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Effect of sex steroids on soleus muscle response in hypocalcemic medium (*in vitro*)

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ABSTRACT

Purpose: Postoperative hypocalcemia is a frequently encountered complication of thyroid surgery. Since hypocalcemic symptoms are closely associated with sex, the aim of this study is to investigate the effects of sex steroids on muscle tissue under hypocalcemic conditions.

Methods: Six groups consisting of control male (M), control female (F), gonadectomized male (M−), gonadectomized female (F−), estradiol-applied gonadectomized male (MX), and testosterone-applied gonadectomized female (FX) rats were used. Contraction recordings were obtained from soleus muscle flaps. Maximal tension (PT), frequency required for 50% of PT (F50), contraction velocity at F50 (V50), and changes in contraction values (d[PT], d[F50], d[V50]) between normocalcemic and hypocalcemic conditions were calculated.

Results: d[PT], d[F50], and d[V50] were significantly higher in M− and MX groups compared with control M group. Whereas d[PT], d[F50], and d[V50] parameters of the F− group were significantly higher than control F group, d[F50] and d[PT] of the FX group showed no significant change and d[V50] for the FX group was significantly lower. A comparison of control groups showed that d[PT], d[F50], and d[V50] of the F group were significantly higher than those of the M group.

Conclusion: Whereas absence of both testosterone and estradiol caused an increase in hypocalcemia-induced changes in contraction parameters of rat skeletal muscle, presence or application of testosterone clearly stabilized contraction parameters.

1. Introduction

Hypocalcemia caused by temporary or permanent hypoparathyroidism is one of the leading complications of thyroidectomy [1–3]. Despite improvements in surgical approach, postoperative hypocalcemia remains an important problem. Current literature states an incidence of 1.7%–68% for hypocalcemia [4]. Postoperative hypocalcemia increases hospital stay and causes loss of labor, and the required additional biochemical investigations increase the cost of treatment [5,6].

The most common cause of hypoparathyroidism is damage to the parathyroid glands during thyroidectomy. The

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signs and symptoms of hypocalcemia are caused by neuromuscular excitability from reduced plasma-ionized calcium. Early manifestations include perioral numbness and tingling in the fingers. Anxiety or confusion can follow. Anxiety often results in hyperventilation, which can then lead to respiratory alkalosis and a further reduction in the serum calcium level. Tetany, marked by carpopedal spasm, convulsions, or laryngospasm (or any combination of the three), may follow and can be fatal. Physical examination includes testing for a Chvostek sign, which is contraction of the facial muscles after tapping on the facial nerve anterior to the ear. Approximately 15% of normal individuals have a positive Chvostek sign, however [7].

Although hypocalcemia in blood tests of patients following total thyroidectomy is not rare, not all cases become symptomatic. Though female patients with laboratory hypocalcemia usually exhibit symptoms of hypocalcemia, male patients rarely show symptoms. This clinical observation made us hypothesize that there might be sex-dependent differences in the neuromuscular system affecting the response to hypocalcemia.

Sex steroids estradiol and testosterone are known to affect many organs and systems, but their effects on the neuromuscular system and their role in the occurrence of hypocalcemic symptoms in particular are not yet fully investigated. The effects of these steroids on cardiac muscle and vascular smooth muscle have been the subject of many studies [8–11], and several mechanisms affected by sex steroids were identified. However, there are limited numbers of investigations on the effects of sex and sex steroids on skeletal muscle tissue. According to these studies [12–16], sex steroids affect the structure and function of skeletal muscle. A study [13] comparing male and female gastrocnemius muscle flaps reported that due to the higher proportion of fatigue-resistant units, muscles of female rats were determined to be more resistant than those of male rats. Another study comparing genioglossus muscles of aged male rats found that testosterone treatment decreased fatigue resistance [17]. The anabolic property of androgens, and in particular testosterone, is one of the best-known aspects of sex steroids. Use of testosterone is associated with increased muscle mass and power but not fatigability in healthy older men [18]. A relationship between size of skeletal muscle fibers and gonadal steroids has also been shown [16]. These hormones also have significant effect on cellular energy metabolism. It has been reported that ATP, creatine phosphokinase, and myokinase activities decrease following gonadectomy and significantly increase after testosterone application [14]. Cellular transportation of ions (calcium and sodium channels) is also known to be affected by testosterone (calcium and sodium ion channels) [8–10,15].

Testes in males and ovaries in females are main sources of testosterone and estradiol. Although estrogens and androgens are also produced in some other tissues as well, these other sources are minimal. For instance, the adrenal glands secrete at least five androgens, although the total masculinizing activity of all these is normally so slight (less than 5% of the total in the adult male) that even in women they do not cause significant masculine characteristics, except for causing growth of pubic and axillary hair. And in the normal nonpregnant female, estrogens are secreted in significant quantities only by the ovaries, although minute amounts are also secreted by the adrenal cortices [19].

The ionized fraction of the blood calcium is very important for neural and musculoskeletal function, and it is tightly regulated by parathyroid glands. Of the total circulating calcium, the ionized fraction is 50%; the remainder is bound to serum proteins, primarily albumin, and, to a lesser extent, is complexed with anions such as citrate or sulfate. Only the ionized fraction is physiologically important, and this component is regulated on a minute-to-minute basis [20]. Extracellular fluid calcium concentration normally is 9.4 mg/dL, which is equivalent to 2.4 mmol calcium per liter. Thus, the plasma and interstitial fluids have a normal calcium ion concentration of about 1.2 mmol/L [19]. Ionized calcium in citrated blood samples is reported as 1.23 ± 0.0 mmol/L [21].

Symptomatic hypocalcemia is caused by neuromuscular excitability from reduced plasma-ionized calcium. We designed an experimental study to observe the differences of female and male skeletal muscle in response to hypocalcemia and the effects of absence and cross application of gonadal steroids (estradiol and testosterone) on this response.

2. Material and methods

Experiments were carried out after obtaining approval from Selçuk University Ethics Committee for Animal Studies (Registration Number: 2008/29). Twenty-one female and 21 male adult Sprague-Dawley rats were used. Animals were housed five rats per cage, in an ambient temperature of $22^{\circ}C \pm 2^{\circ}C$, 51% humidity, and a 12-h light-dark cycle during the experiment. All animals were allowed free access to food and water.

Six groups, each with seven rats, were formed. Control groups (female: F; male: M) were sham operated and received only vehicle (olive oil) injections. To assess the effect of absence of gonadal steroids (estradiol and testosterone), gonadectomized female (F–) and gonadectomized male (M–) groups were gonadectomized and also received vehicle injections. To observe the effects of testosterone and estradiol on opposite sexes deprived of their own gonadal steroid, rats in the cross hormone female (FX) group were gonadectomized and received 100 μg/100 mg/d testosterone (dihydrotestosterone) injections and those in the cross hormone male (MX) group were also gonadectomized and received 5 μg/100 mg/d estradiol injections starting the day after surgery. These doses of estradiol and testosterone were shown to supply normal serum hormone levels [14]. Estradiol and testosterone injections were prepared inside vehicle. Hormones and vehicle were injected intraperitoneally. No infection was observed during follow-up.

Animals were prepared under ketamine (35 mg/kg) and xylazine (5.0 mg/kg) anesthesia. Under aseptic conditions the lower left soleus muscle strips were dissected. Isolated muscle strips were excised under binocular microscope (SMZ800, Nikon Inc, USA). The animal was euthanized following the preparation of the muscle strip. Prepared muscle strip was placed in a recording chamber and pinned down at one end with a stimulating electrode while the second end was connected to a force-displacement transducer (FT-03; Grass Instruments). Dimensions of the soleus muscle strips were