Cell specific response of cardiac poly ADP-R and DNA synthesis to circulatory stress[1]

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Summary. Aortic coarctation induces a large increase in poly ADP-R synthetase activity in non-cardiocyte nuclei, and in cardiocyte nuclei inhibition occurs, suggesting a differentiation dependent regulation of polymer metabolism. In non-cardiocyte nuclei DNA and poly ADP-R (polyadenosine diphosphoribose) synthesis exhibit positive correlation.

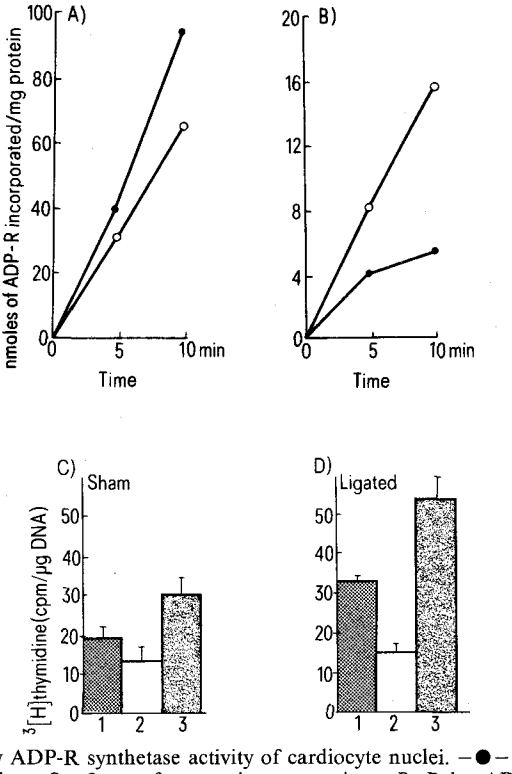
In cardiocytes of adult rats circulatory stress induces increased RNA and protein synthesis[3-5] whereas in cardiac cells other than cardiocytes DNA synthesis also is augmented[6]. The response to stress in cardiocytes exhibits a superimposed ontogenic dependence as manifested by the induction of DNA synthesis in cardiocytes of embryonic or neonatal cardiac tissue[7]. Although the differentiation related cessation of cardiocyte DNA synthesis is well documented[8], molecular mechanisms related to the control of DNA synthesis and to stress induced RNA synthesis are hitherto undefined.

We have observed a characteristically age dependent variation of nuclear poly ADP-ribosylation of predominantly non-histone proteins in cardiocyte nuclei[9]. Because of the prevalently non-histone protein selectivity of in vivo poly ADP-ribosylations[10,11] our interest became oriented towards phenomena related to differentiated cell specific functions relevant to cardiac pathophysiology. It is presently assumed that non-histone chromosomal proteins play an important role in the regulation of cellular specificity of macromolecular biosyntheses[12], although details of this control system are unknown. Poly ADP-ribosylation may serve as a selective physiological probe in experiments concerned with non-histone proteins and if a positive correlation between cell specific regulatory processes and poly ADP-ribosylation of specific non-histone proteins can be established this may lead to the recognition of the physiological function of poly ADP-R, a problem that despite extensive trials[13] has escaped clarification.

An inverse relationship between the triiodothyronine induced increase of cardiocyte RNA synthesis and poly ADP-R synthetase activity exists[14], implying a physiologically operative restriction of RNA synthesis by poly ADP-ribosylation mainly of non-histone nuclear proteins[10,11]. Based on these observations we postulated that cell type specific reactions to stress should be detectable not only in the control of RNA synthesis but also in the regulation of DNA synthesis and a correlation with rates of poly ADP-ribosylations should also be found experimentally.

Sustained circulatory stress in male Sprague-Dawley rats (200–215 g b.wt) was induced by silk ligature coarctation of the abdominal aorta[6] and sham operated animals served as controls. All animals before and after surgery were kept on standard Purina Rat Chow and tap water. On the 8th postoperative day, after 16 h of food deprivation 1 μCi/g b. wt [^{3}H]-methylthymidine (72 Ci/mmole) was injected i.p. into 9 rats which had had aortic coarctation and into 6 sham operated controls. 4 h after in vivo labeling nuclei of cardiocyte and non-cardiocyte origin were isolated by the 1 step isopycnic density gradient centrifugation procedure[15] and the incorporation of [^{3}H]-methylthymidine into DNA was determined following the isolation of DNA[15] from both nuclear subsets. Rates of poly ADP-R synthesis in isolated nuclear subsets were determined as described earlier[9]. Nuclear subsets for poly ADP-R synthetase assays were pooled from 9 rats which had been subjected to aortic coarctation and from 6 sham operated controls.

As illustrated in the figure (A) the rates of in vitro synthesis of poly ADP-R were significantly depressed in cardiocyte nuclei isolated from animals exposed to aortic coarctation. The inhibition of poly ADP-R synthetase activity produced by sustained circulatory stress is reminiscent of the effect of triiodothyronine and steroid hormone treatment[14,17,18] and it cannot be ruled out that the biochemical mechanism elicited by circulatory stress resulting in the depression of poly ADP-R synthetase of cardiocytes may have an endocrine effector component. It is of interest that steroid hormones (hydrocortisone and aldosterone) exert an in vivo inhibitory effect on cardiocyte poly ADP-R synthetase[18]. It is also known that sustained stress produces significant over-production of glucocorticoids[19]; therefore the participation of developmental hormones in the circulatory stress induced depression of cardiocyte poly ADP-R synthetase activity is probable. The opposite consequences of circulatory stress on poly ADP-R synthetase activity of cardiocyte and non-cardiocyte nuclei (fig., B) clearly illustrate the differentiation dependence of the regulation of this enzyme system. Poly ADP-R synthetase activity of



A Poly ADP-R synthetase activity of cardiocyte nuclei. —●—●—, control; —○—○—, after aortic coarctation. *B* Poly ADP-R synthetase activity of non-cardiocyte nuclei. —●—●—, control; —○—○—, after aortic coarctation. *C* [^{3}H] methyl thymidine incorporation into cardiac nuclei. 1, in homogenates; 2, in cardiocyte nuclei; 3, non-cardiocyte nuclei of control (sham operated) animals. *D* [^{3}H] methyl thymidine incorporation into DNA of cardiac nuclei after aortic coarctation. 1, in homogenates; 2, in cardiocytes; 3, in non-cardiocyte nuclei.

The accuracy of the poly ADP-R synthetase assay[9] is within ±5% (fig. A and B) and the SD of in vivo analyses of thymidine incorporation is shown in figure C and D by error bars.

non-cardiocyte nuclei is only about 5% of that of cardiocyte nuclei and sustained aortic coarctation greatly increases enzymatic activity. It is apparent that the increase in enzymatic activity parallels the in vivo augmentation of thymidine incorporation into DNA (fig., D), which phenomenon is an index of cellular hyperplasia; therefore poly ADP-ribosylation in cellular proliferation behaves as is it would directly correlate with DNA synthesis. In vivo thymidine incorporation into DNA (fig., C and D) clearly shows that cardiocytes respond to circulatory stress with hypertrophy[5,6] and hyperplasia is confined to cells other than cardiocytes. The inverse correlation between poly ADP-R synthetase activity and rates of DNA synthesis in various cell types has been noted earlier[20] as it was found that nondividing cardiocytes exhibit a much larger poly ADP-R synthetase activity than liver cells, which are known to undergo mitosis. Characteristically non-cardiocyte cell types which possess the potential of proliferation also exhibit a much lower poly ADP-R synthetase activity.

The present results emphasize the differentiation dependence of regulation of both poly ADP-R and DNA synthesis potential of the 2 cell types within the same organ, and are consistent with a regulatory (inhibitory) effect of poly ADP-ribosylation on RNA synthesis[14]. De-repression of RNA synthesis is induced by inhibition of poly ADP-ribosylation in cardiocytes, resulting in hypertrophy[9,14,17]. Poly ADP-ribosylation is non-cardiocyte nuclei parallels DNA synthesis and it is assumed that induction of poly ADP-ribose synthetase constitutes a terminating signal for DNA synthesis in cell types capable of proliferation because poly ADP-ribosylation is known to inhibit also DNA synthesis[21-24]. Developmental hormones are probable mediators of stress on the poly ADP-ribose regulated systems[14,18].

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Inhibition of potassium (^{86}Rb) influx in Ehrlich ascites cells by bilirubin and ouabain[1]

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Summary. Bilirubin inhibited influx of potassium into Ehrlich ascites cells without altering efflux. The data showed that compared with ouabain, net potassium influx components were impaired in a higher degree by bilirubin. The reversal of this effect was shown, in our experimental conditions, only for ouabain.

Potassium is an essential ion for the function of all animal cells. Cellular membrane transport processes regulate the intracellular concentration of potassium within certain limits[3-6]. Potassium influx is influenced by a) active pump (primary) and cotransport (secondary) mechanisms, b) exchange processes, and c) diffusion through selective channels[7-9]. Organic compounds have been used extensively to study cellular ionic transport mechanisms[10-17]. Recently, unconjugated bilirubin was founds to inhibit the Na-K ATPase in the microsomal fraction of the brain[18] and the potassium influx into Ehrlich ascites cells[19]. The present study was carried out to obtain further information on the effects of bilirubin on potassium fluxes. A comparison was made between the effects of bilirubin and of ouabain, a well-established inhibitor of potassium transport processes[8,10].

Materials and methods. The general methodology used to study potassium influx and intracellular ion content has been described in detail previously[19]. Briefly, radioactive rubidium (^{86}Rb) was used as a marker for potassium. An aliquot (10-15 µl) of a solution of ^{86}RbCl of high specific activity (10-15 Ci/g) was added to cellular suspensions in the range of 5×10^6-1×10^7 cells/cm^3. 1-ml samples of the suspensions were taken at intervals and centrifuged for 30 sec at 2500 × g to obtain a cell pellet. The cells were washed thrice by resuspension in 0.3 osM $MgCl_2$ and then centrifuged again for 30 sec at 2500 × g. The radioactivity of packed cells and aliquots of incubation medium was deter-