Deoxyribonucleases and Nucleic Acid Content of Rat Liver under the Influence of Single Toxic Diethylnitrosamine Doses

When diethylnitrosamine (DENA) and many other dialkylnitrosamines are given in single high doses, widespread liver necroses are observed[1–3]. As measured by the incorporation of thymidine into DNA, regeneration begins between 12 and 24 h after administration of DENA and reaches a maximum 24 to 48 h later[3–5]. Assuming a closer relationship between both deoxyribonucleases (DNases; DNase I, EC 3.1.4.5, DNase II, EC 3.1.4.6) and DNA synthesis[6], one may expect that the activities of both enzymes are influenced by DENA in a consistent manner. Changes may be reversible, because within comparable periods a single high dose of DENA is less likely to induce liver tumours than the prolonged feeding of low doses[7]. These assumptions have been supported by the following investigations.

Material and methods. To 32 female Sprague-Dawley-rats (200–250 g, Wiga, Versuchstier-Zuchtanstalt W. Gassner, Sulzfeld) 210 mg/kg DENA (N-nitroso-diäthylamin, Merck Nr. 820907) were administered intragastrically; 15 animals served as controls and received drinking water in the same manner. At specific time intervals, some animals were killed by decapitation. DNA, RNA, protein, DNase I-, and DNase II-activities of liver homogenates and nuclei were assayed as previously described[6]. Nuclei were prepared from liver homogenates according to the method of DOUNCE[8] with modifications[9]. The isolated nuclei were resuspended in cold 0.14 *M* NaCl and homogenized at high speed in a waring blendor (Bühler, Tübingen).

Results. 24 h after administration of DENA, RNA content and DNase I activity of liver homogenates were diminished significantly by about 50% (Table). DNA content and total DNase II activity of the liver increased by 87 and 197% resp. Maximum effects were reached 24 h (RNA), 2 (DNase I), and 8 (DNA, DNase II) days after administration of DENA. All values turned to normal within some days (RNA), 28 (DNase II), and 49 (DNA, DNase I) days resp. – In close relationship to DNA content, the ratio of DNase II- to DNase I-activities increased by 170, 200, and 370% within 24 h, 2, and 8 days resp. As to the effect of DENA on liver nuclei, DNase II activity was increased reversibly by about 800% within a period of 2–8 days (Figure), whereas DNase I was influenced only in an insiginificant manner.

Discussion. It is commonly accepted that liver necrosis is accompanied by loss of enzyme activities. The fall of RNA- and protein-synthesis (1, 2, 4, 10–13) as well as of RNA- and DNase I-content (Table) may therefore be partly an expression of loss of viable hepatic cells, though more specific effects of dialkylnitrosamines on the mechanism of protein synthesis are probable[4,10–13].

After an initial fall in DNA synthesis[4,5] elevated incorporation of thymidine into DNA – as measured 1–7 days after administration of DENA[3–5] – and an increase of DNA content (Table) probably reflect the replacement of necrotic tissue by newly formed cells in the course of regeneration[3–5]. When compared to DNases (Table), DENA-induced elevation of DNA synthesis is preceeded and/or accompanied by a sharp decrease of DNase I-activity within the homogenates and a distinct increase of nuclear DNase II activity. As to the latter, similar results have been obtained by SCHULZE[14]. With respect to normalization, only DNA synthesis[3,4] and nuclear DNase II activity (Figure) show a certain time-dependent parallelism. Nuclear DNase II may therefore be more specifically implicated in regulating DNA synthesis. Possible relationships between this enzyme and

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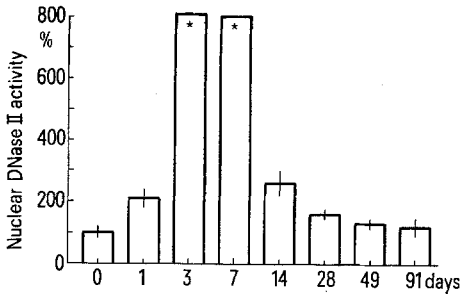
Wet weight (g/1.0 g body-weight), DNA-, RNA- and protein content of the liver (mg/1.0 g body wt.), and total DNase activities (µg DNA-P liberated by liver-DNases/min/1.0 g body wt.) within the homogenates under the influence of 210 mg DENA/kg

Days	0	1	2	8	28	49	71
Wet weight	0.046 ± 0.005	0.040 ± 0.001	0.042 ± 0.006	0.053 ± 0.005	0.046 ± 0.002	0.042 ± 0.002	0.036 ± 0.002[a]
DNA	0.106 ± 0.009	0.120 ± 0.016	0.130 ± 0.008[a]	0.198 ± 0.058[a]	0.175 ± 0.046[a]	0.100 ± 0.008	0.112 ± 0.018
RNA	0.329 ± 0.077	0.167 ± 0.028[a]	0.197 ± 0.034[a]	0.373 ± 0.085	0.382 ± 0.069	0.282 ± 0.020	0.324 ± 0.045
Protein	8.96 ± 0.73	8.96 ± 0.67	8.81 ± 1.18	8.68 ± 0.87	7.39 ± 0.80	8.35 ± 0.62	9.26 ± 0.98
DNase I[d]	2.16 ± 0.29	1.10 ± 0.23[b]	1.05 ± 0.11[c]	1.13 ± 0.34[b]	1.44 ± 0.15[b]	1.82 ± 0.06	2.51 ± 0.36
DNase II	1.48 ± 0.34	2.08 ± 0.31	2.29 ± 0.20[a]	4.40 ± 1.45[a]	1.43 ± 0.15	1.57 ± 0.35	1.27 ± 0.17
Q[e]	0.7 ± 0.3	1.9 ± 0.3[b]	2.1 ± 0.4[b]	3.3 ± 0.7[b]	1.9 ± 0.7[a]	0.9 ± 0.2	0.7 ± 0.2

n = 3–4 animals/group. [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$. [d] DNA (Na-salz, MG 8900000, aus Kalbsthymus, EGA-Chemie Nr. 16820-3, after denaturation by heat. [e] DNase II/DNase I-activities.



Nuclear DNase II-activity (expressed as percent of control value) of livers of rats after single p.o. administration of 210 mg/kg DENA; $n = 8$ (controls) and 2–3 (DENA-groups) resp. Control values: Protein: 3.79 ± 1.28 mg/1.0 mg DNA, DNase I: 0.029 ± 0.010, DNase II: 0.032 ± 0.006 µg DNA-P liberated/min/mg DNA. * To obtain sufficient material for preparation of nuclei, livers were pooled.

a calcium-dependent endonuclease described by HEWISH and BURGOYNE[15] remain to be elucidated.

Zusammenfassung. Durch einmalige p.o. Gabe von 210 mg/kg Diäthylnitrosamin an Ratten wurden die RNA-Konzentration sowie die DNase I-Aktivität der Leber vermindert und die DNase II-Aktivität erhöht. Die Aktivität der Zellkern-DNase II vergrösserte sich bis zum 8fachen der Norm.

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The Effect of Oxotremorine on the Acetylcholine Output from the CSF Containing Spaces

The perfusion technique was widely used for estimation of ACh output from the CNS[1], as well as for the measurement of ACh absorption from the cerebrospinal fluid (CSF)[2]. The present investigation is a study of the pharmacological possibility of influencing this output.

Materials and methods. 33 cats of both sexes weighing 2.0–4.5 kg were anaesthetized with 30 mg/kg pentobarbitone sodium (Sombital, Rafa) intravenously. Cannulas were inserted in the femoral vein to enable further injections and in the trachea to ensure ventilation.

Perfusions of the CSF containing spaces were performed in the cerebral (a) and spinal (b) CSF containing spaces. The inflow in (a) was by a modification of the FELDBERG and SHERWOOD[3] cannula, in which the tip was replaced by a 10 mm No. 18 hypodermic needle. The cannula was inserted as described by BHATTACHARYA and FELDBERG[4], but since the tip was shorter it reached about 1–3 mm above the ventricle. This cannula served as a guide for a specially prepared needle, which was inserted into the ventricle. This modification was performed to ensure proper flow. The outflow cannula was placed in the cisterna magna as described by BHATTACHARYA and FELDBERG[4]. The inflow cannula in (b) was inserted into the cisterna magna and the outflow cannula into the lumbosacral subarachnoid space as described by EDERY and LEVINGER[5].

The osmotic pressure of the artificial CSF[6] was compared to the natural CSF collected from a cannula placed in the lumbosacral subarachnoid space, using an Advanced Instruments Inc. Wide Range Osmometer. This was done in order to evaluate the effects of osmotic pressure differences of the diffusion of substances into the spaces.

The perfusion procedure was similar to that described by EDERY and LEVINGER[5], whereas the perfusion rate was kept constant at 12 ml/h. 5 mg/100 ml eserine were added to the perfusion fluid. The effluent was collected during the first 5 min and in subsequent 20 min samples into graduated test tubes containing 0.2 ml 0.3 *N* HCl. Oxotremorine (OTMN) was added either to the perfusion fluid (0.01 gr/kg/h) or administered i.v. (10 gr/kg). 2-(2,6-Xylidino)5,6-dihydro-4H-1,3-thiazinhydrochloride (Bay Va 1470) was added by i.v. injections (2 mg/cat).

The estimation of ACh in the effluent was performed, after its neutralization, on the isolated guinea-pig ileum, treated with mipafox and morphine[7].

Results and discussion. Table I shows the osmotic pressures of artificial and natural CSF. Many authors used the artificial CSF, which is isoionic to the natural CSF, under the assumption that the effect of the low protein content was negligible[8]. It is seen that the individual variations, as well as the differences between

Table I. The osmotic pressures and their means (mOSM) of subsequent samples collected from the lumbosacral subarachnoid spaces of 3 cats, compared to 4 samples of artificial CSF

Animal 1	Animal 2	Animal 3	Artificial CSF
355	385	319	336
331	338	346	346
328	342	339	342
344	328	326	332
349	330	326	
334	331	335	
336	330	346	
mean = 339	mean = 340	mean = 333	mean = 339

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