crease, this was also computed as 100%, as no higher voltages were tested to avoid damaging the insects.

The effect of injections of arginine, morphine, naloxone and mixtures of them on the voltage threshold was studied (table). We confirmed our earlier results[8] showing that the dose of morphine which produced an increase of 50% in the stimulus threshold 2 h after injection (ED_{50}) was 0.35 mg/g of insect. Arginine also inhibited the deimatic reaction in a dose dependent manner, increasing the stimulus threshold. The same concentration of naloxone antagonised the effect of morphine and arginine at their ED_{50}. Naloxone injected alone had no significant effect on the stimulus threshold until concentrations of 64 µg/g or more were injected[8].

These results show that arginine exerts an action in the praying mantis similar to that of morphine. The fact that arginine also affects memory consolidation suggests some similarity to the effect that opiates have in vertebrates[4], indicating that this amino acid could have a neural activity in insects like that of endorphines and other opiates in vertebrates, although the effect of opioids on memory in insects is not yet known. The concentration of arginine that facilitates memory consolidation in the praying mantis[7] is the same as the ED_{50} found here. The actual amount reaching the nervous tissue is unknown, since the substances were injected into the thoracic cavity of the insects. This fact could explain the need for high doses of these drugs in our experiments and in those with shrimps and bees, although other explanations are also possible[2,3,8]. These findings taken together suggest a neuro-modulator role for arginine which was formerly unexpected.

1 We acknowledge the technical help of Alberto Meza and Alfonso Tablante and critical comments from Dr Carlo Caputo and Dr Erika Jaffe.

2 Maldonado, H., and Miralto, A., J. comp. Physiol. *147* (1982) 455.
3 Nuñez, J., Maldonado, H., Miralto, A., and Balderrama, N., Pharmac. biochem. Behav., in press.
4 Belluzzi, J.D., and Stein, L., Ann. N.Y. Acad. Sci. *398* (1982) 221. McGaugh, J.L., Martinez, J.L., Messing, R.B., Liang, K.C., Jensen, R.A., Varquez, B.J., and Rigter, H., in: Regulatory Peptides: from Molecular Biology to Function, p. 123. Eds E. Costa and M. Trabucchi. Raven Press, New York 1982.
5 Maldonado, H., Z. vergl. Physiol. *68* (1970) 60..
6 Jaffe, K., and Maldonado, H., J. Insect Physiol. *25* (1979) 319. Maldonado, H., J. Insect Physiol. *26* (1980) 339.
7 D'Alessio, G., DiDonato, A., Jaffe, K., Maldonado, H., and Zabala, N.A., J. comp. Physiol. *147* (1982) 231.
8 Zabala, N.A., Jaffe, K., and Maldonado, H., Acta cient. venez. *33* (1982) 353. Zabala, N.A., Miralto, A., Maldonado, H., Nuñez, J.A., Jaffe K., and Calderon, L. de C., Pharmac. biochem. Behav. *20* (1983) in press.

0014-4754/84/070733-02$1.50 + 0.20/0

Hydrogen bond catalysis of mononucleotide ethylation supports non-random DNA alkylations by N-ethyl, N-nitrosourea

K.-W. Stahl[1] and F.E. Köster

Zentrum Biochemie, Hannover School of Medicine, D-3000 Hannover 61 (Federal Republic of Germany), 18 March 1983

Summary. The axial (ax.) and equatorial (eq.) diastereomeric forms of phosphate triesters resulting from reactions of N-ethyl, N-nitrosourea with 3 cyclic mononucleotides were analyzed by column liquid chromatography (CLC). Evidence is presented that the 2'OH group of 3', 5'cAMP essentially contributes to the stereoselective eq. alkyl substitution, most probably by hydrogen bonding catalysis. The neighboring group direction of ethylation gives substantial support to non-random DNA alkylations by NEU.

Recently it has been shown that when intact cells are exposed to 2 polycyclic hydrocarbons[2,3] and N-acetoxy-acetylaminofluorence (AAAF)[4], these compounds bind preferentially to sequences of chromosomal DNA involved in various functions of genetic control. This is in keeping with the molecular model of tumorigenesis[5] suggesting that mutagenic agents reacting with promotor and/or supressor sequences could turn on transforming genes. Also, a number of authors agree on the fact[6-11] although not on the type[6-10] of non-random DNA alkylations by N-nitroso compounds. In contrast to the genotopic selectivity of bulky substituents[2-4], for which steric accessibility to chromatin components[12,13] was assumed to be an essential factor, no chemical reason has been forwarded so far to explain how certain DNA regions could be hyperreactive to methylating and ethylating N-nitroso compounds. We have investigated stereoselective phosphate alkylation of 3', 5' cAMP, 3', 5' c(2'0-ethyl)AMP, 3', 5' c(2'-deoxy) AMP and adenylyl (3' → 5') adenosine (ApA) by N-ethyl, N-nitrosourea (NEU). In this report we present evidence that the 2'OH group catalyzes the ethylation reaction of NEU. Such neighboring group catalysis may have implications for the mechanisms of non-random DNA alkylation by N-nitroso compounds.

It was shown 30 years ago[14] that phosphate groups of DNA could be esterified by alkylating agents and that the triester so formed alkylated nucleobases in a second step reaction[15]. After Loveless[16] had described O^6-alkylated products from in vitro reactions of deoxyguanosine with NEU and 2 other mutagens, it was gradually recognized in the 1970s, and finally become well established, that over 80% of NEU modification of nucleic acids is on oxygens[17].

When we reacted 0.2 M 3', 5' cAMP (^{14}C uniformly labelled, approximately 80 µCi/mmol^{-1}) with 1 M NEU in 200 µl ethanol containing 20% 1 M triethylammonium hydrogencarbonate buffer pH 7.2 for 5 h under gentle shaking at 20 °C we obtained an approximate yield of 25% of alkylated nucleotide and more than 90% of the NEU was decomposed. This was monitored by thin layer chromatography (stationary phases: cellulose (TLC_A) and silica gel (TLC_B); mobile phases: iso-propanol −1% $(NH_4)_2SO_4$ = 2/1, v/v (TLC_A) and chloroform-methanol = 17/3, v/v (TLC_B)) and high efficiency column liquid chromatography (CLC). 92% of total alkylated 3', 5' cAMP was substituted in the phosphate (with the consequence of a hetero-tetracoordinated phosphorus), 8% in the ribose (2'-OH) and no detectable amount in the purine base moiety. This was in keeping with our previous results from adenine nucleotide reactions with NEU[18].

When we synthesized the neutral P-O-ethyl ester of 3', 5' cAMP according to Preobrazhenskaya et al.[19] by activation with di-phenyl-phosphorochloridate and subsequent alcoholysis with ethanol we found an approximately 1:1 ratio of both

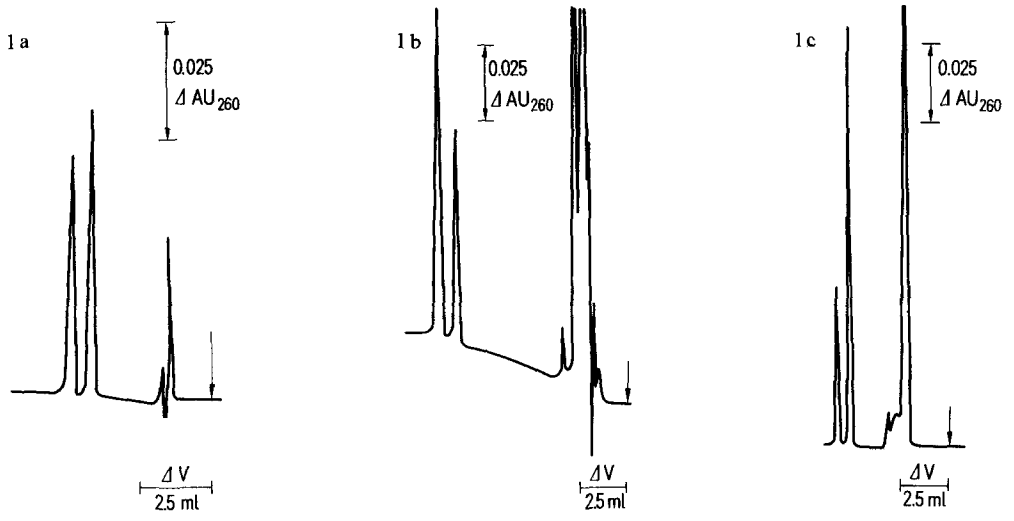

Figure 1. CLC analysis of 3′, 5′ cAMP (1a, 1c) and 3′, 5′c(2′O-C_2H_5)AMP (1b) reacted with either di-phenyl-phosphorochloridate and ethanol[17] (1a) or NEU (1b, 1c). Based on a technique previously developed for the separation of diastereomeric α-thiophosphate analogues of adenine nucleotides[21] the 2 diastereomeric phosphate triesters of the cyclic nucleotide presenting the exocyclic ethyl group either in the axial (ax.) or in the equatorial (eq.) position were separated on a stationary phase (5 µm Nucleosil SA 5, Machery & Nagel, Düren, FRG) with strong cation exchange and reversed phase properties. CLC was carried out using glass columns (Riedel de Haen, Seelze, FRG) of 300 mm length and 3 mm inside diameter which had been 'balanced-slurry' packed under maximum pressure (200 bar) and a DMP-1515 Orlita pump with a home-made damping device[21] and a PM 4 variable wave length photometer from Zeiss with an 8-µl cuvette. Quantiative peak analysis was performed on line with the Spectra Physics system I integrator. The mobile phase was 20% ethanol in 0.2 M CH_3COONH_4 adjusted to pH 4.5 with 1 M CH_3COOH. Under the anion exclusion conditions, negatively charged cyclic nucleotides and charge-free urea are eluted close to the dead volume (V_0 = 1.4–3.7 ml) of the column. The P-O-ethyl esters of the cyclic nucleotides are eluted as 'twin' peaks with differing partition coefficients (K) for the 2 diastereoisomers ($K_{eq.} < K_{ax.}$). Conformational assignments of the 2 diastereomeric forms was effected by ^{31}P-NMR measurements[20,22] (d_6-acetone, δ = ppm for PO_4^{3-}) showing $\delta_{ax.}$ = 6.941 ppm and $\delta_{eq.}$ = 5.110 ppm for the axial and equatorial forms respectively.

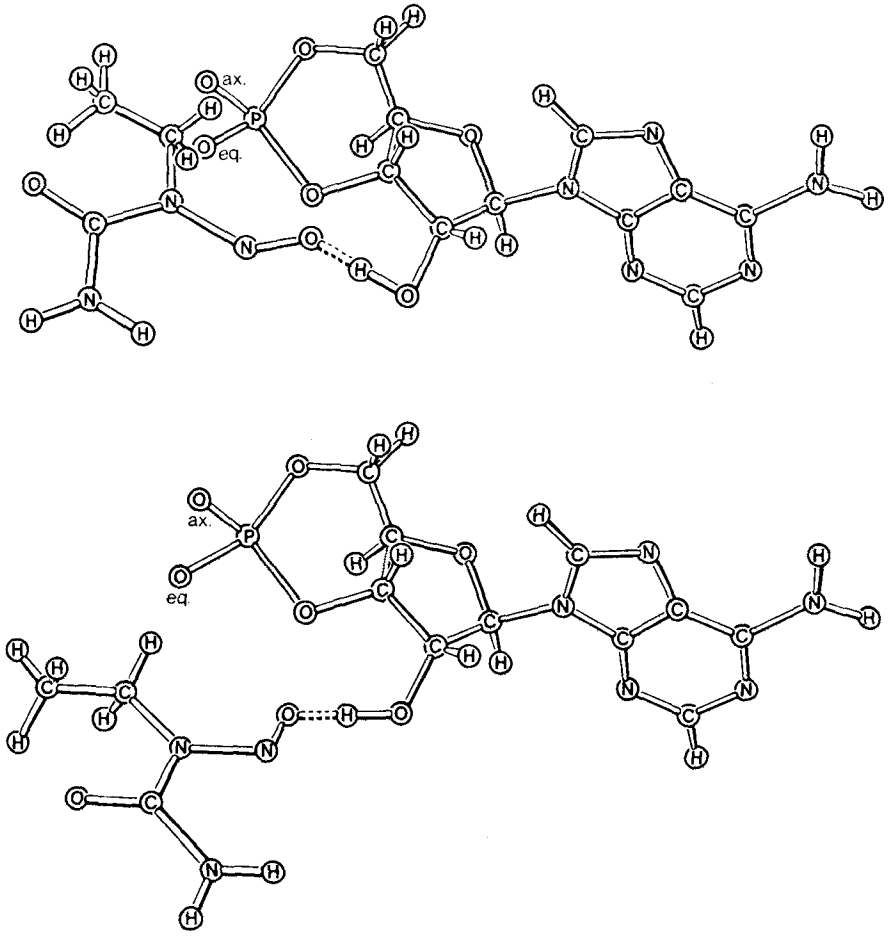

Figure 2. Molecular model of NEU reacting with 3′, 5′ cAMP through 2′OH-group-activation and hydrogen bond formation. It can be clearly seen that the equatorial-positioned P→O nucleophile (top) is a preferential carbonium ion acceptor in this reaction.

triester isomers (48% axial (ax.) and 52% equatorial (eq.) as shown in chromatogram (fig. 1a)). This equilibrium of the diastereoisomers was to be expected, as the large di-phenyl-phosphorochloridate substituent forces the 6-membered 1,3,2-dioxaphosphorinane ring to form a 'chaise longue' conformation with a significant loss of preferential stability of one of the 2 isomer configurations[20]. A 1:1 ax./eq. ratio was also obtained (results not shown) when we reacted the di-adenosinemonophosphate ester, ApA, with NEU under the standard conditions described for 3', 5' cAMP.

The amount of ax. (30%) and eq. (70%) conformers of the neutral P-O-ethyl ester of 3', 5' cAMP which resulted from repeated incubations with NEU is shown in the CLC chromatogram (fig. 1c).

This finding was in complete contradiction to the results of Engels and Pfleiderer[23]. In keeping with the greater thermodynamic stability of axial O-alkyl in 2-substituted 1,3,2-dioxaphosphorinanes[20] as a consequence of the gauche-effect[24] they presented evidence for a 7:3 ax./eq. ratio from the analysis of data obtained from NMR spectra of the phosphotriesters. This ratio corresponds exactly to the values we found (fig. 1b), when we reacted the 2-ethyl ether 3', 5' cAMP (which we had previously synthesized by alkylation with ethyl-iodate accordingly to Tazawa et al.[25]) and 3', 5'c-d2'-AMP (results not shown) with NEU according to our standard procedure.

Our results are straightforward and, in our view, their explanation seems to be obvious. In the SN_1-type reaction of diazoalkane and NEU the carbonium ion reacts with the P→O nucleophile which in the case of the 2-substituted 1,3,2-dioxaphosphorinane shows a greater thermodynamic stability in the $P\lesssim{}^{O}_{O}{}^{ax.}$ than in the $P\lesssim{}^{O}_{O}{}_{eq.}$ position and favors the formation of P-O-ethyl$_{ax.}$ stereoisomer. It can be seen from the molecular model shown in figure 2 that the hydrogen bond between the 2'-OH group of the ribose moiety and the N→O group of NEU reacting with 3', 5' cAMP will induce a labilization of the N-alkyl bond in NEU and thus favor the formation of the ethyl carbenium. This carbonium will then react preferentially with the closest nucleophilic site which is the equatorially positioned P→O. When neighboring group catalysis of the 2'-OH cannot possibly occur as the hydrogen is either substituted by an alkyl residue (3', 5'c(2' O-C_2H_5)AMP) or missing (3', 5'c d2'-AMP), then oxygen substitution in the phosphate moiety of cyclophosphate nucleotide leads to the thermodynamically favored equilibrium of ax.: eq. = 7:3.

At present it may be difficult to prove hydrogen-bond-catalysis in chromatin alkylations by N-nitroso compounds. But in theory, it can be expected that histones and other nuclear proteins determining tertiary DNA structure contain a variety of appropriate hydrogen-bond donator groups. In hyperreactive genome regions their effect on alkyl transfer to nucleophilic DNA sites might be compared to the catalysis of active centers from transferase enzymes.

Despite tremendous research effort in this field our knowledge of the qualitative and quantitative relationships between the tumorigenicity of N-nitroso compounds and DNA alkylations[26] and also of the persistence of promutagenic DNA lesions[27] remains unsatisfactory. It may yet be possible to relate tumorigenicity to DNA alkylations but only when the genotopic selectivity of the promutagenic lesions can be consistently analyzed and better understood.

1 We acknowledge the helpful cooperation of Dr Fritz Eckstein, M.P.I. for Exp. Medicine, Göttingen, who determined the ^{31}P-N.M.R. spectra of the diastereomeric forms of the neutral ethyl-ester of 3', 5' cAMP. This work has been supported by a personal grant to one of us (Sta 131/6 PAN) from the Deutsche Forschungsgemeinschaft. Reprint requests to K.-W. Stahl.

2 Shoyeb, M., Proc. natl Acad. Sci. *75* (1978) 5841.
3 Jack, P., and Brookes, P., Int. J. Cancer *25* (1980) 789.
4 Beard, P., Kaneko, M., and Cerutti, P., Nature *291* (1982) 84.
5 Dulbecco, R., Umschau *82* (1982) 262.
6 Ramanathan, R., Rajalakshmi, S., Sarma, D.S.R., and Farber, E., Cancer Res. *36* (1976) 2073.
7 Galbraith, A.I., Chapleo, M.R., and Itzhaki, R.F., Nucl. Acids Res. *5* (1978) 3357.
8 Sudhakar, S., Tew, K.D., Schein, P.S., Woolley, P.V., and Smulson, M.E., Cancer Res. *39* (1979) 1411.
9 Cox, R., Cancer Res. *39* (1979) 2675.
10 Berkowitz, E.M.L., and Silk, H., Cancer Lett. *12* (1981) 311.
11 Rajalakshmi, S., Rao, P.M., and Sarma, D.S.R., Chem.-biol. Interact. *35* (1981) 125.
12 Kootstra, A., and Slaga, T.J., Biochem. biophys. Res. Commun. *93* (1980) 954.
13 Kootstra, A., MacLeod, M.C., Iyer, R., Selkirk, J.K., and Slaga, T.J., Carcinogenesis *3* (1982) 821.
14 Alexander, P., Nature *169* (1952) 52.
15 Alexander, P., Lett, J.T., and Parkins, G., Biochim. biophys. Acta *48* (1961) 423.
16 Loveless, A., Nature *223* (1969) 206.
17 Singer, B., Nature *264* (1976) 333.
18 Stahl, K.-W., Köster, F.E., and Schlimme, E., Synthesis *6* (1974) 426.
19 Preobrazhenskaya, N.N., Sheibarova, Z.A., and Profof'ev, M.A., Dokl. Akad. Nauk SSSR *174* (1967) 100.
20 Mosbo, J.A., and Verkade, J.G., J. Am. chem. Soc. *95* (1973) 4659.
21 Stahl, K.-W., Schlimme, E., and Bojanowski, D., J. Chromat. *83* (1973) 395.
22 Köster, F.E., Ph. D. thesis, Technische Universität Hannover 1978.
23 Engels, J., and Pfleiderer, W., Tetrahedron Lett. *21* (1975) 1661.
24 Wolfe, S., Acc. chem. Res. *5* (1972) 102.
25 Tazawa, J., Tazawa, S., Alderfer, J.L., and Ts'o, P.O.P., Biochemistry *11* (1972) 4931.
26 Peterson, A.R., Peterson, H., and Heidelberger, Ch., Cancer Res. *39* (1979) 131.
27 Kleihues, P., Bamborschke, St., and Doerjer, G., Carcinogenesis *1* (1980) 111.

0014-4754/84/070734-03$1.50 + 0.20/0

Collagenolytic activity from circulating polymorphonuclear leucocytes of patients with asbestosis[1]

I. Lemaire, C. Grondin and R. Bégin

Laboratoire de physiologie cellulaire, Unité de recherche pulmonaire, Faculté de médecine, Université de Sherbrooke, Sherbrooke (Québec, Canada J1H5N4), 19 August 1983

Summary. Levels of collagenolytic activity produced by circulating polymorphonuclear leucocytes (PMN) of patients exposed to asbestos and patients with asbestosis were found to be similar to those of normal controls.

Asbestosis is an occupational lung disorder characterized by chronic inflammation of the alveolar structures and progressive diffuse interstitial fibrosis[2]. The asbestotic lung displays extensive fibrosis with emphysematous changes[3] sug-