R.G.).

Animals Female Sprague Dawley rats (Südd.Versuchstierfarm, Tuttlingen, F.R.G.) were used. Food was withdrawn 18 h before the experiments. For one experiment with NDBA rats were pretreated 3 days with 4 doses (2 doses on the last day) of gentamicin (40 mg/rat) and cefoxitin (100 mg/rat).

Perfusion of rat intestinal segments Segments of about 10 cm length from proximal jejunum and distal ileum were reperfused in a modified all-glass perfusator from the mucosal side (3). Perfusate: 50 ml Tyrode's solution, pH 7.4, 15 mM glucose and one of the four nitrosamines in a concentration range of 10^{-7} to 10^{-3}M. The carbogen gassing the perfusate was extracted in 2 wash bottles containing n-butanol and methoxyethylamine for trapping volatile nitrosamines and CO_2, respectively. After a 2 h perfusion total radioactivity as well as the percentage of unmetabolized nitrosamine was determined in samples of perfusate, absorbate, tissue homogenate and the content of the 2 wash bottles. Glucose and electrolytes were measured in perfusate and absorbate in order to control the viability of the segments.

Analytical techniques Samples of perfusate and absorbate were analysed for unmetabolized nitrosamines by HPLC using a backflush technique (4). Samples of 0.05-5 ml were injected directly onto a concentrating column (10x4.6 mm, RP18 30µ) and eluted with acetonitril/water onto an analytical column (125x4.6 mm, RP18 5µ). With samples of tissue homogenate metabolites were separated on C18 Bond Elut extraction columns (Analytichem, Frankfurt, F.R.G.). The amount of tissue bound radioactivity was determined in the pellet after exhaustive extraction with polar and apolar solvents.

Abbreviations: NDEA, NDPA, NDBA and NDAA stand for N-Nitroso-diethylamine, -di-n-propylamine, -di-n-butylamine and -di-n-pentylamine, respectively.

RESULTS AND DISCUSSION

Nitrosamines did not influence physiological parameters of rat small intestinal segments such as water transport and transfer of glucose and electrolytes. Transport and metabolism of nitrosamines were clearly dependent upon chain length, dose and the intestinal segment used. The results obtained with a low and a high dose are given in table 1.

Table 1. Distribution of radioactivity after 2h perfusion of rat jejunal and ileal segments with one of three nitrosamines[1]

		Conc[2]	n	perfusate	absorbate	tissue	CO_2	cov.b.[3]
NDPA	Jej.	1.18	7	69.3±0.8(25)[4]	4.6±0.5(60)	2.3±0.3(29)	5.07±0.63	0.08
	Il.		6	75.3±1.5(14)	1.2±0.2(22)	3.3±0.3(5)	0.59±0.11	0.02
	Jej.	387	5	76.0±1.9(6)	2.8±0.4(9)	2.2±0.3(3)	1.08±0.17	0.02
	Il.		5	79.5±3.41(4)	1.1±0.2(5)	3.9±0.5(1)	0.12±0.01	<0.01
NDBA	Jej.	0.98	6	69.7±2.7(40)	10.0±0.8(97)	3.0±0.1(70)	2.56±0.46	0.09
	Il.	0.79	6	63.3±2.5(55)	1.8±0.3(94)	3.3±0.3(50)	0.43±0.11	0.02
	Jej.	230	5	47.5±5.4(14)	2.4±0.5(32)	9.3±0.5(8)	3.12±0.49	0.12
	Il.		8	44.8±3.2(8)	1.6±0.7(20)	12.2±0.6(2)	0.21±0.04	0.01
NDAA	Jej.	0.59	7	31.6±3.5(66)	12.0±1.4(86)	9.6±1.0(40)	5.08±0.85	0.20
	Il.		5	47.9±7.0(66)	5.5±0.8(88)	14.4±1.3(38)	0.73±0.04	0.06
	Jej.	350	5	46.1±3.8(15)	2.3±0.3(39)	14.9±1.2(29)	0.78±0.11	0.05
	Il.		5	35.6±4.6(12)	0.5±0.1(28)	16.5±2.1(19)	0.18±0.05	0.02

[1] Values are given as percent of the initial dose, mean±S.E.M.
[2] Concentration of nitrosamines in perfusate, µmol/l
[3] Covalent binding, mean values only
[4] Mean percentages of total metabolites are given in parentheses

Whereas metabolism of NDEA was negligible(not shown in the table), NDBA and NDAA were degraded during absorption to more than 90% at concentrations below 10 µM. Metabolism was accompanied by accumulation of radioactivity leading to maximally 2, 6,and 11 times higher concentrations in absorbate as compared to perfusate with NDPA, NDBA and NDAA, respectively. With increasing doses the metabolic capacity decreased more rapidly in the ileum than in the jenunum. However, the most striking differences between jejunal and ileal segments were found in CO_2 production and in covalent binding. Oral pretreatment with the antibiotics gentamicin and cefoxitin stimulated the metabolism of NDBA at a concentration of 2.24 µM to about 150%.

These results clearly support the importance of intestinal mucosa in the first pass metabolism of xenobiotics. Studies are underway to identify the metabolites formed by rat small intestine. Preliminary results suggest that NDBA is mainly oxidized at the ω-position to give N-nitrosobutyl-(4-hydroxybutyl)amine and -(3-carboxypropyl)-amine which are potent carcinogens producing selectively tumours in the urinary bladder (5). Therefore, rat intestinal metabolism seems to result in a toxification rather than a detoxification of NDBA.

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