Changes in rat ventricular myosin in old age

I. Syrový[1]

Institute of Physiology, Czechoslovak Academy of Sciences, Vídeňská 1083, CS-14220 Prague-4-Krč (Czechoslovakia), 7 February 1983

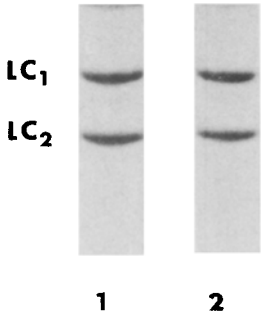
Summary. Myosin was isolated from rat ventricular myocardium, and its properties were compared in adult and very old animals. Ca^{2+}-ATPase activity of ventricular myosin was found to be lower in very old animals as compared with adult ones; K^+-ATPase activity, however, does not change with the aging process. Neither were there any differences between the two age groups in the pattern of ventricular light chains of myosin.

It is known that ventricular myosin exists in 3 iso-forms, which differ in their ATPase activities[2-4]. The ratio of these isoenzymes is species- and age-dependent[5]. In a given species, the developmental changes in ATPase activity of ventricular myosin correlate with the changes in distribution of individual myosin isoenzymes[6]. This fact, and also the observation that the isoenzymic pattern can be changed under the influence of the thyroid hormone or after chronic mechanical overloading[2,3,7], point to the physiological significance of myosin isoenzymes in cardiac muscle. It has been shown that individual isoenzymes differ in their peptide maps and the antigenic structure of their heavy chains[5,8-11]. The postnatal developmental changes in cardiac myosin are not accompanied by differences in the pattern of myosin light chains. It was found that cardiac myosin of rabbits 9-3 days before birth contains light chains not present in adult cardiac muscle myosin[12]. Ventricular myosin of fetal rats has a light chain corresponding to the skeletal muscle embryonic light chain[11]. In human fetal ventricles, an additional light chain has been described, whose electrophoretic properties are identical with those of the adult atrial LC_1[13], and in fetal rabbit ventricle an additional light chain was also observed, which had the same mobility in two-dimensional electrophoresis as atrial LC_1[14].

Thus it seemed to be of interest to compare the basic properties of ventricular myosin isolated from adult and very old animals. We therefore examined ventricular myosin of adult and senile rats by electrophoresis in the presence of sodium dodecyl sulfate (SDS) and by measuring ATPase activity.

Ca^{2+}- and K^+-ATPase activity of myosin from rat ventricles. Activity is given as μmol P_i/mg protein/min. Each value represents the mean ± SD of the number of experiments given in parentheses

Source of myosin	Ca^{2+}-ATPase	K^+-ATPase
Adult rat	1.02 ± 0.02	0.79 ± 0.03 (3)
Senile rat	0.68 ± 0.03	0.77 ± 0.04 (3)



Electrophoretic fractionation of light chains of ventricular myosin in the rat. 10% polyacrylamide, 50 μg. *1* Ventricular myosin from adult rat. *2* Ventricular myosin from senile rat.

Female albino rats (Wistar) aged 4 months and 29 months were used. For preparation and purification of myosin the method of Perry[15] was used and modified after Syrový and Gutmann[16]. The modification was based on initial washing out of sarcoplasmic proteins with dilute phosphate buffer and on the addition of β-mercaptoethanol (2 mM, final concentration) to all solutions used during the isolation procedure. Ca^{2+}-activated ATPase was measured in a medium containing 0.05 M Tris-HCl, pH 7.5, 10 mM $CaCl_2$, 0.025 M KCl, 5 mM ATP and 0.2 mg of protein/ml, 27 °C, K^+-activated ATPase in a medium containing 1 mM EDTA, 0.5 M KCl, 5 mM ATP, 0.1 M Tris-HCl, pH 7.5. SDS-gel electrophoresis was performed according to Weber and Osborn[17] in 10% polyacrylamide gel. 50 μg of myosin were loaded and light chains of myosin were subsequently detected using Coomassie blue.

The activities of the ATPases of adult and senile rat ventricular myosin are compared in the table. The Ca^{2+}-ATPase is 1.5 times higher in adult rats than in very old animals. On the other hand, K^+-activated ATPase activity of myosin from adult rats does not differ from that of senile rats.

The figure illustrates the electrophoretic patterns of the light chains of ventricular myosin. The electrophoretic mobilities of light chains of ventricular myosin from adult rat and senile rat are identical.

Our results have shown that Ca^{2+}-ATPase activity is higher in old rats than in senile individuals, whereas K^+-ATPase remains unchanged. The electrophoretic pattern of light chains of myosin is the same in both the myosins compared. From this it can be postulated that the change of myosin ATPase activity (Ca^{2+}-ATPase) of myosin is connected with a change in the structure of heavy chains of the myosin molecule.

It is evident from recent studies[5,6,18] that V_1 and V_3 isoforms of ventricular myosin do not differ in the composition of their light chains. Our results suggest that both myosins compared differ in the ratio of ventricular myosin isoforms. It was shown by Lompre et al.[5] and by Watras[18] that the ratio of cardiac myosin isoenzymes in the rat and also in other mammals changes during postnatal development and that these changes correlate with the postnatal changes in Ca^{2+}-activated myosin ATPase[6,18]. Thus the decrease of Ca^{2+}-ATPase of ventricular myosin suggests that myosin isoforms are redistributed during old age, probably by an increase of the V_3 isoform and/or by a decrease of the V_1 isoform, which has higher Ca^{2+}-ATPase activity than the V_3 isoform. It should be noted that the average heart weight of experimental animals was 710 mg in adult and 1200 mg in senile rats.

The fact that myosin isolated from the ventricles of the adult rats has the same K^+-ATPase activity as myosin prepared from senile rats is not surpsising, because K^+-ATPase of ventricular myosin also does not change in 2-day to 12-week-old rats, whereas Ca^{2+}-ATPase increases 1.6 times[18].

If it should be proved that the proportion of ventricular myosin isoforms changes during old age, this would

strengthen the idea that isoenzymic shifts underlie the mechanism of cardiac adaptation to new functional demands.

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Inhibition of tumor promotion by a lecanoric acid analogue[1]

K. Umezawa, T. Matsushima, T. Sawa, T. Takeuchi and I. Hirono

Departments of Molecular Oncology, and Carcinogenesis and Cancer Susceptibility, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108 (Japan), and Institute of Microbial Chemistry, 3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141 (Japan), 5 April 1983

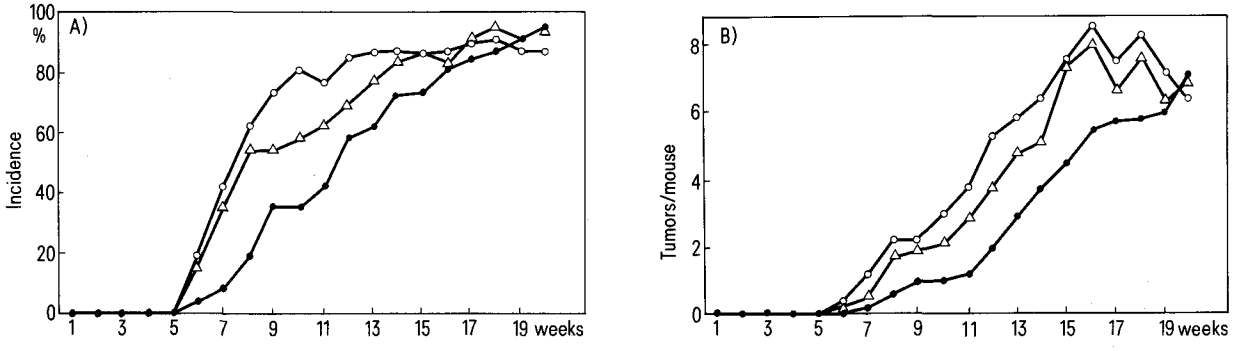
Summary. 3′,5′-Dichloro-2,4′-dihydroxybenzanilide, an inhibitor of histidine decarboxylase, inhibited skin tumor promotion induced by 12-O-tetradecanoylphorbol-13-acetate in mice.

Lecanoric acid has been isolated from culture filtrates of *Streptomyces* as an inhibitor of histidine decarboxylase[2]. Because lecanoric acid was easily metabolized in animals, a number of its analogues containing a peptide bond in place of an ester bond were synthesized, and were shown to inhibit histidine decarboxylase, arachidonic acid release and prostaglandin synthetase[3]. Since 12-O-tetradecanoylphorbol-13-acetate, a tumor promoter in mouse skin, is known to enhance these biological activities[4,5], lecanoric acid analogues were expected to inhibit tumor promotion. Therefore, we studied the inhibitory effects of lecanoric acid analogues on in vivo tumor promotion.

Materials and methods. Female CD-1 mice were purchased from Charles River Japan Inc. Lecanoric acid analogues were kindly supplied by the Central Research Laboratory of Sanraku-Ocean Co., Ltd. TPA was purchased from Consolidated Midland Corporation.

The backs of 7-week-old mice were shaved and 0.1 mg of 7,12-dimethylbenz(a)anthracene (Tokyo Kasei Co., Ltd) in 0.1 ml of acetone was applied. From 1 week later TPA (0.01 mg) and 1 mg of 3′,5′-dichloro-2,4′-dihydroxybenzanilide (Product number SD-170) or 4′-methoxy-4-methyl-2-hydroxy-benzanilide (SD-702) or TPA alone dissolved in 0.1 ml of acetone were applied twice a week for 20 weeks. Each group consisted of 26 female CD-1 mice.

Results and discussion. Data on the incidence of tumor bearing mice and the number of tumors are shown in figure A and B, respectively. Two mice, one from the control group in week 19 (keratoacanthoma) and the other from the SD-702 group in week 16 (thymic lymphoma), were lost during the experiment. Application of SD-170 decreased both the incidence and number of tumors, as shown in figure A and B. The table shows that the tumors were smaller in the SD-170 group than in the control group. SD-



Effect of lecanoric acid analogues on mouse skin tumor promotion. Mice were painted with TPA alone (○), TPA and SD-170 (●) or TPA and SD-702 (△) twice a week. *A* Incidence of tumor bearing mice. *B* Number of tumors per mouse.