INHIBITION OF DNA SYNTHESIS IN DIFFERENTIATING CARDIAC MUSCLE BY NAD

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1. Introduction

DNA synthesis in differentiating cardiac muscle of the rat decreases progressively following birth and essentially ceases by the middle of the third week of postnatal development [1]. The activity of nuclear poly(ADP-ribose) polymerase (an enzyme which catalyzes a reaction involving the sequential transfer of the ADP-ribose moiety of NAD to chromosomal proteins resulting in the formation of a homopolymer of ADP-ribose residues and the release of nicotinamide) and the intracellular concentration of NAD increase progressively in cardiac muscle during this same period of development and it was suggested that NAD and poly(ADP-ribose) polymerase were involved with the repression of DNA replication in this tissue [2]. The studies reported in this communication were undertaken in order to investigate this possibility.

2. Materials and methods

Timed pregnant rats were obtained from Holtzman (Madison, Wisconsin) on the 14th day of gestation. They were housed in individual cages and maintained on water and standard laboratory chow ad libitum. Neonatal rats were raised in litters of ten.

Tissue slices of ventricular cardiac muscle were prepared with a Stadie-Riggs tissue slicer. Approximately 150 mg of tissue was incubated at 37°C in 5 ml of Eagle's minimum essential medium containing Hanks' salt base with 5 μCi/ml [^{3}H]thymidine (spec. act. of 17 Ci/mmol, Amersham/Searle). At the end of the incubation period the tissue was homogenized in 0.15 M NaCl and DNA synthesis was estimated by measuring the incorporation of [^{3}H]thymidine into DNA as previously described [1]. [^{3}H]Thymidine incorporation under these incubation conditions is linear for at least 4 h.

Thymidine transport was determined by measuring the uptake of [^{3}H]thymidine into the acid-soluble fraction of cardiac muscle. Slices were incubated as above then washed twice in 0.15 M NaCl, blotted dry and homogenized in 2.0 ml of 0.15 M NaCl. An equal volume of ice-cold 10% perchloric acid was added and the acid-soluble radioactivity was measured as described [1].

3. Results

NAD added to tissue slices of differentiating cardiac muscle inhibits the incorporation of [^{3}H] thymidine into DNA in a concentration-dependent manner (fig.1). A time study of this inhibition and the effect of NAD on thymidine transport are shown in fig.2. NAD inhibits [^{3}H]thymidine incorporation into DNA progressively during the incubation period but has little effect on [^{3}H]thymidine transport. The inhibition of [^{3}H]thymidine incorporation by NAD is specific since other related pyridine nucleotides have little effect on incorporation (table 1). The inhibition observed with NADH may be due to its oxidation to NAD.

4. Discussion

The terminally differentiated ventricular cardiac muscle cell of the adult mammal exists in a repressed

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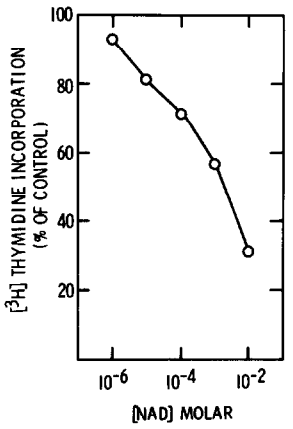

Fig.1. Effect of NAD on [^{3}H]thymidine incorporation into DNA of tissue slices of differentiating cardiac muscle. Tissue slices were prepared from 5-day-old neonatal rats and incubated for 2 h.

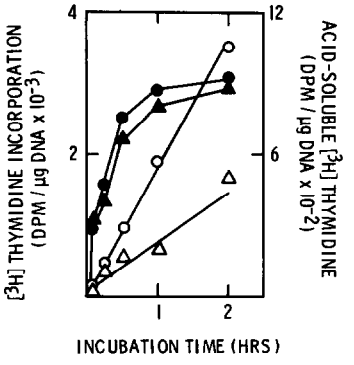

Fig.2. Effect of NAD on [^{3}H]thymidine transport and incorporation into DNA of tissue slices of differentiating cardiac muscle. Tissue slices were prepared from 6-day-old neonatal rats. Closed symbols represent thymidine transport; open symbols represent thymidine incorporation into DNA. Circles = controls, triangles = addition of 1 mM NAD.

state in that it has irreversibly lost the ability to replicate its DNA, undergo mitosis and divide [1,3,4]. Little is known about the mechanisms responsible for this loss of synthetic activity. Recently it was observed that as the rate of DNA synthesis decreases in differentiating cardiac muscle intracellular levels of NAD are elevated and the activity of the nuclear enzyme which converts NAD to poly(ADP-ribose) is increased [2]. Data presented in the present communication show that NAD inhibits DNA synthesis when added to tissue slices of differentiating cardiac muscle.

Table 1
Effect of NAD, related pyridine nucleotides and nicotinamide on [^{3}H]thymidine incorporation into DNA of tissue slices prepared from differentiating cardiac muscle.

Addition	[^{3}H]thymidine incorporation (% of control)
NAD	69
NADH	88
NADP	98
NADPH	92
NMN	98
Nicotinamide	97

Tissue slices were prepared from cardiac muscle of 4-day-old neonatal rats and incubated for 2 h with the indicated compound at a final concentration of 0.1 mM.

The mechanism for this inhibition remains to be determined. In rat embryonic cells incubated with NAD labelled at the adenosine moiety with tritium and processed for autoradiography, silver grains are found almost exclusively over the nucleus indicating that NAD, or at least the ADP-ribose moiety, is concentrated in the nucleus [5]. NAD has been reported to depress DNA synthesis and template activity of chromatin towards exogenous DNA polymerase in isolated nuclei and this inhibition is related to the ADP-ribosylation of nuclear proteins [6–8]. Because of the above observations and the data presented in this report it is suggested that NAD may play a role in repressing DNA synthesis and cell proliferation in cardiac muscle during terminal differentiation. This repression could occur by the chemical modification of chromosomal proteins or possibly nuclear enzymes [9–11] by ADP-ribosylation.

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