Caffeine – DNA Interactions: Biochemical Investigations Comprising DNA-Repair Enzymes and Nucleic Acid Synthesis

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Chicken embryo cells were treated with caffeine (0.5–8.0 mM) alone or combined with various chemical and physical DNA – and/or chromatin-interactive agents. Analytical procedures comprised scheduled (SDS) and unscheduled (UDS) DNA synthesis, RNA synthesis (RNS), the activities of O^6-alkylguanine-DNA alkyltransferase (AT) and poly (ADP-ribose) polymerase (PARP) as well as nucleoid sedimentation. Additional investigations were done in rat thymic and splenic cells. The effect of caffeine on DNase-I activity served as an in vitro-model system.- When present in the PARP-, SDS-, UDS- and RNS-assays, caffeine inhibited the corresponding tracer (^{14}C-NAD, dT-^{3}H, ^{3}H-U) incorporation in a dose-dependent manner. The AT activity was slightly stimulated. At concentrations of 0.06–0.3 mM, caffeine inhibited DNase-I activity by excess substrate. No specific effects of caffeine could be shown by nucleoid sedimentation.- Besides the reduced permeability of the cells to nucleic acid precursors, the results obtained with the PARP- and DNase-I assays give evidence for the formation of a DNA-caffeine adduct as a prominent mechanism of cellular caffeine effects including DNA repair inhibition.

Indroduction

As evidenced by vast literature, caffeine, at extremely high concentrations (within the range of 0.25–10 mM), has various effects on mammalian cells damaged by DNA – interactive physical or chemical agents (for review see, e.g., Garattini, 1993; Müller *et al.,* 1996). Physical agents are UV-light (Schroy and Todd, 1975; Link *et al.,* 1995), X-irradiation (Schroy *et al.,* 1980; Painter, 1980; Müller *et al.,* 1996), and heat (Coss and Smith, 1991). The chemical agents comprise, e.g., carcinogens (Friedlos and Roberts, 1978; Goth-Goldstein and Painter, 1981), and anti-cancer drugs such as adriamycin (Sadzuka *et al.,* 1995), bleomycins (Allen *et al.,* 1985), and cisplatin (Yasutake *et al.,* 1995). Despite of numerous investigations as to the combined exposure of caffeine and DNA-interactive agents, the molecular basis of the observed effects is not well understood. Various mechanisms of action are postulated (Kihlmann, 1977; Kihlmann *et al.,* 1974; Müller *et al.,* 1996; in particular p.59).

Previous results suggested that chicken embryo cells offer a simple, rapid and inexpensive short-term nucleotoxicity test which, combined with in-vitro investigations in DNA-directed enzymes, even allow suggestions as to the mechanism(s) of DNA-interactive agents (Ignatius and Tempel, 1992; Ignatius *et al.,* 1994). Therefore, it was the objective of the present investigation to characterize, *in vitro*, the interaction of caffeine with three DNA-directed enzymes, i.e. deoxyribonuclease I (DNase I), O^6-alkylguanine – DNA alkyltransferase (AT), and poly (ADP-ribose) polymerase (PARP), and to compare the results obtained with those of other commonly used cytotoxicity short-term tests, i.e. DNA and RNA synthesis being performed in chicken embryo brain and liver cells and, though in a preliminary form, thymic and splenic cells of the rat. Physico-chemical analyses comprised the nucleoid sedimentation technique. It turned out that direct interactions between DNA and caffeine are most probably implicated in the effects observed.

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Materials and Methods

Chemicals

[^{3}H] Methyl-thymidine (dT-^{3}H, specific activity 3.0 TBq/mmol) was a NEN^R-product (Dupont de Nemours, Belgium, N. V.-S. A.). [5.6-^{3}H] Uridine (U-^{3}H, specific activity 1.48 TBq/mmol) and nicotinamide [U-^{14}C] adenine dinucleotide (^{14}C-NAD, ammonium salt, specific activity 11.0 GBq/mmol) were purchased from Amersham Buchler, Braunschweig, Germany). Bleomycin and doxorubicin were gifts from Mack (Illertissen, Germany) and Farmitalia Carlo Erba (Freiburg, Germany). Caffeine was from Sigma (München, Germany, No. C 0750). The other test substances and chemicals were from Aldrich (Steinheim, Germany), Merck (Darmstadt, Germany), Serva Heidelberg, Germany), and Sigma (München, Germany).

Cells

We used fertile eggs from White Leghorn chickens from a conventional breeding and female Wistar rats (180–200 g body weight). The embryos were removed from the eggs and sacrified by decapitation at the stage of 15 days after beginning of incubation (37.8 °C, 60% humidity, the eggs rotated six times a day).

Liver-and brain-cells were prepared as described previously, liver tissue solution being performed by collagenase (0.1% in Hank's solution, Ca^{2+}- and Mg^{2+}-free) (for further details see, e.g., Ignatius *et al.,* 1992). Thymic and splenic cells of the rat were prepared as described in a previous paper (Tempel and Spath, 1987). The cell suspensions were adjusted to final concentrations of 10^6-10^7 cells/ml Hank's medium (Ca^{2+}- and Mg^{2+}-free). The liver-, thymic-, and spleen- cell suspensions showed a rather uniform cell picture, whereas the brain-cell preparations, as expected, comprised many cell types which were not identified further. Cell viability was determined by trypan blue exclusion. After 60 min incubation at 37 °C, for instance, 89.9±5% of the thymic cells and 80±6% of the chicken embryo liver-cells were microscopically intact.

Treatment of the cells

To test caffeine, stock solutions of the substance were freshly prepared in Hank's solution and added to the cell suspensions either during a 30 min pre-incubation period, preceding the tracer incorporation assays, or concomitantly with the addition of dT-^{3}H or U-^{3}H following a 30 min pre-incubation without caffeine. When combination effects of caffeine and various nucleotoxic agents were studied, the cells first were treated with these agents, i.e., 30 min by chemical agents or 8 sec with UV-light and 32 min with hyperthermia at 43 °C and X-irradation. Immediately after this pretreatment period, the cell suspensions were further incubated in the presence of caffeine for 90 min, i.e., during the tracer (dT -^{3}H, ^{3}H-U) incorporation period. For X-irradiation of the cells the Kristalloflex 710H X-ray machine (Siemens, Erlangen, Germany) was used (55 kV, 40 mA, half value layer 0.16 mm Cu, filtration with 0.16 mm Cu) at a dose rate of 1 Gy/min (calibrated with a Siemens dosimeter in the center of the useful X-ray beam). During exposure the cell suspensions remained within micro test tubes placed on ice at the beginning of irradiation. The control preparations were sham-irradiated. For UV-irradiation, the cell suspensions were spread out on ice-cooled Petri dishes (0.045 ml/cm^2) and exposed (or sham-exposed) to a germicidal tube (Phillips TUW 30 W, delivering its most energy at 254 nm at an incident fluence of 4 J m^{-2} s^{-1}).

Analytical procedures

The enzymatic analyses comprised *DNase-I-, AT-* and *PARP-* activities. The criterion for *DNase-I* activity was the hyperchromic effect of the assay, measured at 22 °C during an incubation period of 2.5–20 min (Ignatius et al., 1994). Calf thymus DNA (Na salt, MW = 8900,000, Aldrich) was used as substrate.

AT activity was determined in chicken embryo brain extracts as described in detail elsewhere (Ignatius *et al.,* 1994). Briefly, the method is based on the transfer of ^{3}H-labelled methyl groups from the O^6-position of guanine of the substrate DNA to the acceptor protein of the tissue extract. Enzyme activity is represented by the amount of radioactivity in the protein fraction being determined by liquid scintillation counting.

PARP activity was determined in permeabilized cells by the incorporation of the adenosine diphosphate (ADP)-ribose moiety of ^{14}C-NAD into the

acid (trichloroacetic acid)- insoluble precipitates (Junod *et al.,* 1989), the details of the procedure being described in a previous paper (Ignatius *et al.,* 1992). *Scheduled DNA synthesis* and *RNA synthesis* were measured *in vitro* by the incorporation of dT-^{3}H and U-^{3}H, respectively, into the perchloric acid precipitate (6%) of the cell suspensions (for details see, e.g., Ignatius *et al.,* 1994). In order to determine *unscheduled* (repair-induced) *DNA synthesis*, scheduled DNA synthesis was suppressed by hydroxyurea (10^{-1} M) which was added to the Hank's medium during the cell preparation and the following incubations. Concomitantly with the dT-^{3}H and U-^{3}H-activities of the acid-insoluble fraction the *radioactivity of the acid-soluble* fraction was assayed. Preceding precipitation by perchloric acid, the cells were washed 3-times with Hank's solution to terminate the entry of nucleic acid precursors in the cells.

The nucleoid sedimentation technique (Cook and Brazell, 1975; Cook and Brazell, 1976; Mattern *et al.,* 1987) was performed as described previously (Ignatius *et al.,* 1994).

In general, each data point represents the mean of at least three independent experiments (± the standard deviations). The significance was tested by Student's t-test.

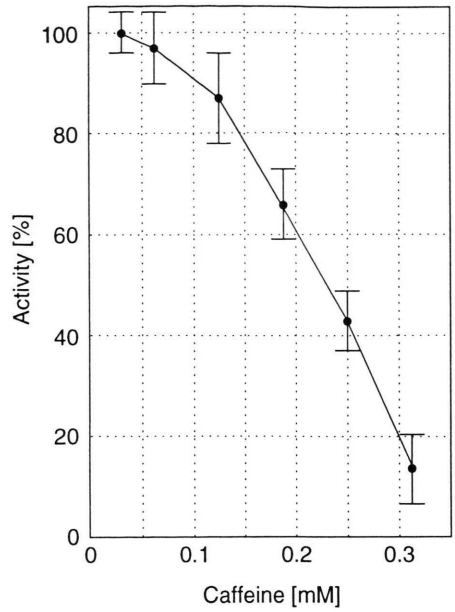

Fig. 1. DNase-I activity in the presence of caffeine. The enzyme assay consisted of 700 µl Tris(hydroxymethyl)-aminomethane/HCl-buffer, pH 7.4, 100 µl DNase I – solution (1.357 Kunitz units) in aq. bidest., 50 µl of an aqueous solution of 124 µg DNA, 50 µl 4 M $MgCl_2$, and 100 µl of an aqueous solution of caffeine being 30 min pre-incubated with the enzyme-free reaction mixture. Values given in per cent of the caffeine-free control assay. Control values: Increase in absorbance of the reaction mixture: 0.5±0.05/20 min at 22 °C.

Results

Caffeine inhibited the *DNase-I* reaction in a dose-dependent manner, the DE_{50}-value being approximately 0.23 mM (Fig. 1). At DNA concentrations of 31.3–250 µg/assay (1.1 ml), inhibition by excess of substrate was evident (Fig. 2).

Before the influence of caffeine on *AT* activity was investigated, the sensitivity of the AT assay was tested by incubating the enzyme extract with two substances known to inhibit AT activity in a definite manner, i.e., ethidium bromide (EB) (Link and Tempel, 1991) and DNA (Bhattacharyya *et al.,* 1990). The inhibiting activity of EB and DNA was evident at the concentrations used, i.e., > 6.3 x 10^{-6} and > 0.06 x 10^{-8} M, respectiviely, and showed a distinct dose-relationship, with DE_{50}-values being about 17.4 µM EB and 1.4 nM DNA (Table I).

Under the same conditions caffeine, at concentrations of 2–8 mM, did not inhibit AT activity. However, at concentrations of 2–4 mM the substance exerted a slight, though significant, stimulating effect on the enzyme activity (Table I).

Table I. O^6-Alkylguanine-DNA alkyltransferase (AT) – activity of chicken embryo brain cells following exposure to caffeine, ethidium bromide, and DNA. Values given in per cent of the controls. Control values: 70 fmol O^6-methylguanine transferred per 1 g protein of the AT assay.

Caffeine		Ethidium		DNA	
mM	%	µM	%	nM	%
2	110±1	6.34	77±4	0.7	72±5
3	120±3	12.68	57±3	1.4	51±4
4	112±5	25.36	39±4	2.8	28±3
6	102±4	50.72	14±4	5.6	18±2
8	96±5	---	---	11.2	12±2

Like its inhibiting effect on DNase-I activity (Fig. 1), caffeine diminished *PARP*-activity in a concentration-dependent fashion (Table II). Caffeine concentrations of about 2.0 mM were needed

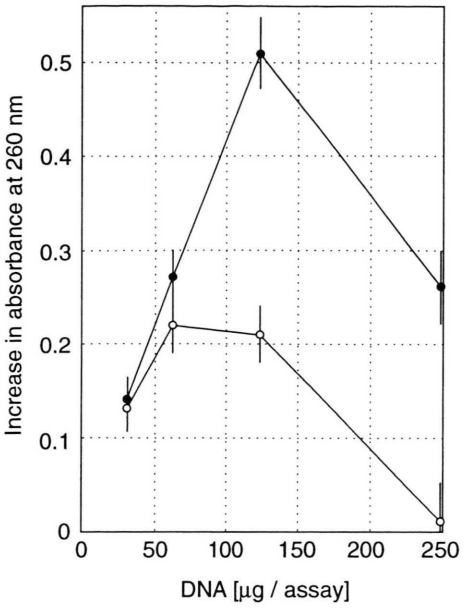

Fig. 2. DNase-I activity in the presence of 0.25 mM caffeine in dependence on substrate concentration; rate of reaction as increase in absorbance at 260 nm during an incubation period of 20 min; ■ controls, ○ with caffeine.

Table II. Poly(ADP-ribose)polymerase (PARP) – activity of chicken embryo brain cells following exposure to caffeine, 3-aminobenzamide, and ethidium bromide. Values given in per cent of the controls. Control values: 144±59 pmol ^{14}C-NAD/mg DNA.

Caffeine		3-Aminobenzamide		Ethidium	
mM	%	mM	%	mM	%
0.5	96±16	0.016	85±6	0.03	105±9
1.0	68±12	0.032	58±4	0.15	66±7
2.0	51±10	0.064	33±4	0.30	50±7
4.0	36±5	0.128	26±3	1.50	20±7
8.0	21±3	0.256	21±5	3.00	7±2

to inhibit the enzyme activity by 50%. By using 3-aminobenzamide, a specific PARP-inhibitor, and ethidium bromide the same effect was obtained by 0.03–0.04 and 0.3 mM, respectively (Table II).

The *incorporation of the nucleic acid precursors*, dT-^{3}H and U-^{3}H, into the acid insoluble fraction of the cells remained unchanged, even at the highest caffeine-concentrations used (6 mM), when the cells were preincubated with the xanthin derivate and washed thereafter in caffeine-free Hank's medium (Table III). A distinct and dose-dependent inhibition of nucleic acid synthesis, however, could

Table III. Scheduled DNA synthesis of chicken embryo brain – and liver-cells (B-and L-cells, respectively) as well as of rat thymic and splenic cells (T-and S-cells, respectively) under the influence of 1.0 mM caffeine. A: Exposure of the cells during a 30 min pre-incubation period (prior to the addition of dT-^{3}H). B: Addition of caffeine concomitantly with dT-^{3}H. Values given in per cent of the controls. Control values of dT-^{3}H incorporation: B-cells: (1.1±0.2)x10^6 dpm/µg DNA, L-cells: (0.8±0.15)x10^6 dpm/µg DNA, T-cells: (6.5±1.5)×10^6 dpm/µg DNA, S-cells: (1.5±0.5)x10^6 dpm/µg DNA.

	B-Cells	L-Cells	T-Cells	S-Cells
A	97±7	96±8	96±5	95±11
B	77±11	58±9	59±6	41±11

be observed when caffeine was present in the dT-^{3}H or U-^{3}H incorporation assay, the sensitivity ranging in the order scheduled DNA synthesis (SDS) > RNA synthesis (RNS) > unscheduled DNA synthesis (UDS) (Table IV). At caffeine-concentrations of 6 mM, for instance, SDS, RNS, and UDS decreased by about 60, 50, and 35%, respectively. No principal differences existed between the cell preparations used, i.e., chicken embryo brain and liver cells as well as thymocytes and splenocytes of the rat (Table III).

The radioactivity of the acid-soluble fraction decreased as well, the corresponding dT-^{3}H values being always significantly lower than those of the acid-precipitate. The most significant differences between both fractions were seen with respect to the scheduled DNA synthesis. At 1.0 mM caffeine, e.g., the incorporation of dT-^{3}H into the acid-insoluble and into the acid-soluble fraction was dropped to 74 and 10 per cent, respectively (Table IV).

To obtain additional information as to the mechanism of caffeine action, brain cell suspensions were first treated with a number of chemical and physical agents of known, though different, action mechanism. Following pre-exposure, SDS was determined either in the presence or in the absence of caffeine. From the results given in Table V, a strictly additive effect of caffeine and the agents used is suggested. Especially, no indications as to synergistic interactions were obtained.

The *nucleoid sedimentation* technique was used to measure DNA strand breaks. Chicken embryo brain cells were treated either with caffeine alone or with a combination of caffeine and ethidium

Table IV. Scheduled (SDS) and unscheduled (UDS) DNA synthesis, and RNA synthesis (RNS) of chicken embryo brain cells under the influence of caffeine. Values given in per cent of the controls. Control values: SDS: $(1.1 \pm 0.2) \times 10^6$ dpm/µg DNA, UDS: $(0.33 \pm 0.06) \times 10^6$ dpm/µg DNA, RNS: $(0.22 \pm 0.019) \times 10^6$ dpm/µg DNA.

Caffeine	Scheduled DNA synthesis		Unscheduled DNA synthesis		RNA synthesis	
[mM]	insoluble	soluble	insoluble	soluble	insoluble	soluble
0.5	86±10	49±8	100±9	85±7	90±8	90±10
1.0	74±10	10±6	90±11	70±7	81±8	88±9
2.0	55±7	7±4	79±9	55±9	63±7	79±4
4.0	42±8	5±2	68±11	45±8	53±7	63±8
8.0	37±7	3±3	62±8	38±5	48±9	48±6

Table V. Scheduled DNA synthesis (SDS) of chicken embryo brain cells under the influence of caffeine (1 mM) and various DNA- and/or chromatin-interactive agents. With respect to the dose-effect-relationships determined previously (Tempel *et al.*, 1992), the agents were tested at concentrations (doses) resulting in a SDS-inhibition by < 50%. Values given in per cent of the controls.

Agent	Concentration	SDS with		SDS with caffeine + agent	
		caffeine	agent	Calculated	Determined
	[µg/ml]	%	%	%	%
3-Aminobenzamide	1089	70±5	106±5	74±8	76±9
Bleomycin	500	74±6	67±6	50±7	57±8
Cysteine.HCl	300	77±5	77±10	59±7	64±9
Dideoxythymidine	100	70±8	70±8	49±9	58±5
Doxorubicin	5	77±5	50±5	39±7	38±11
Ethidium	15	78±5	69±4	54±8	51±7
Hoechst 33258	30	78±5	61±5	48±9	49±7
Hydrogenperoxide	1.7	77±10	62±6	48±9	48±10
Methylnitrosourea	258	74±8	69±4	51±8	53±5
Novobiocin	250	70±5	63±6	44±7	42±7
Hyperthermia	43°	73±5	79±7	58±8	61±3
UV-Light	32 Jm^{-2}	68±4	86±6	58±6	57±5
X-rays	8 Gy	72±10	73±3	51±8	53±11

bromide (EB), X-rays, UV-light, or hyperthermia. The results are given in Table VI. Whereas nucleoid sedimentation remained unchanged at the caffeine concentrations tested (0.125–4.0 mM), nucleoids of EB-pretreated brain cells showed a reduced sedimentation rate, the differences between controls and caffeine-treated cells being significant at concentrations of 4.0 mM (Table VI).

To investigate the potential DNA repair inhibiting activity of caffeine, brain cells were exposed to three different physical agents, i.e., X-rays, UV-light, and hyperthermia. Even at the highest concentrations used caffeine did not exert a significant effect.

Table VI. Nucleoid sedimentation of chicken embryo brain cells under the influence of caffeine (0.5–4.0 mM) and ethidium bromide (200 μg/ml), X-rays (16 Gy), UV-light (32 J m^{-2}), and hyperthermia (30 min at 43 °C). Values given in per cent of the controls. Control values: Sedimentation distance 1.81±0.17 cm; *: $p < 0.05$.

Caffeine [nM]	0.5	1.0	2.0	4.0
Hank's	100±4	102±6	103±8	100±4
Ethidium	96±4	96±7	90±6	86±6*
X-Rays	102±5	102±3	96±2	94±7
UV-Light	100±5	104±4	113±10	100±6
Hyperthermia	102+6	101+6	101+7	102±5

Discussion

At extremely high concentrations, within the range of 0.25–10 mM ("corresponding to several hundred cups of coffee or tea per day"), caffeine is considered to be a DNA repair inhibitor (Müller *et al.,* 1996). Despite a great number of investigations performed since the sixties the mechanism(s) of action is (are) not well understood. As summarized recently by Müller *et al.,* interactions of caffeine with DNA repair enzymes, and/or DNA precursor pools have been suggested (for references see, e.g., Müller *et al.,* 1996). A prominent and almost generally accepted mechanism of radiosensitization by caffeine is the ability of the drug to prevent the radiation-induced G_2-delay thereby reducing the time "available for repair before final fixation of damage during mitosis" (Müller *et al.,* 1996; Bernhard *et al.,* 1996; further references in these papers).

To get further insight into the mechanism of caffeine, the interactions of the substance with DNase-I, O^6-alkylguanine-DNA alkyltransferase (AT) and poly (ADP-ribose) polymerase (PARP), nucleic acid synthesis and chicken embryo brain nucleoids, were studied.

Irrespective of the molecular biology of DNases (Weir, 1993), it could be shown that caffeine inhibits *DNase-I* activity by excess of substrate (Fig. 1 and Fig. 2) which confirms the interaction of caffeine with DNA *in vitro,* otherwise evidenced by physico-chemical methods (Pohle and Fritzsche, 1981; Tornaletti *et al.,* 1989; Kunicka *et al.,* 1990).

Up to now no conclusions can be drawn as to the potential caffeine-induced inhibition of the endonucleolytic DNA catabolism *in vivo.* Treating human TK6 lymphoblastoid cell lines with 2 mM caffeine, either before or up to 3 hours after Gamma-irradiation *in vitro,* eliminated the degradation of DNA and decreased the percentage of apoptotic cells (Zhen and Vaughan, 1995). On the other side, reducing the radiation-induced G_2-delay of HeLa cells caused the cells to undergo apoptosis instead of mitotic death (Bernhard *et al.,* 1996).

AT protects cells against the genotoxic effects of monofunctional alkylating agents by converting O^6-alkylguanine residues into guanine. The cysteine of an active site-cysteine residue is alkylated stoichiometrically and thereby the AT protein is inactivated and not regenerated ("suicide mechanism"), the mechanism of action of AT-proteins being represented as an S_{N2}-reaction (Pegg *et al.,* 1993; Pegg *et al.,* 1995). Though it might be speculated that caffeine influences the correct alignement (Tenud *et al.,* 1970) of the nucleophile (cysteine thiolate) and the substrate (O^6-alkylguanine), the extent of AT stimulation by caffeine is to small (Table I) as to allow further suggestions.

Many substances, including methylxanthines, act as *PARP*-inhibitors (Ujiie and Goshima, 1988; ibid further references). Poly (ADP-ribosyl)-ation is a post-translational protein modification which is catalyzed by PARP. PARP is a highly conserved nuclear enzyme using nicotinamide-adenine dinucleotide (NAD) as substrate. Since the DNA-binding domain of the enzyme specifically binds to DNA-strand breaks, PARP activity is increased by DNA-strand breaking agents, such as hydrogenperoxide, bleomycin, or ionizing radiation (for review see, e.g., DeMurcia *et al.,* 1994). On the other hand, masking DNA-strand breaks by, e.g., intercalating agents such as ethidium bromide may decrease the enzyme activity (Table II) (Ignatius *et al.,* 1992). In analogy to the results obtained by using the DNase assay (Fig. 1 and Fig. 2), it may be suggested, therefore, that caffeine inhibits PARP-activity *in vitro* by masking DNA-strand breaks being constitutively present in chicken embryo cells (Tempel *et al.,* 1992). As shown by, e.g., Malanga and Althaus, two enzymes, PARP and poly (ADP-ribose)glycohydrolase, may function cooperatively "as a histone shuttle mechanism at breakage sites on DNA" and "establish local DNA accessibility for proteins involved in DNA repair" (Malanga and Althaus, 1994). With respect to the prevention of the radiation-induced G_2-block, one may conclude, therefore, that caffeine, by binding

to DNA, decreases the accessibility of DNA to damage recognition proteins regulating the cell cycle following DNA damage (for review see., e.g., Kaufmann and Paules, 1996; Lohrer, 1996).

The *incorporation of nucleic acid precursors* into DNA and RNA of chicken embryo brain- and liver-cells as well as of thymic and splenic rat cells remained unchanged when the cells were preincubated with caffeine and washed thereafter in caffeine-free Hank's medium. When present during the precursor incorporation phase, caffeine inhibited the incorporation of dT-^{3}H and U-^{3}H into the acid-insoluble fraction of the cells (Tables III and IV). As shown by the results presented in Table IV, this inhibition was dose-dependent and comprised scheduled (SDS) and unscheduled (UDS) DNA synthesis as well as RNA synthesis (RNS) in the order of SDS> RNS> UDS. Waldren *et al.* evidenced that caffeine inhibits de novo-synthesis and reutilisation of purines as well as the synthesis of pyrimidines in cultured mammalian cells (Waldren *et al.,* 1983). Purine- and pyrimidine-nucleosides enter cells by facilitated-diffusion carrier mechanisms exhibiting a rather broad specificity (Young and Jarvis, 1983). Since rather heterogenous drugs are able to inhibit nucleoside transport (ibid), the possibility must be considered that caffeine, at the concentrations used, interferes with the metabolism of the nucleic acid precursors, i.e., dT-^{3}H and U-^{3}H. Therefore, the incorporation of dT-^{3}H into the acid-soluble fraction was measured in brain cells. As shown in Table IV, the acid-soluble ^{3}H-activity decreased sharply and in a dose-dependent manner under the influence of caffeine. Since the corresponding dT-^{3}H values were always significantly lower than those of the acid-insoluble fraction, it is very probable that the apparent inhibition of nucleic acid synthesis reflects a decrease in cellular permeability of the nucleic acids precursors. This is confirmed also by the fact that the caffeine-effect on nucleic acid synthesis could only be shown when caffeine was present during the precursor incorporation phase (Table III) and the strictly additive character of the combination effects of caffeine and various DNA-(chromatin-) interactive agents of different action mechanisms (Table V).

The *nucleoid sedimentation* technique was used to reveal possible changes in chromatin structure (Cook and Brazell, 1975; Cook and Brazell, 1976; Mattern *et al.,* 1987). A decrease in nucleoid sedimentation could only be shown when chicken embryo brain cells were treated with ethidium bromide (EB) and caffeine. Because EB, at the concentration used, induces chromatin condensation as reflected by an increase in nucleoid sedimentation (ibid., Tempel, 1996), it follows from the combination effects that caffeine diminishes the EB effect possibly by decreasing the access of the intercalator to DNA due to the formation of a DNA-caffeine adduct, by decreased cellular permeability of EB when combined with caffeine (Kimura and Aoyama, 1989) and/or by the formation of caffeine complexes with DNA intercalators, including EB (Larsen *et al.*,1996; Traganos *et al.,* 1991). Since these three phenomena, i.e., binding of caffeine to DNA (Fig. 2), reduced EB-permeability, caffeine-DNA-intercalator complexes are evidenced (ibid.), the sedimentation analyses present no definite conclusions.

All in all, the most prominent results may be summarized as follows:

(I) At concentrations inhibiting DNA-repair, caffeine is able to influence the activities of various DNA-directed enzymes.

(II) The inhibition of a mammalian DNA endonuclease, i.e. DNase I, which could be shown for the first time, is one of the most sensitive parameters for the caffeine action.

(III) From the enzymatic analyses, a caffeine-DNA adduct is suggested which may prevent the accessibility of DNA to damage recognition proteins regulating radiation-induced G_2-block as well as inhibition of replication initiation normally caused by, e.g., ionizing radiation and ultraviolet light.

(IV) At rather low concentrations, caffeine decreases cell permeability to nucleic acid precursors.

(V) Because all caffeine effects mentioned above are observed in the mM range which is hardly to attain in a living body, the present results are of experimental rather than of physiological and/or toxicological relevance.

(VI) Whereas the concentrations needed to inhibit DNA-repair precludes therefore caffeine as a substance to sensitize tumor cells to anticancer agents *in vivo,* other methylxanthines might give more promising results and should be investigated further.

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