EFFECT OF CADMIUM ON RNA-POLYMERASE AND PROTEIN SYNTHESIS IN RAT LIVER

Humberto A. HIDALGO, Vasantha KOPPA and Sara E. BRYAN
Department of Biological Sciences, University of New Orleans New Orleans, Louisiana 70122 USA

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1. Introduction

By studying the intracellular movement of cadmium we have shown that the metal enters rat liver nuclei and attains a maximum concentration (3.0×10^{-3} μmol Cd/mg DNA) after one hour in rats received 20 μmol of cadmium/kg [1] and have correlated the uptake and disappearance of cadmium from the nucleus with the appearance of a cytoplasmic cadmium binding protein (CdBP). Other investigators [2,3] have shown that RNA polymerase (a nuclear bound enzyme) is either completely or severely inhibited by adding cadmium to nuclei at concentrations which are 30–800 times greater than those found in vivo. Weser and Hübner [2] have reported that cadmium does not inhibit the RNA polymerase activity of rat liver nuclei 10 hours after animals were injected with 10 μmol of Cd/kg. In an attempt to reconcile these in vivo and in vitro data, we have studied the effect of cadmium on RNA polymerase activity prior to and after 10 hours of exposure. In this report we show that cadmium inhibits RNA polymerase activity in vivo. The metal also inhibits, perhaps independently, protein synthesis in the liver. Our data suggest that RNA polymerase activity is dependent on nuclear cadmium levels up to seven hours after intraperitoneal cadmium injection, while at eleven hours RNA polymerase activity is no longer a function of nuclear cadmium levels under our given experimental conditions.

2. Materials and methods

2.1. *Animals*

Female albino rats weighing 200–250 g were obtained from Pel-Freez, Arkansas, USA. Animals had free access to Purina Rat Chow and water. Experimental animals were injected intraperitoneally with cadmium chloride (20 μmol/kg rat), and/or labeled [^{3}H]leucine (40 μCi/kg rat) before the animals were sacrificed by cervical dislocation at the time intervals indicated in fig.1. For nuclear cadmium measurements laboratory bred Fisher strain male rats were injected intraperitoneally with 40 μCi ^{115}Cd/20 μmol Cd/kg.

2.2. *Chemicals*

^{115} Cd (0.152 mCi/mg), [^{3}H]Leucine (60 Ci/mmol) and ^{14}C-ATP (45 mCi/mmol) were obtained from New England Nuclear USA. All other chemicals were obtained from sources already described [6].

2.3. *RNA polymerase*

RNA polymerase assays were carried out as described previously [3,5]. The isolation of purified nuclei was carried out as already described [1,6] with the modification that no $CaCl_2$ or detergents were used. DNA was measured as previously described [4].

2.4. *[^{3}H]Leucine labeling*

10 μCi of [^{3}H]leucine were injected intraperitoneally 30 min prior to sacrificing the animals. A cytoplasmic and nuclear fraction were obtained as already described [1]. To 0.5 ml of cytoplasmic fraction 5 ml of cold 10% TCA was added, the precipitate was collected on a filter and washed twice with 10% TCA and ethanol: ether (1:3 v/v). All samples (nuclei and cytoplasmic proteins) were dissolved with a tissue solubilizer (Eastman USA) prior to scintillation counting in a Beckman Model LS-1000 counter. Protein was measured by the modified Lowry method [7].

2.5. *Nuclear cadmium (^{115}Cd) measurements*

The levels of ^{115}cadmium in rat liver nuclei were measured as previously described [1].

3. Results

3.1. *Inhibition of RNA polymerase by cadmium in vivo*

The observed RNA polymerase activity in rat liver nuclei following a pulsed cadmium exposure is plotted as a function of time in fig.1. RNA polymerase activity varies from control at all times except at 11 h. This correlates well with previous work showing practically no RNA polymerase inhibition at 10 h, when a smaller cadmium dose (10 μmol/kg) was administered. We find maximal inhibition (1½–2 h after cadmium injection with a continuous increase from 2–16 h thereafter.

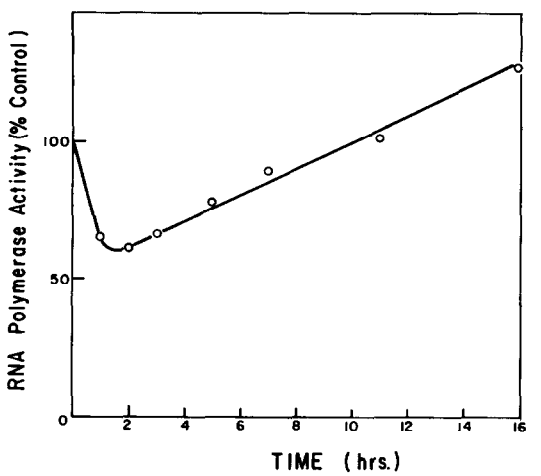

Fig.1. DNA-dependent RNA-polymerase activity of freshly isolated rat liver nuclei. 20 μmol of cadmium chloride/kg rat were injected intraperitoneally before the animals were sacrificed at the times indicated. Each point represents the mean value of the nuclear RNA polymerase activity obtained in triplicate from 4–5 separately injected animals. The complete incubation mixture contained (in 0.35 ml): 50 μmol of Tris-HCl, pH 8.2; 2.5 μmol of $MgCl_2$; 3.0 μmol of NaF; 35 μmol of KCl; 0.3 μmol each of GTP, CTP, UTP; 0.015 μmol of ATP; 0.005 μmol of [^{14}C]ATP and 0.1 ml of nuclear suspension (400–600 μg of DNA). Activities were obtained as pmoles of AMP incorporated/15 min/mg of DNA. Average control incorporation was 1516, sample standard error is 41 pmol AMP/15 min/mg DNA.

3.2. *RNA polymerase activity and nuclear bound cadmium*

Changes in RNA polymerase activity in vivo following cadmium injection suggested that the decrease in activity could be a direct result of the presence of cadmium in the nucleus. To test this idea we measured the levels of nuclear cadmium following challenge, and plotted (fig.2) the percentage of nuclear bound cadmium (from a maximum concentration at 1 h of 3.0×10^{-3} μmol Cd/mg DNA) against the change (from control) in RNA polymerase activity. The activity of this polymerase seems to be a function of nuclear cadmium up to a time at which 70–80% of the cadmium has been removed from the nucleus (6½–8 h after challenge). At this point another set of events appears to make RNA polymerase activity independent of nuclear cadmium levels. Extrapolation of the curve in fig.2 (dotted line) indicates that the RNA polymerase activity would be the same as control (no change) if there was no cadmium present in the nucleus. The nuclear cadmium concentration at which RNA polymerase seems to be independent of nuclear cadmium levels corresponds to a time at which most of the intracellular cadmium is bound to newly synthesized CdBP in the cytoplasm [1].

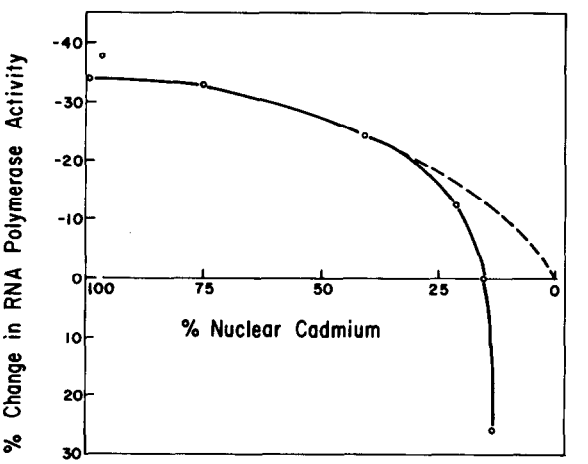

Fig.2. Change in RNA-polymerase activity as a function of nuclear cadmium. The percent nuclear cadmium is derived from the nuclear to cytoplasmic ratio of cpm/mg DNA: cpm/mg protein [1]. Percent change in RNA polymerase activity is obtained by the factor: percent RNA polymerase activity −100.

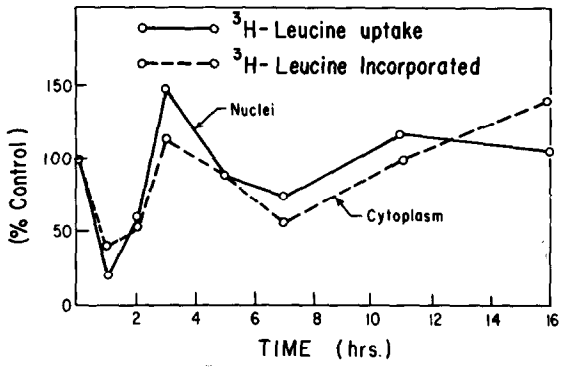

Fig.3. Leucine uptake by cytoplasmic protein and nuclei. Rats were injected intraperitoneally each with 10 μCi of [^{3}H]leucine 30 min before they were sacrificed; the animals were also injected with cadmium chloride (20 μmol/kg) at the times indicated. The acid-insoluble cytoplasmic fraction and purified nuclei were analyzed for uptake of labeled leucine. Activities were obtained as dpm/mg protein. Average control cytoplasmic protein and nuclear leucine uptake correspond to 405 dpm/mg protein and 257 dpm/mg protein, respectively.

3.3. *Effect of cadmium on protein synthesis*

Animals were injected with labeled leucine to measure incorporation and uptake of label into cytoplasmic proteins and purified nuclei, respectively. Our results indicate (fig.3) cytoplasmic protein synthesis is maximally inhibited one hour after cadmium injection and recovers thereafter, increasing continuously from 7–16 h. The increase in labeled leucine in the nucleus follows a similar pattern in the cytoplasm, although nuclear uptake appears to level off at later times (11 and 16 h), as compared with cytoplasm. The initial decrease in cytoplasmic protein synthesis seems to be independent of the effect of the metal on RNA synthesis; it occurs one hour prior to maximum polymerase inhibition. The increased cytoplasmic protein synthesis, at seven hours after challenge and onward, parallels increased levels of RNA synthesis.

4. Discussion

CdBP protein synthesis is completed, following a single pulsed cadmium exposure, within the relatively short time period of 3–10 h after challenge [1]; CdBP mRNA synthesis is thought to occur prior to 3 h and to be induced by cadmium [1,8]. The present report shows that cadmium also generally affects RNA polymerase activity and protein synthesis. This effect can be correlated, in the case of RNA polymerase, with nuclear cadmium levels. That other workers [3] (assaying nuclei of challenged animals) have not detected in vivo inhibition, can be explained by the time point they chose to study: at 10 h when RNA polymerase activity is probably no longer a function of nuclear bound cadmium. The subsequent increase over control in RNA synthesis (after 11 h) is not a result of CdBP mRNA synthesis because the protein synthesis itself (CdBP) is about 90% complete at this time; CdBP mRNA is apparently synthesized while overall RNA polymerase activity is at a minimum. We suggest that increased RNA and protein synthesis following cadmium challenge may be the result of a recuperative process which is only possible after most of the intracellular cadmium is sequestered by CdBP. This is in agreement with the proposal [1,8] that intracellular cadmium becomes biologically inert upon binding to CdBP. Apparently, early after exposure cadmium depresses both RNA and protein synthesis by separate mechanisms since maximum inhibition of protein synthesis precedes maximal RNA synthesis inhibition.

This work illustrates that the biological consequences of exposure to toxic metals, such as cadmium, can be observed at the biochemical level by probing the dynamic effects of metals on cellular processes. A better understanding of these effects has become a necessity because of increased industrial and technological applications of metals which has increased the risk of environmental exposure for man and other organisms.

Acknowledgement

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