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The risk of life-threatening ventricular arrhythmias in presence of high-intensity endurance exercise along with chronic administration of nandrolone decanoate

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ABSTRACT

Anabolic steroids used to improve muscular strength and performance in athletics. Its long-term consumption may induce cardiovascular adverse effects. We assessed the risk of ventricular arrhythmias in rats which subjected to chronic nandrolone plus high-intensity endurance exercise.

Animals were grouped as; control (CTL), exercise (Ex): 8 weeks under exercise, vehicle group (Arach): received arachis oil, and Nan group: received nandrolone decanoate 5 mg/kg twice a week for 8 weeks, Arach + Ex group, and Nan + Ex. Finally, under anesthesia, arrhythmia was induced by infusion of 1.5 μg/0.1 mL/min of aconitine IV and ventricular arrhythmias were recorded for 15 min. Then, animals' hearts were excised and tissue samples were taken.

Nandrolone plus exercise had no significant effect on blood pressure but decreased the heart rate ($P < 0.01$) and increased the RR ($P < 0.01$) and JT intervals ($P < 0.05$) of electrocardiogram. Nandrolone + exercise significantly increased the ventricular fibrillation (VF) frequency and also decreased the VF latency ($P < 0.05$ versus CTL group). Combination of exercise and nandrolone could not recover the decreasing effects of nandrolone on animals weight gain but, it enhanced the heart hypertrophy index ($P < 0.05$). In addition, nandrolone increased the level of hydroxyproline (HYP) and malondialdehyde (MDA) but had not significant effect on glutathione peroxidase of heart. Exercise only prevented the effect of nandrolone on HYP.

Nandrolone plus severe exercise increases the risk of VF that cannot be explained only by the changes in redox system. The intensification of cardiac hypertrophy and prolongation of JT interval may be a part of involved mechanisms.

1. Introduction

Nandrolone, 19-nortestosterone, is a famous synthetic anabolic androgenic steroid (AAS) that derived from testosterone. In addition to androgenic properties, AASs have remarkable anabolic activity. For this reason, 19-norsteroids are used to improve muscular strength and performance in human athletics and also to accelerate weight gain and to improve the feed efficiency in animals [1,2]. However, the anabolic and androgenic activities are associated with some undesirable side effects in mammals' body systems. The most cardiovascular adverse effects of these agents are hypertension, myocardial infarction, dysrhythmias, hypertrophic cardiomyopathy, and sudden cardiac death [1–3].

The cardioprotective effects of regular courses of exercise as the only practical method have been proved. It is confirmed that exercise can improve the heart tolerance against ischemia/reperfusion injury [4] and can preserve the heart's mechanical function following ischemia/reperfusion periods in animals [5,6]. Regular physical exercise can decrease the death rates by reduction of the major cardiovascular risk factors such as plasma cholesterol levels, blood pressure, overweight and glucose intolerance [7]. Because of the harmful effects of anabolic steroids, efficacy of exercise training along with consumption of high amounts of these drugs is controversial and debatable. Some studies reported that nandrolone can

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eliminate the protective effects of exercise training on cardiovascular system [8–10]. Acute injection of nandrolone can increase the possibility of cardiac arrhythmias in animals with ischemic hearts [8]. In addition, the occurrence of sudden cardiac death among athletics using anabolic steroids, especially nandrolone, is reported [11–13]. In two separate studies we recently showed that nandrolone consumption in presence of low and moderate intensity endurance exercise may increase the risk of fatal ventricular arrhythmia in rat [14,15]. However, to our knowledge, so far no study has examined the effect of chronic nandrolone deaconate consumption along with high intensity exercise on the development of lethal cardiac arrhythmia. Therefore, in the present study we tested the ventricular arrhythmogenic possibility following to chronic administration of nandrolone deaconate along with high intensity swimming exercise in rat.

2. Materials and methods

The study procedures were performed in accordance with the guidelines for the care and use of laboratory animals provided by Ethic committee permission No. K/93/272, Kerman University of Medical Sciences, Kerman, Iran and were carried out on 48 male Wistar rats weighing 200–250 g. The animals were housed in controlled humidity and room temperature and a 12 h light/dark cycle with ad libitum access to standard rat chow and water and were randomly divided to six experimental groups: (1) sedentary group (CTL group); (2) trained group with exercise by swimming training (Ex group); (3) sedentary vehicle-treated (Arach group) (vehicle = 0.2 mL/kg Arachis oil as solvent of nandrolone decanoate twice a week, intramuscularly, for 8 weeks; (4) sedentary nandrolone-treated (Nan group, received nandrolone decanoate 5 mg/kg body weight twice per week, intramuscularly, for 8 weeks) [14]; (5) trained vehicle-treated (Arach + Ex group, received Arachis oil and swimming training); and (6) trained nandrolone-treated (Nan + Ex group received nandrolone decanoate and swimming training). The selected dose of nandrolone in this study is comparable to the dosage that has been reported to be frequently used by heavy AAS abusers [10,16].

2.1. Swimming training

The high intensity swimming training protocol was done 5 days/week for 8 weeks. It was executed in a pool adapted for rats with the depth of 30–50 cm that contained warm water (30–32 °C). The swimming time in the first round was 20 min, which was increased daily by 10 min until 60 min came on the fifth day. From the second week until the end of eighth week, exercise duration was 60 min/day for 5 days/week. In addition, from the second week the animals were worn caudal dumbbells that weighed 2% of their body weight. The caudal weight was gradually increased until it was 15% of body weight on the sixth week and was thereafter kept constant. This method is a modifier of methods that previously used [14,17,18]. When each animal was exhausted and could not sustain the swim, it was removed from the water and the rest was given for two minutes. Then to continue the practice, it was thrown into the water. All of the rats were weighed weekly to adjust the weight of the dumbbells. Sedentary rats were placed in the pool for 10 min, twice a week to mimic the water stress of the protocol [17].

2.2. Blood pressure and electrocardiogram recording and arrhythmia induction

The methods of heart rate (HR), blood pressure (BP), and electrocardiogram (ECG) recordings and also arrhythmia induction were described previously [19]. At the end of experiment period, the animals were anesthetized with sodium thiopental (50 mg/kg, IP) [20]. The right common carotid artery was cannulated by a polyethylene 50 tube and the HR and BP were continuously recorded by a pressure transducer and a Power Lab system (AD Instruments). The animals were artificially ventilated with room air at 50 strokes per minute during arrhythmia induction by a rodent Harvard ventilator. Recovery time from surgery was considered 15 min. After this time, basal ECG (limb lead II) and BP were recorded. Exclusion criteria were the presence of cardiac arrhythmia or a sustained drop in mean arterial BP to <70 mmHg after the recovery time. For arrhythmia induction, aconitine was infused in the tail vein through an angiocatheter (G-24) with a syringe pump at a velocity of 0.1 mL/min (15 μg/mL in saline) for 10 min. The hemodynamic parameters and ECG were simultaneously recorded during the infusion and for another 5 min after the infusion period ended [19].

2.3. Measured parameters

The mean arterial pressure (MAP) was calculated using the MAP = diastolic arterial pressure + (systolic arterial pressure − diastolic arterial pressure)/3 formula. The PR interval (the interval time from the P-wave onset to QRS complex onset) and QT interval (the earliest Q or R-wave onset to the end of T wave) of basal ECG were determined by a mean of 2 min of ECG recorded strip. To prevent the dependence of QT interval on heart rate, corrected QT (QTc) interval was measured using Bazett's formula normalized as $QTcn\text{-}B = QT/(RR/f)^{1/2}$, where RR is R–R interval and $f = 150$ ms [21,22].

From aconitine injection onset for 15 min the episodes of premature ventricular contraction (PVC), ventricular tachycardia (VT) and ventricular fibrillation (VF) were counted. The latency and duration of PVC, VT and VF were also measured in seconds. According to the Lambeth conventions, ventricular arrhythmias were defined as: Ventricular premature beat (VPB; equal to PVC) is defined as a ventricular electrical complex (complete electrical event: QRS, RS, QRST or RST) that is different in shape (voltage and/or duration, i.e., height and/or width) from the preceding (non-VPB) ventricular complex and is premature in relation to the preceding ventricular complex, VT; is a run of four or more consecutive ventricular premature beats, VF; is defined as a sequence of a minimum of four consecutive ventricular complexes without intervening diastolic pauses, in which the intrinsic shape, the peak–peak interval and the height vary, and the variation between each is non-progressive [23]. The latency period for each type of ventricular arrhythmia was the time interval between the onset of aconitine injection until the moment of its occurrence [19].

Finally, the animals were sacrificed, their hearts were removed and rinsed with cold saline and then weighed. The heart weight (HW, left and right ventricle) was normalized by total body weight of the animal (HW/BW in mg/g). This ratio was considered as an index of cardiac hypertrophy [14,15,24]. Heart apex was dissected, weighed and homogenized in 5 mL of 0.1 MTris–HCl buffer (pH 7.4) in ice-cold condition. After centrifuging, the clear supernatant solution was taken for the biochemical analysis. Total proteins were measured by using the Lowry et al. method [25]. Malondialdehyde (MDA) levels, as an index of lipid peroxidation, were estimated by the concentration of thiobarbituric acid reactive substances (TBARS) in heart tissue [26]. Glutathione peroxidase (GPx) of the heart tissues was determined using their relative Randox assay kits, respectively (according to the manufacturer's protocol) [27]. The value of hydroxyproline of heart tissue was assessed by the use of hydroxyproline kit (Shanghai Crystal day Biotech Company) according to its protocol [28].