CHANGES IN THE CYCLIC AMP CONTENT IN MUSCLE IN EXPERIMENTAL DEXAMETHASONE-INDUCED MYOPATHY

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The active participation of cyclic AMP in the regulation of metabolism and the mechanism of relaxation and contraction of muscle tissue necessitates the investigation of cyclic AMP in various forms of neuromuscular diseases [2, 3, 7]. Changes in the cyclic AMP level and in activity of enzymes regulating its metabolism have been found in genetically determined diseases of muscle tissue: progressive muscular dystrophies and myotonias. However, it has not been established in these diseases whether changes in the cyclic AMP content are decisive factors in the development of the muscle dystrophy. More informative data on the possible role of cyclic AMP in the development of muscular dystrophies can be obtained by the study of cyclic AMP in induced muscular dystrophies. Analysis of changes in the cyclic AMP content in these conditions is facilitated by the presence of a specific factor, in this case dexamethasone.

Among the various forms of induced myopathies, steroid myopathy is the commonest. The frequency of cases of steroid myopathy, following the extensive clinical use of glucocorticoid preparations, increases year by year. The cyclic AMP concentration in muscle in steroid myopathy has not hitherto been studied. There are only scattered items of information which give evidence of an increase in its content after a single injection of glucocorticoids [5].

The cyclic AMP content in rabbit skeletal muscle was studied in experimental steroid myopathy.

EXPERIMENTAL METHOD

Fifteen male rabbits aged 5-6 months and weighing 2.5-3.5 kg were used. Myopathy was induced by administration of dexamethasone (from "Galenica," Yugoslavia) in a dose of 0.8 mg/kg body weight daily. The development of myopathic symptoms was assessed from the total range of active movements, ability of the animals to turn over from the supine to the prone position, muscle tone, strength, and state of nutrition of the skeletal muscles. A biopsy specimen of the thigh muscles was taken with forceps for analysis of cyclic AMP on the 1st, 7th, and 14th days of the experiment. The muscle tissue was weighed and then homogenized by a glass homogenizer in 1 ml of EDTA-Na_2 solution. To remove proteins, the homogenate was placed in a boiling waterbath for 3 min, cooled, and centrifuged. The supernatant was collected and kept at −20°C until determination. Cyclic AMP was determined directly in the aqueous extract of the muscles by the method of competitive binding with protein [13], using the standard kit from the Radiochemical Centre, Amersham, England. The cyclic AMP concentration in the muscle was expressed in picomoles/100 mg tissue. The results of the investigation were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Prolonged administration of dexamethasone led to the development of characteristic symptoms of steroid myopathy in the rabbits. The first symptoms of myopathy appeared on the 7th day of the experiment: the animals became fatigued while moving about the room, and had difficulty in turning over from the supine to the prone position. On the 9th day the myopathic symptons increased. Reduced mobility and increased fatigue at rest were observed, and the rabbits dragged their hind limbs when walking. On the 14th day of the experiment marked atrophy of the hind limbs was observed; the animals could not walk about unaided and remained in a state of immobility.

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By the first day of the experiment a sharp increase was observed in the cyclic AMP concentration in the muscle. Whereas in intact rabbits its value was 32.8 ± 4.8 pmoles/100 mg tissue, after the first injection of dexamethasone, i.e., on the 1st day, it reached 60.3 ± 4.8 pmoles/100 mg ($P < 0.01$). On the 7th and 14th days, during the period of manifestation of a clinical picture of steroid myopathy, the cyclic AMP concentration was not significantly changed, with values of 61.8 ± 7.3 and 64.1 ± 8.2 pmoles/100 mg respectively.

The increase in the cyclic AMP concentration could be connected with an increase in activity of the enzyme regulating its synthesis or a decrease in activity of the enzyme destroying it. The enzyme synthesizing cyclic AMP from ATP is adenylate cyclase, which is built into the cell membrane and is activated by chemical effector signals from the external medium [4]. The hormones which modify adenylate cyclase activity include adrenalin, glucagon, insulin, and other polypeptide hormones. Glucocorticoids act on cyclic AMP indirectly through other hormones. However, synthetic glucocorticoids can probably also exert a direct influence on adenylate cyclase, for they contain certain ions which change its activity. One such preparation is dexamethasone, the molecule of which contains fluorine ions. It has been shown experimentally that fluorine anions have a marked stimulating effect on adenylate cyclase [8]. Fluorine anions are considered to accelerate the conversion of the inactive form of cyclic AMP into the active form. Glucorticoids themselves act more strongly on another enzyme regulating the stable cyclic AMP concentration, namely phosphodiesterase [5, 10].

It can thus be concluded on the basis of data in the literature that elevation of the cyclic AMP level in skeletal muscle in dexamethasone-induced steroid myopathy may be connected with the combined action of dexamethasone on the enzyme systems determining the cyclic AMP concentration. Of all the steroid preparations dexamethasone and triamcinolone are known to have the severest side effects on muscles and to give rise to steroid myopathy more frequently than the other steroids in clinical practice [6].

The increase in the cyclic AMP concentration as a result of prolonged administration of dexamethasone is characterized by a whole series of simultaneous pathological changes in the muscle, and leads ultimately to the development of severe muscular dystrophy. An increase in the cyclic AMP concentration is known to stimulate the activity of many intracellular enzymes and to induce various types of cellular activity [1]. In particular, an increase in cyclic AMP may be associated with the increased glycogen concentration in the muscle discovered previously [11, 12]. Changes in the glycogen content may be connected with the direct effect of cyclic AMP on gluconeogenesis. It has been suggested that the more intensive formation of cyclic AMP from ATP disturbs the acceptor function of the ATP-calcium complex, as a result of which calcium is liberated and forms a complex with other acceptors, including membrane phospholipids [9]. This, in turn, may contribute to the disturbance of the structural integrity of the membranes and of their function, and may also influence the velocity of enzymic reactions dependent on Ca^{++} [2].

It can be concluded from the analysis of these experimental data that an increase in the cyclic AMP concentration in muscle, which is found long before clinical manifestation of the disease, may be one of the possible trigger mechanisms of steroid myopathy.

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