# MUSCLE ENZYME ACTIVITY IN HUMAN BLOOD VESSEL WALLS UNDER NORMAL CONDITIONS AND IN ESSENTIAL HYPERTENSION

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Changes are found in the activity of some enzymes in the musculature of the renal arteries in persons with severe arterial hypertension: activity of myofibrillary ATPase is sharply increased, while activity of cholinesterase and hexokinase is lowered.

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The purpose of this investigation was to study the activity of a number of enzymes in the muscular tissues of the human blood vessel wall (myofibrillary adenosinetriphosphatase and acetylcholinesterase, and also hexokinase) at various age periods under normal conditions and in essential hypertension.

## EXPERIMENTAL METHOD

The renal arteries obtained from human cadavers not more than 24 h after death were studied. According to some data [6-8], the protein fractions and enzyme activity of the blood vessel wall, like those of other tissues with a relatively low level of metabolism, remain unchanged for 24 h or even longer if the material is kept at a low temperature.

Renal arteries from cadavers of persons dying from severe essential hypertension without well marked signs of atherosclerosis were investigated. Similar arteries from persons not suffering from cardiovascular diseases during life were used as controls. Altogether about 40 arteries from patients with hypertension and the same number of controls were studied.

The ATPase (3.6.1.3) activity was determined in extracts of myofibrillary proteins by the usual method based on the increase in content of inorganic phosphate at pH 9.1, and expressed as $Q_p$. To detect cholinesterase (3.1.1.8) activity in the same extracts, hydrolysis of acetylcholine was carried out for 1.5 h at pH 7.8, the acetylcholine concentration in the sample after hydrolysis being determined by Hestrin's method and expressed in μg acetylcholine hydrolyzed by 1 mg protein per hour. Hexokinase (2.7.1.1) activity was determined from the lowering of the glucose concentration in a buffered solution containing ATP, Mg,, KCl, and KF. The glucose concentration in the samples was determined by the glucose–oxidase method [4].

## EXPERIMENTAL RESULTS

Adenosinetriphosphatase. ATPase activity in extracts of myofibrillary proteins obtained from persons aged 15-75 years who had not suffered from hypertension was extremely low. Expressed as $Q_p$, its mean value was 30.94±4.4, and no clear differences associated with age could be detected. The coefficient of corrleation between age and ATPase activity (r) was −0.02 (t = −0.087, n = 21). Activity of this enzyme in extracts from the renal arteries of persons with essential hypertension, as Table 1 shows, was much higher (by 311%) than the results obtained in control experiments for the same age groups ($P < 0.01$; n=17).

Cholinesterase. Cholinesterase activity in extracts of myofibrillary proteins of control vessels taken from persons of different ages averaged 226.2±26.4 μg acetylcholine hydrolyzed by 1 mg protein per hour (Table 2).

A statistically significant negative correlation was found between age and enzyme activity in the arteries (r = −0.51, t* = −2.8, n = 23).

*Calculated from the formula $t = r\sqrt{\frac{n-2}{1-r^2}}$ [9].

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TABLE 1. Activity of ATPase ($Q_p$) in Extracts of High Ionic Strength from Human Renal Arteries

| Age (in years) | Arteries from persons without hypertension | | | Arteries from persons with hypertension | | |
|---|---|---|---|---|---|---|
| | *n* | *M* | ±*m* | *n* | *M* | ±*m* |
| 15—29 | 7 | 34,50 | 9,5 | | | |
| 30—39 | 5 | 30,50 | 9,0 | | | |
| 40—59 | 5 | 30,96 | 3,7 | 9 | 96,3 | 14,82 |
| 60—75 | 4 | 25,20 | 4,1 | 8 | 88,8 | 13,50 |

TABLE 2. Cholinesterase Activity in Extracts of High Ionic Strength from Muscle of Human Renal Arteries

| Age (in years) | Arteries from persons without hypertension | | | Arteries from persons with hypertension | | |
|---|---|---|---|---|---|---|
| | *n* | *M* | ±*m* | *n* | *M* | ±*m* |
| 15—29 | 9 | 337,5 | 42,6 | | | |
| 30—39 | 6 | 217,8 | 55,4 | | | |
| 40—59 | 4 | 180,3 | 32,9 | 7 | 104,7 | 21,1 |
| 60—75 | 4 | 169,4 | 9,5 | 11 | 139,1 | 25,7 |

TABLE 3. Hexokinase Activity in Muscle of Human Renal Arteries

| Age (in years) | Arteries from persons without hypertension | | | Arteries from persons with hypertension | | |
|---|---|---|---|---|---|---|
| | *n* | *M* | ±*m* | *n* | *M* | ±*m* |
| 16—29 | 7 | 0,221 | 0,094 | | | |
| 30—39 | 8 | 0,253 | 0,070 | | | |
| 40—49 | 4 | 0,625 | 0,100 | 3 | 0,091 | 0,051 |
| 50—59 | 4 | 0,449 | 0,090 | 6 | 0,165 | 0,041 |
| 60—75 | 4 | 0,569 | 0,070 | 14 | 0,192 | 0,029 |
| Total 16—75 | 27 | 0,380 | 0,050 | 23 | 0,149 | 0,040 |

The cholinesterase activity in arteries obtained from persons aged 40-59 years with essential hypertension was much lower (57.7%) than in control vessels of persons of the same age group ($P = 0.05$; $n = 7$). However, this parameter in arteries of persons dying from hypertension at an older age (60-75 years) was only slightly lower than the control, and this difference was not statistically significant.

The results of these investigations thus indicate that myofibrillary proteins of blood vessels obtained from cadavers of persons dying from essential hypertension have lower cholinesterase and considerably (almost three times) higher ATPase activity than controls. In conjunction with the observations of Yur'ev [1, 5], who found an increase in the content of contractile protein in the renal arteries in essential hypertension, they evidently indicate an increase in contractile power of the muscle of the blood vessel wall in this disease.

Hexokinase. Hexokinase activity in extracts of low ionic strength obtained from control vessels taken from cadavers of persons aged 16-75 years averaged 0.38±0.05 μmole glucose converted by 1 mg protein in 20 min (Table 3). A definite tendency was observed for enzyme activity to increase with age, the coefficient of correlation between age and hexokinase activity (r) being +0.54 ($t = 3.2$, $n = 27$).

According to the literature [7, 10] tissue respiration in animals decreases with age and lactic acid production in the aortic wall increases. The results of this investigation, demonstrating a marked increase with age in hexokinase activity—one of the enzymes limiting the rate of glycolysis [3]—in the muscle of human blood vessels likewise confirm the increased role of glycolysis as the source of energy in blood vessel walls at a more advanced age. Adaptation evidently takes place in vivo to energy production under conditions of a reduced oxygen supply to the inner layers of the thickened vessel wall.

Hexokinase activity was much lower in the renal arteries of persons with essential hypertension. In patients aged 40-49 years it was only 14% of the activity of control vessels taken from persons of the same age ($P < 0.01$, $n = 3$). Admittedly, in older patients (50-75 years) this difference was much smaller, their enzyme activity being 35% of the control value ($P < 0.01$, $n = 20$).

The results now described, as well as those of previous investigations [1, 2, 5], show that marked biochemical changes take place in the muscle tissue of the blood vessels in persons with essential hypertension. In addition to an increase in content of the fraction containing proteins of the actomyosin complex, the ATPase activity is considerably increased and the cholinesterase activity of the myofibrillary proteins slightly reduced. Hexokinase activity of the vascular muscles is appreciably reduced in essential hypertension. The changes detected thus concern both the biochemical substrate of the contractile reaction of the blood vessels and also the energy metabolism in the muscle tissue. These changes in the muscle of the blood vessels may evidently play an essential role in the genesis of essential hypertension.

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