INVESTIGATIONS ON THE INFLUENCE OF TESTOSTERONE PROPIONATE VERSUS ACTIVITY OF 5'-NUCLEOTIDASE AND ADENYLPYROPHOSPHATASE IN THE AORTIC WALL OF NORMAL AND THYROIDECTOMIZED MALE ALBINO RATS

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Baló and co-workers (7) have established in the aorta of humans a highly active adenylpyrophosphatase. The latter enzyme splits the ortophosphate from adenosine triphosphate and adenosine diphosphate and is contained in the arterial tissue only. Adenosine monophosphate is produced as the end product of the enzyme action. In 1950, Reis (12) proved also the presence of 5'-nucleotidase in the tissue of the aorta, which is capable of splitting adenosine monophosphate.

The adenylpyrophosphatase plays an important role in supplying the tissues with energy (6, 11). Its high activity in the arterial tissue of man is of great interest, especially when considering the low oxidation metabolism at this particular level (9).

On the contrary, 5'-nucleotidase (5'-ribonucleotide-phosphohydrolase 3 . 1 . 3 . 5) reveals a low activity in the human arterial tissue (12). This enzyme is important for the regulation of tissue calcification and plays a significant role in the formation of the ground substance during the metabolism of mucopolysaccharides (15).

With advancing of age, 5'-nucleotidase activity in the arterial tissue is significantly increased (9). The problems of hormonal regulation of these two enzymes within the vascular wall (15) are of particular interest.

Furman (8) makes reference to a very interesting hypothesis concerning the atherogenic action of the androgenic hormones.

Considering the important role ascribed to 5'-nucleotidase and adenylpyrophosphatase in the aortic wall, in the course of the atherosclerotic process (6, 15), we set out to investigate the influence of testosterone propionate on the activity of this particular enzyme. Since the development of the atherosclerotic process is influenced also by the function of the thyroid gland (11), the studies were conducted on normal rats and on rats with removed thyroid. Literature data on similar investigations were not found.

Material and Methods

The studies were carried out on 56 young, sexually mature unbred male white rats, weighing from 140 to 180 g, distributed in the following series: group I — control (intact) — 14 rats; II — treated with testosterone pro-

pionate — 10 rats; III — thyroidectomized — 12 rats; IV — thyroidectomized, treated with testosterone propionate — 11 rats; V — pseudothyroidectomized — 9 rats.

The thyroidectomized rats (III and IV) were investigated one and a half months after the operation. It is well known (2) that the thyroid hormones have comparatively prolonged period of semi-disintegration and are being conserved in the organism for several days after thyroidectomy. In the work of Lebedeva (1), recommendation is made that experimentation with thyroidectomized animals should be conducted not prior to 30—35 days after the operation. The pseudothyroidectomized animals were studied in the course of the same terms.

Testosterone propionate was administered in the form of oil solution at dose 1 mg/100 g weight, with three-fold injection over a period of 10 days.

The animals under investigation received bread and milk ad libitum. Those operated upon received water solution of calcium lactate instead of water, aiming reduction of mortality rate in the postoperative period (owing to involvement of the parathyroid glands). Intramuscular injections were also made with 10% solution of calcium gluconate (0.1 ml/100 g body weight). Calcium lactate was also given to the control animals.

Before the investigation, the animals were kept for 15 hours on a fasting regime. They were killed on the same hour of the day (11.00 a. m.). The aorta was promptly removed, cleansed from the blood and periaortal tissues and homogenized for 15 min at 0° C with saline solution (1 ml solution per 10 mg aortic tissue). Centrifugation was performed for 10 min (3000 rev/min). The activity of the two studied enzymes was determined in the supernatant liquid.

The method of Ahmed and Reis (4) as modified by Zemplenyi and assoc (14), was resorted to for 5′-nucleotidase, and of Banga and Novotny (6) — for adenylpyrophosphatase.

The activity of 5′-nucleotidase was determined with substrate adenosine-5′-monophosphate, at pH 7.5, of adenylpyrophosphatase at substrate ATP and pH 9.0. Veronal buffer was used for both enzymes. The incubation at 37° C lasted 30 minutes.

The amount of released inorganic phosphate was determined with SPECOL at 650 millimicrons. The activity of the enzymes was measured by means of increasing the inorganic phosphate in milligrams per 100 mg fresh tissue.

Results and Discussion

The results of the investigations are illustrated in Table 1.

The data in Table 1 demonstrate:

1. The introduction of testosterone propionate in normal male rats provokes statistically reliable rise of 5′-nucleotidase activity ($t=3.09$, $P<0.01$) and reduction with 10.4% of adenylpyrophosphatase activity ($t=3.15$, $P<0.01$).

2. After thyroidectomy no statistically reliable changes in the activity of 5′-nucleotidase occur ($t=1.11$, $P>0.25$), whereas the activity of adenylpyrophosphatase reveals a statistically reliable reduction ($t=4.63$, $P<0.001$).

Tab

Activity of 5′-nucleotidase and adenylpyrophosphatase in the aortic wall of normal and thyroidectomized male albino rats

Incubation for 30 min at 37° C
Increase of inorganic phosphate — mg/100 mg fresh tissue

Series	Number	5′-nucleotidase (pH=7.5)	Adenylpyrophosphatase (pH=9.0)
I — Control	14	0.388+0.029	2.775+0.192
II — Treated with testosterone propionate	10	0.529±0.035	2.494±0.185
III — Thyroidectomized	12	0.455±0.055	1.765±0.142
IV — Thyroidectomized, treated with testosterone propionate	11	0.346±0.041	1.335±0.133
V — Pseudothyroidectomized	9	0.353±0.063	2.037±0.220

3. The treatment of thyroidectomized male rats with testosterone propionate (Series IV) does not account for statistically reliable changes in the activity of 5′-nucleotidase ($t=1.56$, $P<0.01$) and causes a new reduction in the activity of adenylpyrophosphatase ($t=2.19$, $P<0.05$), statistically reliable. The decrease in relation to control animals (I) is still greater —51.8%.

4. In the animals subjected to pseudothyroidectomy (V) no statistically reliable changes are established as regards 5′-nucleotidase ($t=0.63$, $P<0.05$). The activity of adenylpyrophosphatase shows statistically reliable reduction ($t=2.51$, $P<0.05$). Most likely, in this case a stress reaction is involved (5).

It is a well known fact that the hormones of the thyroid gland exert a stimulating action on the tissue oxidation (5). It may be assumed that the inhibition of the oxidation processes within the aortal tissue in thyroidectomy is externally manifested also by the lowered activity of the adenylpyrophosphatase, specific for this tissue, as demonstrated by our personal data. There are literature references (3, 13) concerning the influence of the thyroid hormones in general on the ATP-ase activity in the organism.

In accordance with our data, the testosterone propionate exerts variable effect on the activity of the two enzymes. It inhibits the activity of adenylpyrophosphatase, with the inhibition being more strongly pronounced in the thyroidectomized animals. Ganga and Novotny (6) have established that in the arteriosclerotic aortal tissue, the activity of adenylpyrophosphatase is lower than in normal tissue. Probably, the effects of testosterone propionate and thyroid hormones upon the adenylpyrophosphatase, established by us, might be explained by certain aspects of these hormones' participation in the atherosclerotic process (8).

Unlike adenylpyrophosphatase activity, the activity of 5′-nucleotidase shows statistically reliable increase after treatment of normal rats with testosterone propionate. This is a further indication for the easier occurrence of the atherosclerotic process since it is well known (15) that in higher 5′-nucleotidase activity, the formation of the ground substance, which is a prerequisite for subsequent lipoid depositions, is accomplished at a higher degree.

Inferences

1. After treatment of normal male rats with testosterone propionate, a statistically reliable increase of the activity of 5′-nucleotidase and reduction of the activity of adenylpyrophosphatase are established.

2. Thyroidectomy causes an increase of the adenylpyrophosphatase activity, without altering significantly the activity of 5′-nucleotidase.

3. The treatment of thyroidectomized male rats with testosterone propionate provokes a still greater reduction in the activity of adenylpyrophosphatase, without anyway exerting a statistically reliable effect on 5′-nucleotidase.

4. In pseudothyroidectomized rats, the activity of adenylpyrophosphatase is lowered, whilst that of 5′-nucleotidase shows no statistically reliable changes whatsoever.

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ИЗУЧЕНИЕ ВОЗДЕЙСТВИЯ ТЕСТОСТЕРОНПРОПИОНАТА НА АКТИВНОСТЬ 5'-НУКЛЕОТИДАЗЫ И АДЕНИЛПИРОФОСФАТАЗЫ В СТЕНКЕ АОРТЫ НОРМАЛЬНЫХ И ТИРЕОИДЭКТОМИРОВАННЫХ САМЦОВ БЕЛЫХ КРЫС

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РЕЗЮМЕ

Исследованию подверглось 56 самцов белых крыс, созревших в половом отношении, которые были сгруппированы в следующие серии: I — контрольные (интактные); II — получавшие тестостеронпропионат; III — тиреоидэктомированные; IV — тиреоидэктомированные, получавшие тестостеронпропионат; V — псевдотиреоидэктомированные. Оперированных животных исследовали через полтора месяца после операции. Тестостеронпропионат вводился в общей дозе 1 мг/100 г веса трехкратно в течение 10 дней. Статистическая обработка данных показывает, что тестостеронпропионат вызывает снижение активности аденилпирофосфатазы, причем это снижение значительно заметнее у тиреоидэктомированных, чем у нормальных животных. Тиреоидэктомия также снижает активность энзима.

5'-нуклеотидаза повышается после введения тестостеронпропионата нормальным крысам. После тиреоидэктомии уровень ее не изменяется статистически достоверно.