

AGE PECULIARITIES OF NON-MYOSIN AND MYOSIN ADENOSINE TRIPHOSPHATASE ACTIVITY OF RABBIT VENOUS WALL

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Investigations of metabolic heterogeneity of endothelial and smooth muscle cells (EC and SMC, respectively) of the vascular wall in different physiologic and pathological conditions promise to become a perspective research trend for clarifying the participation of the vascular wall in the complex process of circulation autoregulation (5, 6). From this point of view, knowledge of adenosine triphosphate degradation enzymes of the venous wall is of special interest. This knowledge is of immediate importance for clarifying of the underestimated and lending hard itself to investigation role of the venous wall in this process. According to literature data available, these enzymes are located in vascular EC and SMC (9, 13). However, their participation in the metabolism of these cells is not completely decoded yet (4, 10). We are insufficiently acquainted with the age-dependent changes of enzyme activity in the venous wall and vascular wall at all, too. This conceives our intention to investigate the age-dependent changes of non-myosin and myosin adenosine triphosphatase (ATP-ase) activity in veins of different size and structure type.

Material and methods

Material from v. portae, mesenteric, posterior caval and femoral veins taken from 16 rabbits aged 7, 21, 42, and 80 days as well as 5—6 months was examined in our study. The latter were sexually mature. Myosin ATP-ase was ascertained after the calcium-cobalt method of Padykula and Herman (14) at pH 9.2 but non-myosin one — after Wachstein—Meisel's lead method (19) at pH 7.2. Both methods were applied in modification of Loida et al. (3). The specificity of methods used was controlled by means of: 1. Incubation without substrate for more accurate definition of background reaction; 2. Heating of sections prior to incubation in distilled water at 80 °C for 10 min in order to exclude non-enzymatic ATP hydrolysis; 3. Parallel incubation in equimolar 2-glycerophosphate (β -glycerophosphate) concentration as substrate to exclude non-specific phosphomonoesterases participation in ATP hydrolysis; 4. Preincubation with 0.0363 per cent water solution of L-cysteine at 37 °C for 30 min and postincubation with added L-cysteine in concentration of 0.0363 per cent to exclude alkaline phosphatase participation in ATP hydrolysis.

Results and discussion

Our results obtained show that these reactions for ascertaining of both myosin and non-myosin ATP-ases are positive in EC and SMC of all veins studied. There are some differences in the intensity of reactions applied in both cell kinds (EC and SMC) when animals of the same age group are concerned. The compari-

son of veins from animals of different age reveals a definite age-dependent dynamics of these differences as follows:

a) **N o n - m y o s i n A T P - a s e — E C.**

Reaction for non-myosin ATP-ase in EC is very strongly positive in 7-day old rabbits. It seems that it is more positive in basal cellular parts (fig. 1). Reaction does not show any significant changes during individual growth when EC are concerned.

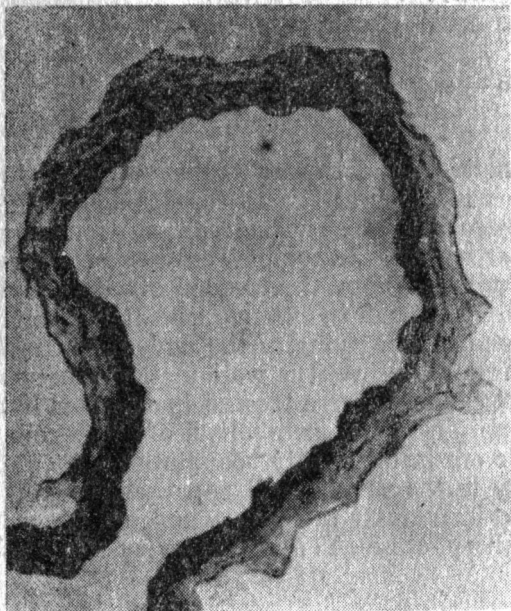


Fig. 1. Posterior caval vein of 7-day old rabbit. Reaction for non-myosin ATP-ase. Microphoto: projective 8:1, ob. 100

S M C.

Reaction is slightly positive in the shape of diffusely sedimentized product in media SMC and especially of *v. portae* specimens in adventitia SMC, too, when 7-day old rabbits are studied (fig. 1). In comparison with EC of the same age group the reaction is considerably less expressed. It becomes more and more positive during individual growth (fig. 2). It is strongly positive and prevails over that one in EC when sexually mature animals are examined (fig. 3). Besides, the manner of precipitation of the reaction product changes — the diffuse background remains and even increases but together with that precipitation product of reaction outlines more distinctly the borderlines between single SMC (fig. 3). There are certain intensity variations in single veins of animals from the same age group as well as in certain cases of

one and the same animal. However, these variations have not any definite regular character. Controls indicate that in some cases they are due to non-specific factors.

b) **M y o s i n A T P - a s e — E C.**

Reaction of EC is positive in 7-day old rabbits. A certain enhancement of reaction intensity sets in during individual growth and reaction is more intensive in sexually mature animals than that of younger ones.

S M C.

SMC located in media mainly but in single segments of *v. portae* also in adventitia show a slightly positive reaction in 7-day old animals. However, reaction intensity is weaker than that of corresponding EC. It increases during individual growth and is considerably stronger in sexually mature animals in comparison with that in newborn ones (fig. 4). However, the rising of reaction intensity is considerably quicker in SMC as compared with that in EC. That is why reaction in SMC is significantly stronger than that in corresponding EC when sexually mature rabbits are examined. A tendency appears with advancing age consisting in reaction product precipitations in the shape of granules in place of diffuse background. Sometimes SMC membranes can be distinguished, too (fig. 4).

In a previous paper (13) it has been reported that positivity of reactions for non-myosin and myosin ATP-ases in single tunics of veins from sexually mature

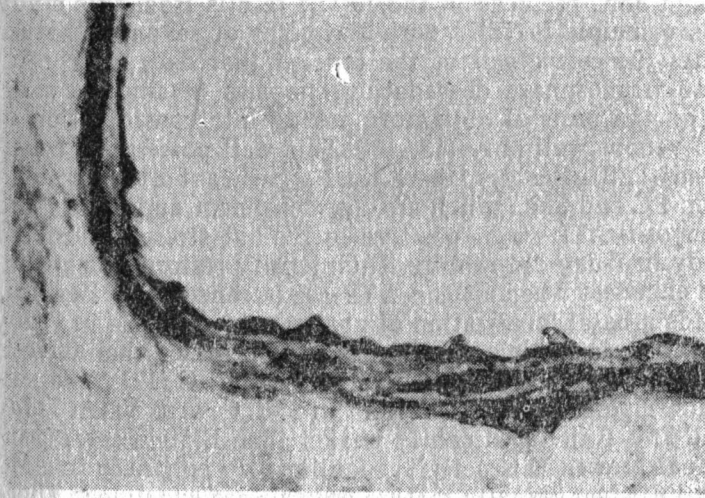


Fig. 2. *V. portae* of 21-day old rabbit. Reaction for non-myosin ATP-ase. Microphoto: oc. 10, ob. 40

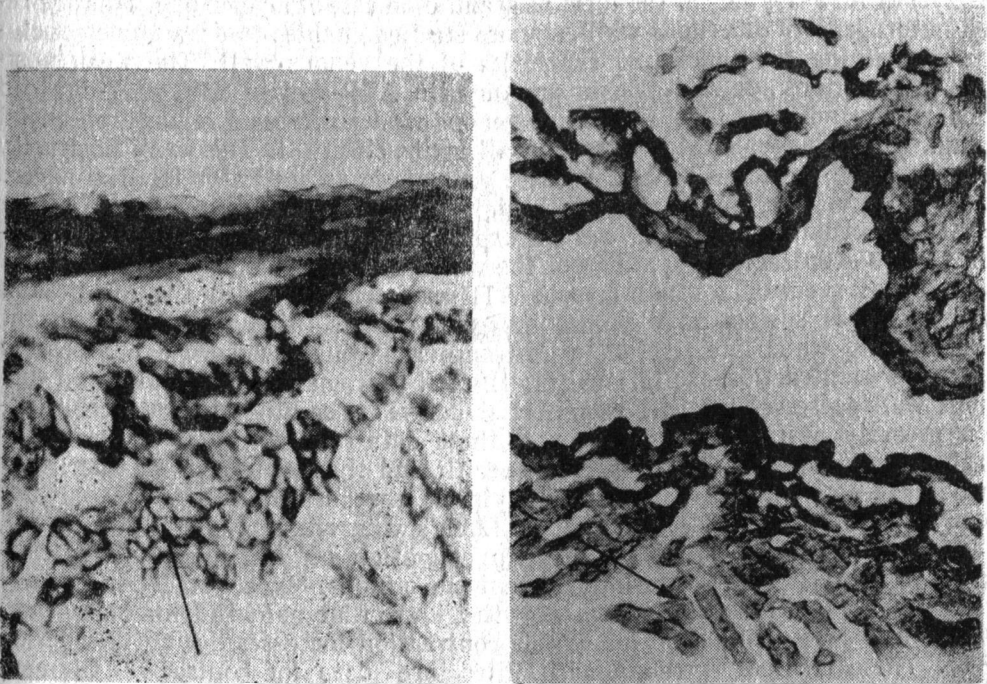


Fig. 3. *V. portae* of sexually mature rabbit. Reaction for non-myosin ATP-ase. Arrow indicates an outlined membrane localization of the reaction product in longitudinal adventitial SMC. Microphoto: oc. 10, ob. 40

Fig. 4. *V. portae* of sexually mature rabbit. Reaction for myosin ATP-ase. Arrow indicates longitudinally oriented adventitial SMC in which there is a tendency towards reaction product sedimentation in the shape of granules. Microphoto: oc. 10, ob. 10

cats is related with EC and SMC presence in them. The results from this study show that in general this principle is valid with the veins of sexually mature rabbits, too. This indicates the correctness of the concept that data interpretation concerning adenosine triphosphate degradation enzymes in single venous wall tunics can be done on the basis of obligatory taking into consideration of structural peculiarities of venous wall only (13, 16). This wall possesses a considerable variability, indeed (1, 2). Besides, there exist significant differences in reactions intensity between EC and SMC which are age-dependent and with fixed dynamics. Reaction for myosin ATP-ase is positive in EC but strongly positive for non-myosin one already in 7-day old rabbits. Its intensity restores during individual growth but even enhances when myosin ATP-ase is concerned. Besides, there exists a trend towards a basal localization of non-myosin ATP-ase in 7-day old animals. This localization is in principle characteristic of epithelial cells and probably related with the nature of metabolic processes in EC (17). As a set-off to that, myosin and non-myosin ATP-ase activity in SMC is weak when 7-day old animals are concerned and even significantly weaker than that in corresponding EC. During individual growth this activity significantly enhances in SMC as it prevails over that in EC when sexually mature animals are examined. There are simultaneous changes of reaction product precipitation. It is diffuse in 7-day old animals and becomes diffuse with outlined membrane localization in case of non-myosin ATP-ase and in the form of granules in case of myosin one. This age-related heterogeneity of venous wall enzymes studied enables to draw some conclusions concerning the functional capacities of the venous wall. The weak positivity of reactions for non-myosin and myosin ATP-ases in SMC of 7-day old animal veins indicates that at this age venous wall possesses a reduced potential for high-energy phosphate uptake for wall contraction (8). The weak positivity of reaction for myosin ATP-ase in these animals is an indicator for diminished capacities of venous myocytes to break high-energy bonds and to utilize ATP for their contraction (6—8). This can be explained with the insufficient development of SMC contractile apparatus of the venous wall at this age (18). The weak positivity of reaction for non-myosin ATP-ase correlates with the insufficient development of surface sarcolemmal invaginations at this age reported in the literature (18) which are related to Ca-transport — one necessary component of the process «contraction-excitation» (11, 12). All that enables the assumption that venous wall possesses a rather imperfect structure concerning its contractile function in early postnatal ontogenesis. If these data obtained after investigation of rabbit veins are not strictly species-specific and thus valid also for man, they can be used as an initial basis for clarifying of the unexplained fact established already by Abt (cited after 15) that children's vein are relatively thick-mural. However, it requires an obligatory investigation of ATP-dissociating enzymes in human venous wall during different age periods. Myosin ATP-ase activity increase in SMC during individual growth shows that potential for macroenergetic ties and ATP utilization for muscle contraction increases during this period (6—8). These changes correlate with literature data for contractile filament enhancement in venous vessel SMC with rabbits during postnatal ontogenesis on the account of reduction of the rest cell organellae (18). Non-myosin ATP-ase activity increase during postnatal ontogenesis established by us correlates with literature data for rising number of surface sarcolemmal invaginations during this period (18). All that demonstrates that during venous wall SMC differentiation into contractile phenotype setting in during growth some metabolic chan-

ges of these SMC occur directed towards perfection of vascular contractile function. The latter presupposes a more active venous wall participation in the processes of venous blood flow autoregulation. This age-differentiated metabolic heterogeneity of venous SMC covers probably a series of other enzyme systems only scantily reported in the literature (16). The profound investigation of this problem can more completely elucidate the role of venous wall metabolism for circulation autoregulation during different age periods of postnatal ontogenesis.

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ВОЗРАСТНЫЕ ОСОБЕННОСТИ АКТИВНОСТИ НЕМИОЗИНОВОЙ И МИОЗИКОВСКОЙ АДЕНОЗИНТРИФОСФАТАЗЫ В ВЕНОЗНОЙ СТЕНКЕ КРОЛИКОВ

Г. Маринов, М. Трошева

РЕЗЮМЕ

Изучены возрастные изменения активности немиозиновой и миозиновой аденозинтрифосфатазы в стенках воротной, брыжеечной, задней полой и бедренной вен 16 кроликов в возрасте 7, 21, 42, 80 дней, а также и половозрелых (5—6 месячных) животных. Установлено, что сдвиг реакции в положительную сторону в отдельных оболочках венозной стенки связан с наличием в них эндотелиальных и гладкомышечных клеток. Интенсивность этой реакции однако показывает зависимость от определенных различий в возрасте. Устанавливается, что реакции слабо положительны в гладкомышечных клетках семидневных животных. В процессе возрастания животных увеличивается и активность реакций. Это показывает, что при дифференциации гладкомышечных клеток венозной стенки в контрактильном фенотипе, наступающей с ростом животных, наступают изменения в обменных процессах венозной стенки, направленные к совершенствованию ее контрактильной функции. Это дает основание предполагать более активное участие венозной стенки в процессах авторегуляции венозного кровотока.