Chilling followed by incubation at 37°C causes a reduction in NAD^+ levels which can be prevented by the poly(ADP-ribose)transferase inhibitor 3-aminobenzamide

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The exposure of cells for 60 min to a serum free medium at ice temperature followed by a return to normal culture conditions (30 min at 37°C) caused a dramatic decrease in NAD^+ levels. This decrease in NAD^+ was prevented by 3-aminobenzamide. Alkaline elution analysis of DNA from cultures that were sisters to the ones utilized for measuring cellular NAD^+ content revealed an absence of DNA breakage. These data suggest that poly(ADP-ribose)transferase may be induced in conditions not involving DNA fragmentation. The induction of this enzyme could therefore represent a cellular emergency reaction and not just a response to DNA damage.

Cold shock *NAD^+* *Poly(ADP-ribose)transferase* *3-Aminobenzamide* *DNA damage*

1. INTRODUCTION

Current knowledge on ADPRT, a chromatin bound enzyme, suggests that the synthesis of ADPRT is stimulated by various types of DNA breaks [1,2] and that histone and non-histone proteins serve as acceptors for ADP-ribose residues [3]. The source for ADP-ribose units is NAD^+ and, therefore, damage to DNA results in a rapid decrease in total size of the cellular NAD^+ pool [4]. Jacobson et al. [5] have demonstrated that the drop in NAD^+ which follows DNA damage is not dependent on a decreased biosynthesis of NAD^+ or increased NAD^+-glycohydrolase activity. The biological role of protein modifications by ADPRT centres around the fact that this enzyme has an absolute requirement for DNA and, as previously mentioned, can be activated by DNA breaks [1,2,6]. Thus, several authors have suggested that ADPRT is involved in the regulation of the activity of one or more enzymes of the DNA repair system [7,8]. However, increasing experimental evidence demonstrates that DNA fragmentation is not a singularly rate-determining factor in controlling ADPRT activity [9,10]. We herein report that cellular NAD^+ content is lowered by returning chilled cells to normal culture conditions (37°C) and that this effect can be prevented by 3-AB. We suggest that ADPRT may be induced by cold shock, a condition which does not involve DNA breakage.

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Abbreviations: NAD^+, nicotinamide adenine dinucleotide; ADPRT, poly(ADP-ribose)transferase; 3-AB, 3-aminobenzamide; CHO, Chinese hamster ovary

2. MATERIALS AND METHODS

2.1. *Cell culture*

CHO cells were grown in McCoy's 5a supplemented with 10% fetal bovine serum, penicillin and streptomycin in a CO_2 incubator at 37°C. Approx. 8.5×10^5 cells were seeded in 50 mm plastic

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Petri dishes and experiments were performed 24 h later.

2.2. *NAD⁺ levels*

Monolayer cultures were washed twice with cold PBS and 1 ml of cold 2.5% perchloric acid was added to each plate. After a 10 min incubation in an ice bath the solution was removed and the extraction procedure was repeated. Cell extracts were neutralized with K_2CO_3 and the precipitate was removed by centrifugation. Aqueous solutions of nucleotides were filtered through 0.22 μm pore size microfilters and analyzed for NAD^+ content by reversed-phase high-performance liquid chromatography. Chromatographic apparatus and conditions have been described [11].

2.3. *DNA damage*

DNA strand breaks were determined in intact cells by the alkaline elution procedure described by Kohn et al. [12] with minor modifications [13].

3. RESULTS

NAD^+ levels were assayed in CHO cells after 60 min of chilling on ice followed by a return to normal culture conditions at 37°C (30 min) and compared with those obtained with control cells (grown at 37°C) or with cells that were chilled but not post-incubated at 37°C. Results shown in table 1 indicate that the return of chilled cells to normal culture conditions resulted in a dramatic decrease in NAD^+ levels (line C) with respect to the ones found in control cells (line A) or in cells that were chilled but not post-incubated at 37°C (line B). In the latter instance a small lowering in NAD^+ content was observed. This decrease, however, was not statistically significant. The results so far presented may be compared with those obtained when 5 mM 3-AB was present during the 30 min incubation at 37°C (table 1, line D). The inhibitor of ADPRT largely prevented the drop in NAD^+ levels. Since a reduction in NAD^+ content is caused by ADPRT induction which follows treatment of cells with various DNA damaging agents [6], we tested for DNA break cells that were cultured in parallel with the ones assayed for NAD^+ content. Under the experimental conditions used in this study, no damage could be detected by the sensitive alkaline elution technique (table 2), whereas H_2O_2 (30 μM for 1 h at 4°C) utilized as a positive control, resulted in extensive DNA breakage (table 2, line E).

Table 1

The effect of chilling followed by incubation at 37°C on NAD^+ levels in cultured CHO cells

Experimental conditions[a]	NAD^+[b]	
	nmol/10^6 cells	% control
A	1.14 ± 0.05	100
B	1.06 ± 0.08	93.0
C	0.65 ± 0.05[c]	57.7
D	1.02 ± 0.06	92.7

[a] Experimental conditions were: A, control cells (grown at 37°C); B, cells exposed for 1 h at 4°C; C, cells exposed for 1 h at 4°C and then for 30 min at 37°C; D, cells exposed for 1 h at 4°C and then for 30 min at 37°C in the presence of 5 mM 3-AB

[b] Cell extracts were prepared as described in section 2 and NAD^+ analysis was performed by reversed-phase HPLC on a Supelcosil LC18 column. Values represent the mean ± SE from 4–5 separate experiments

[c] $p < 0.001$ as compared with controls (Student's t-test)

Table 2

The effect of H_2O_2 exposure or chilling followed by incubation at 37°C on the induction of DNA breakage in cultured CHO cells

Experimental conditions[a]	Strand scission factor[b]
A	–
B	0
C	0.01 ± 0.005
D	0.01 ± 0.006
E	0.81 ± 0.096

[a] Experimental conditions were: A, B, C, and D were the same as described in the legend of table 1; E, 30 μM H_2O_2 for 1 h at ice temperature

[b] CHO cells were analyzed for DNA damage by the alkaline elution technique (see section 2) and strand scission factor (SSF) was calculated from the DNA elution profiles according to the following relationship: $SSF = -\log A/B$, where A = fraction of DNA retained in the filter after 9 h of elution, in treated cells, and B = fraction of DNA retained after 9 h of elution, in untreated cells. Values represent the mean ± SE from 3 separate experiments

4. DISCUSSION

In this study we have shown that the chilling of cells for 60 min followed by a return to normal culture conditions caused a profound decrease in NAD^+ content and that the drop in NAD^+ levels could be prevented by the ADPRT inhibitor, 3-AB. This suggests that an augmented ADPRT activity is responsible for the drop in NAD^+ though the participation of other mechanisms such as a decreased synthesis of NAD^+ or an increased NAD^+-glycohydrolase activity cannot be ruled out. However, the hypothesis that the drop in NAD^+ content is caused by ADPRT induction is supported by the fact that the potent and selective inhibitor of this enzyme, 3-AB [14], was able to prevent the drop in NAD^+ levels. It should be noted that 3-AB does not affect NAD^+-glycohydrolase activity [6]. Nuclear ADPRT is stimulated by a variety of cellular insults resulting in the induction of DNA breakage [6,15–17]. Under our conditions we found no evidence that activation was due to the appearance of DNA strand breaks. An induction of ADPRT in the absence of DNA fragmentation was also reported by Wallace et al. [9] in polyamine depleted cells and by Jackowski and Kun [10] in cardiocytes (cardiocyte nuclei of neonate rats had ten times more ADPRT activity than cardiocyte nuclei from adult rats).

We conclude therefore that ADPRT induction is an event which does not necessarily follow DNA damage. ADPRT may serve to decrease cellular NAD^+ content, thus slowing down energy requiring reactions. Wintersberger and Wintersberger [18] have made an attractive hypothesis on ADPRT induction by DNA damaging agents, suggesting that a slow-down of energy requiring reactions, such as replicative DNA synthesis, would give cells more time to repair DNA damage. Our data indicate that cells may respond to other types of stress in a similar fashion and, as previously hypothesized by Wintersberger and Wintersberger [18], ADPRT may represent a cellular emergency reaction.

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REFERENCES

[1] Benjamin, R.C. and Gill, D.M. (1980) J. Biol. Chem. 255, 10493–10501.
[2] Benjamin, R.C. and Gill, D.M. (1980) J. Biol. Chem. 255, 10502–10508.
[3] Gaal, J.C. and Pearson, C.K. (1985) Biochem. J. 230, 1–18.
[4] Wielkens, K., Schmidt, A., George, E., Bredehorst, R. and Hilz, H. (1982) J. Biol. Chem. 257, 12872–12877.
[5] Jacobson, M.K., Levi, V., Juarez-Salinas, H., Barton, R.A. and Jacobson, E.L. (1980) Cancer Res. 40, 1797–1802.
[6] Shall, S. (1984) in: DNA repair and its inhibition (Collins, A. et al. eds) pp. 143–191, IRL Press, Oxford.
[7] Creissen, D. and Shall, S. (1982) Nature 296, 271–272.
[8] Cleaver, J.E. and Morgan, W.F. (1985) Mutation Res. 150, 69–76.
[9] Wallace, H.M., Gordon, A.M., Keir, H.M. and Pearson, C.K. (1984) Biochem. J. 219, 211–221.
[10] Jackowski, G. and Kun, E. (1981) J. Biol. Chem. 256, 3667–3670.
[11] Stocchi, V., Cucchiarini, L., Magnani, M., Chiarantini, L., Palma, P. and Crescentini, G. (1985) Anal. Biochem. 146, 118–124.
[12] Kohn, K.W., Ewig, R.A.G., Erickson, L.C. and Zwelling, L.A. (1981) in: A Laboratory Manual of Research Procedures (Friedberg, E.C. and Hanawalt, P.C. eds) pp. 379–401, Marcel Dekker, New York.
[13] Cantoni, O. and Costa, M. (1983) Mol. Pharm. 24, 84–89.
[14] Hunting, T.J., Gowans, B.J. and Hendersen, J.F. (1985) Mol. Pharm. 28, 200–206.
[15] Durkacz, B.W., Omidji, O., Gray, D.A. and Shall, S. (1980) Nature 283, 593–596.
[16] Berger, N.A., Sikorski, G.W., Petzold, S.J. and Kurohara, K.K. (1979) J. Clin. Invest. 63, 1164–1171.
[17] Cleaver, J.E., Bodell, W.J., Morgan, W.F. and Zelle, B. (1983) J. Biol. Chem. 258, 9059–9068.
[18] Wintersberger, J. and Wintersberger, E. (1985) FEBS Lett. 188, 189–191.