Physical Exercise and Binding of Digoxin to Skeletal Muscle – Effect of Muscle Activation Frequency

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Summary. Ten healthy subjects who had ingested 0.5 mg digoxin daily for at least 10 days, performed a 1-hour bicycle exercise test on two occasions, 24 h after the latest dose, with the same work load but at two different pedalling rates, 40 and 80 rpm. During exercise the mean digoxin concentration in the thigh muscle increased by 8% at 40 rpm (n.s.) and by 29% at 80 rpm (p<0.01). The serum digoxin concentration decreased by 39% at both pedalling rates (p<0.001). The results suggest that the increase in skeletal muscle digoxin concentration during exercise is related to the neuromuscular activation frequency. The digoxin concentration in erythrocytes was measured in 16 healthy subjects before and 1 minute after a 1-hour bicycle exercise test. The erythrocyte digoxin concentration decreased by 12% (p<0.01) during the exercise indicating that the increased uptake of digoxin in skeletal muscle during exercise influences the digoxin concentration in other tissues.

Key words: digoxin, tissue binding; skeletal muscle exercise, serum digoxin

The binding of digoxin to the ventricular myocardium in dogs and to the right atrial myocardium in humans increases with the myocardial activation frequency [1, 2]. An activity-related binding of digoxin is also seen in skeletal muscle. Thus, skeletal muscle exercise increases the binding of digoxin to skeletal muscle with a concomitant decrease in the serum digoxin level [3]. These changes are related to the intensity of the skeletal muscle work [4].

The mechanism underlying the increased binding of digoxin to cardiac and skeletal muscle as a secondary effect of increased muscle activity, is not known at the present time. According to a theory presented by Lüllmann et al. [5], the membrane-bound Na⁺-K⁺-ATPase activity, which is dependent on the contraction frequency of the heart muscle, determines the concentration of the specific cardiac glycoside receptors at each moment. Thus, only one particular conformation of Na⁺-K⁺-ATPase displays high affinity for the cardiac glycosides. Since this conformation occurs only transiently during each pump cycle, an increasing number of pump cycles per unit of time (as during increasing contraction frequency) would increase the concentration of the cardiac glycoside-binding sites. This implies that a raised heart rate increases the concentration of the specifically bound drug in this organ. In vivo studies on dogs support this hypothesis [6, 7].

If the cited theory is valid for skeletal muscle, such binding of digoxin to muscle should increase when the muscle activation frequency is raised. Since muscle tension and the number and contraction frequency of active muscle fibres have been shown to be interdependent [8], Lüllmann’s theory is also consistent with the finding of a work intensity dependent redistribution of digoxin during exercise.

The significant increase in the binding of digoxin to skeletal muscle during skeletal muscle exercise might indicate that exercise causes a decrease in digoxin content in other tissues than the exercising skeletal muscle. One readily accessible tissue is the erythrocytes, which we chose to study.

Thus, the purpose of the present study was to determine 1) whether an increased muscle activation frequency (increased pedalling rate during bicycle exercise) increases skeletal muscle digoxin binding and 2) whether skeletal muscle exercise influences the digoxin concentration in erythrocytes.

Subjects and Methods

Activation Frequency Study

Ten healthy men (aged 21–34 years) participated in the study, which was approved by the ethical committee of the Karolinska Hospital. They were informed of the procedure and purpose of the study.
and their consent was obtained. All had normal resting and exercising ECGs.

After about two weeks' intake of digoxin (0.5 mg/day), two exercise tests were performed 24 h after the latest dose with 2–7 days in between. Both exercise tests were submaximal with the same work load. Guided by a pre-digoxin exercise test, the load (80–200 W) was selected to give a heart rate of about 140 beats/min after 1 h of exercise. In one of the two tests the pedalling rate was 40 revolutions per minute (rpm) and in the other 80 rpm. The order of the tests was randomized. The subjects rested 1 h in the supine position before ECG recordings, blood sampling (through a cannula in a cubital vein) and exercise. Just before exercise a muscle biopsy specimen was taken from the quadriceps femoris muscle (vastus lateralis), using the percutaneous needle biopsy technique [9], for analyses of skeletal muscle digoxin concentration.

The exercise was then performed in a sitting position on an electrically braked bicycle ergometer (EM 380 B, Siemens-Elema, Stockholm, Sweden) for 1 h. Blood samples were taken after 5, 15, 30, 45 and 60 min of exercise and the ECG was recorded simultaneously. Oxygen uptake was measured after 10 min of exercise.

One minute after exercise a second muscle biopsy specimen was taken from the same muscle as before exercise and with the subject in the supine position. A 1-h resting period then followed with blood samplings and ECG recordings 1, 5, 15, 30, 45 and 60 min after exercise.

Erythrocyte Digoxin Concentration Study

Blood samples (cubital vein) for the determination of digoxin levels in the erythrocytes and serum were taken from 16 healthy subjects (aged 21–44 years) simultaneously with a muscle biopsy specimen from m. quadriceps femoris (vastus lateralis) after 60 min of supine rest before exercise and 1 min after exercise. The subjects performed the exercise on an electrically braked bicycle ergometer (Siemens-Elema) at a pedalling rate of 80 rpm and with a work load individually selected, varying between 100 and 200 W, to give a heart rate of about 140 beats/min after 1 h of exercise. The subjects gave their informed consent and the study was approved by the ethical committee of the Karolinska Hospital.

Methods

Skeletal Muscle, Erythrocyte and Serum Digoxin Concentration. The skeletal muscle specimens were freeze-dried and carefully freed from connective tissue and fat. Two pieces from each biopsy specimen were weighed and homogenized in phosphate buffer, after which digoxin was extracted from the tissue with dichloromethane. After evaporation of the dichloromethane phase the residue was redissolved in human serum and the digoxin concentration was measured by radioimmunoassay [10] using a commercial 125I-RIA Kit (New England Nuclear, Mass., USA). The method has been described in detail previously [2, 11].

To determine the erythrocyte digoxin concentrations we used heparinized blood. After centrifugation of the heparinized blood the erythrocytes were washed twice in saline. One ml of the concentrate of the erythrocytes, 3 ml aqua dest. and 5 ml dichloromethane was shaken for 1 min. After centrifugation and removal of the dichloromethane phase, the extraction procedure was repeated twice and the 15 ml dichloromethane was evaporated to dryness under a stream of nitrogen in a + 50 °C water bath. The residue was redissolved in human serum 50 μl and the digoxin concentration was measured by the 125I-RIA Kit. Recovery of 3H-digoxin added to erythrocytes was 83 ± 3% (mean ± SD). The serum digoxin concentrations were measured without any extraction procedure. The coefficient of variation for duplicate analyses was 11% for skeletal muscle, 8% for erythrocyte and 6% for serum concentration. The skeletal muscle, erythrocyte and serum digoxin concentrations are given as mean values of duplicates. The digoxin concentrations are expressed as nmol/kg dry weight (d. w.) in skeletal muscle and nmol/l in serum and concentrate of erythrocytes.

Electrocardiogram

The resting ECG recordings comprised 6 limb leads and 6 chest leads (V1–V6). During exercise 6 chest-head leads (CH1–CH6) were used. The heart rate was calculated from the ECG recordings over a period of 30 s.

Oxygen Uptake

The oxygen uptake was determined by the Douglas bag technique. Air expired over a 3-min period was collected in the bag. Gas volumes were measured with a spirometer and the gas samples were analysed for oxygen and carbon dioxide by the Scholander method.

Statistical Methods

Mean values ± SD are indicated unless otherwise stated. Student's t-test for paired observations was used to test statistical significance.