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Training increases the concentration of [^{3}H]ouabain-binding sites in rat skeletal muscle

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Exercise is associated with a net loss of K^+ from the working muscles and an increased plasma K^+ concentration, indicating that the capacity for intracellular reaccumulation of K^+ is exceeded. Training reduces the exercise-induced rise in plasma K^+, and an increased plasma [K^+] may interfere with physical performance. Since the clearing of K^+ from the extracellular space depends on the capacity for active K^+ uptake in skeletal muscle, the effects of training and inactivity on the total concentration of ($Na^+ + K^+$)-ATPase was determined. Following 6 weeks of swim training, the concentration of [^{3}H]ouabain-binding sites in rat hindlimb muscles was up to 46% ($P < 0.001$) higher than in those obtained from age-matched controls. Whereas muscle Na^+, K^+ contents remained unchanged, the concentration of citrate synthase increased by up to 76% ($P < 0.001$). Training induced no change in the [^{3}H]ouabain-binding-site concentration in the diaphragm, but in the heart ventricles, the K^+-dependent 3-*O*-methylfluorescein phosphatase activity increased by 20% ($P < 0.001$). Muscle inactivity induced by denervation, plaster immobilisation or tenotomy reduced the [^{3}H]ouabain-binding-site concentration by 20–30% ($P < 0.02$–0.001) within 1 week. In conclusion, training leads to a significant and reversible rise in the concentration of ($Na^+ + K^+$)-ATPase in muscle cells. This may be of importance for the beneficial effects on physical performance by improving the maximum capacity for K^+ clearance.

Introduction

Exercise is known to be associated with an increase in plasma [K^+] resulting from a net loss of K^+ from the working muscles [1,2]. Direct recordings with K^+-sensitive electrodes have shown that in the interfibre space the K^+ concentration may reach 7–10 mM [2,3], and during acute maximal physical performance even the arterial blood plasma [K^+] may reach 7 mM in human subjects [4]. Plasma K^+ concentrations in venous blood of up to around 9 mM and corresponding electrocardiographic abnormalities have been reported in long-distance runners [5]. These observations indicate that the capacity for net reaccumulation of K^+ into the working muscle fibres can be exceeded and the heart exposed to a K^+ concentration sufficient to interfere with excitability and performance.

Training has been shown to reduce significantly the exercise-induced rise in plasma K^+ in human subjects [6,7] as well as the loss of K^+ from exercising human skeletal muscles [8]. The capac-

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ity to clear K^+ from the plasma is to a large extent determined by the concentration of $(Na^+ + K^+)$-ATPase in skeletal muscle. It is interesting, therefore, that following 7 km of treadmill running per day with 6% elevation 5 days per week for 6 weeks, the activity of $(Na^+ + K^+)$-ATPase in terms of μmol P_i/mg protein per hour in purified plasma membrane fractions prepared from the gracilis muscle of dogs was found to be increased by 165% ($P < 0.05$) and that the exercise-induced rise in plasma $[K^+]$ decreased from 1.6 to 0.4 mM [9]. It has been pointed out that measurements on purified membrane fractions determine only a minor fraction (0.2–8.9%) of the total $(Na^+ + K^+)$-ATPase in heart or skeletal muscles [10,11]. Since it is not known whether the enzyme activity obtained after purification is representative for the total population of $(Na^+ + K^+)$-ATPase molecules, a quantitative evaluation of changes in the concentration of $(Na^+ + K^+)$-ATPase in muscle cannot at present be based on measurements on membrane fractions. We have analysed the effects of endurance training as well as inactivity on the total concentration of [^{3}H]ouabain-binding sites in rat skeletal muscle biopsies, since this method allows complete recovery of the $(Na^+ + K^+)$-ATPase [12,13].

Materials and Methods

6-week-old female Wistar rats were trained by daily swimming 5 days a week in a thermostatically controlled (36 °C) water bath – starting by 1 h per day and increasing by 1 h per week until 6 h per day was reached after 5 weeks. This was continued for 1 more week before training was stopped. Groups of five rats were killed after 4 or 6 weeks of training or after 6 weeks of training followed by 1 or 3 weeks of rest. The effect of muscle inactivity was studied in groups of four or five 12-week-old rats either by immobilization by a rapidly fixing plaster (Hexcelite®) or by distal tenotomy of the achilles tendon under diethyl ether anaesthesia. In all cases, groups of age-matched rats were used as controls in order to allow correction for the known age-dependent decrease in [^{3}H]ouabain-binding-site concentration [14].

The animals were killed by decapitation, blood samples were collected for determination of thyroid hormones (T_3 and T_4), and a series of different muscles were prepared for determination of Na^+, K^+ contents by flame photometry or the concentration of [^{3}H]ouabain-binding sites. This was performed using biopsies weighing around 5 mg and a newly developed method based on vanadate-facilitated binding of [^{3}H]ouabain to the $(Na^+ + K^+)$-ATPase [15,12]. Previous studies have shown that this assay gives the same results as measurements performed using intact muscles [12]. Furthermore, measurements of K^+-dependent 3-*O*-methylfluorescein phosphatase, have confirmed that the determinations of [^{3}H]ouabain-binding capacity in muscle biopsies quantifies the total amount of $(Na^+ + K^+)$-ATPase in rat skeletal muscle [16]. For measurements of K^+-dependent 3-*O*-methylfluorescein phosphatase activity in the heart, crude homogenates of the ventricle walls were prepared as earlier described [17,18]. To evaluate the effect of training on skeletal muscles using an independent parameter, the citrate synthase activity, a citric acid cycle marker enzyme, was measured in extensor digitorum longus and diaphragm muscles as earlier described [19].

Results and Discussion

Following 6 weeks of endurance training, the body weight and soleus muscle weight were 7 ($P < 0.01$) and 14% ($P < 0.001$) lower, respectively, than in the age-matched controls. The heart/body weight ratio was increased by 14% ($P < 0.005$). All of these changes were completely reversible following 3 weeks of rest. Body temperature was within 37.0–37.5 °C after swimming and plasma T_3 and T_4 showed no increase with training. These observations argue against the possibility that the effect of training on skeletal muscle [^{3}H]ouabain-binding-site concentration should be a simple outcome of increased thyroid status. This is of particular importance in view of the fact that hyperthyroidism in skeletal muscles is associated with an increase in [^{3}H]ouabain-binding-site concentration of up to 230% [13]. Following 4 and 6 weeks of training, the citrate synthase activity in extensor digitorum longus muscle was increased by 71 ($P < 0.001$) and 76% ($P < 0.001$), respectively, indicating a typical response to the en-

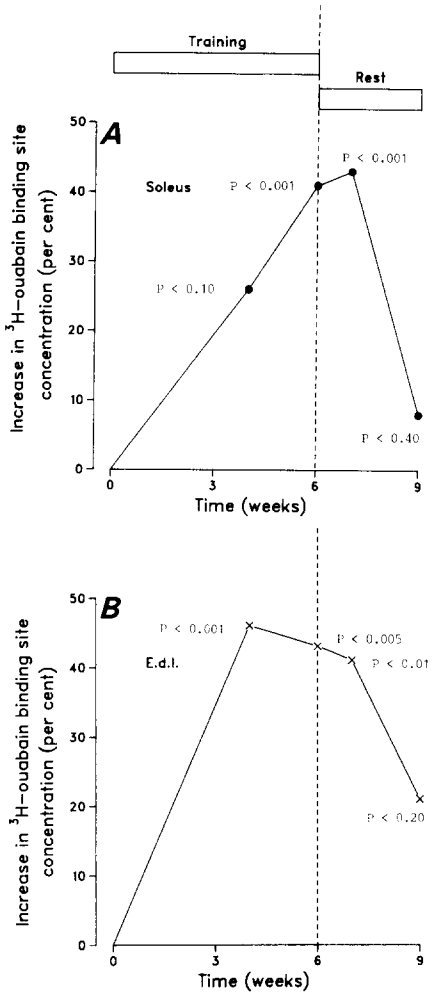

Fig. 1. Time-course of the increase in [^{3}H]ouabain-binding-site concentration in rat soleus and extensor digitorum longus (e.d.l.) muscles during training and subsequent rest. 6-week-old female Wistar rats were trained by swimming for 6 weeks and then allowed to rest for up to 3 weeks. Age-matched controls were kept in the animal room throughout under the same conditions as the trained rats. The animals were killed by decapitation. The muscles were exposed and samples of around 5 mg wet weight were prepared and incubated in buffer containing 1 mM vanadate [12]. Samples were prewashed two times 10 min at 0°C. They were then incubated in buffer containing [^{3}H]ouabain (2.08 μCi/ml) and unlabelled ouabain added to a final concentration of $1 \cdot 10^{-6}$ M for two times 60 min at 37°C. This was followed by a washout for four times 30 min at 0°C to remove [^{3}H]ouabain from the extracellular space. A minor correction for nonspecific retention of [^{3}H]ouabain was based on measurements performed with $1 \cdot 10^{-3}$ M [^{3}H]ouabain and unlabelled ouabain added to the buffer during incubation. The radiopurity of the [^{3}H]ouabain isotope was 95%. This was corrected for. The ^{3}H activity was determined by liquid scintillation counting and the [^{3}H]ouabain-binding-site concentration in the samples is expressed as pmol/g wet wt. [12–14]. Each point represents the mean relative increase in [^{3}H]ouabain-binding-site concentration in five muscle biopsies obtained from five trained rats compared with five age-matched rats. The statistical significance of differences was determined using the two-tailed t-test for non-paired observations and indicated by P.

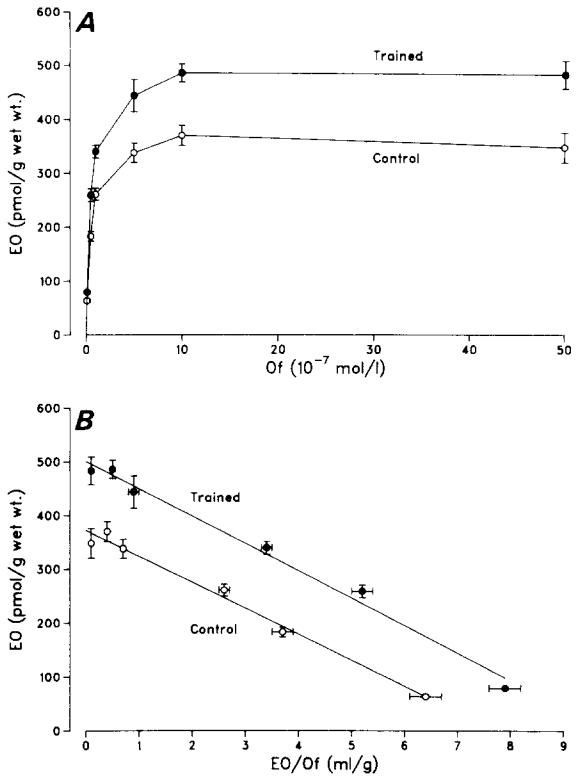

Fig. 2. The effect of endurance training for 6 weeks and the concentration of ouabain on the binding of [^{3}H]ouabain in rat soleus muscle biopsies. Rats, training and [^{3}H]ouabain-binding determination were as described in the legend to Fig. 1 except that samples were incubated at $1 \cdot 10^{-8}$ to $5 \cdot 10^{-6}$ M [^{3}H]ouabain and unlabelled ouabain for four times 60 min. Panel A shows 'bound' (EO) [^{3}H]ouabain as a function of the concentration of [^{3}H]ouabain in the incubation medium (Of). Panel B shows the 'bound' (EO) versus 'bound/free' (EO/Of) [^{3}H]ouabain. After correction for unspecific uptake and retention of [^{3}H]ouabain, values were corrected for radiopurity of the isotope and loss of specifically bound [^{3}H]ouabain during the washout in the cold by factors of 1.04 and 1.21, respectively [13]. The isotherms of the Scatchard-type plot in panel B were constructed using linear regression analysis. The intercepts with the ordinate denote the maximum [^{3}H]ouabain-binding site concentration (EO_{max}). The slopes of the line denote the apparent dissociation constants for receptor-ligand interaction (K_d). Each point represents the mean of observations on five animals with bars denoting 2×S.E.

durance training [20]. This effect was approximately halved after 1 week of detraining and reached the control level after 3 weeks of rest. The Na^+, K^+ contents of soleus and gastrocnemius muscles showed no significant change with training.

Training increased the concentration of [^{3}H]ouabain-binding sites by between 22 and 46% in the soleus (Fig. 1A, Fig. 2), extensor digitorum longus (Fig. 1B, Table I), gastrocnemius and spine muscles (Table I). In contrast, the diaphragm showed no significant change (Table I). In extensor digitorum longus, the rise was somewhat earlier in onset than in soleus, and the return towards the control level was incomplete even 3 weeks after the cessation of training (Fig. 1). The selectivity of the effect of training, i.e., it is demonstrated in extensor digitorum longus, gastrocnemius and spine muscles, but not in the diaphragm, argues against the possibility that the change is due to a universally distributed endocrine factor or general stress. 1 week after cessation of training, the increase in [^{3}H]ouabain-binding-site concentration in skeletal muscles was of the same order of magnitude as immediately after training was stopped (Fig. 1). The slow adaptation to detraining of skeletal muscle [^{3}H]ouabain-binding-site concentration observed in the present study agrees with earlier observations on human subjects of a relatively slow decay of the endurance-training-induced increase in maximal O_2-uptake, skeletal muscle capillarization, citrate synthase and succinate dehydrogenase activities [21]. In agreement with the lack of any major effect of training on the [^{3}H]ouabain binding site concentration in the diaphragm muscle, training did not increase citrate synthase activity in this tissue. In the heart ventricles, 6 weeks of training increased the K^+-dependent 3-*O*-methylfluorescein phosphatase activity by 20% ($P < 0.02$).

To ensure that the increase in [^{3}H]ouabain-binding-site concentration was not the outcome of an increase in affinity of the receptors for ouabain, [^{3}H]ouabain binding was measured in soleus muscle biopsies over a concentration range of labelled and unlabelled ouabain from $1 \cdot 10^{-8}$ to $5.0 \cdot 10^{-6}$ M (Fig. 2). The binding isotherms are given in a Scatchard-type plot (Fig. 2B) where the total specific [^{3}H]ouabain-binding-site concentration (EO_{max}) was 372 and 502 pmol/g wet wt. in muscles from controls and trained rats, respectively, and the apparent dissociation constant (K_d) for receptor-ligand interaction was $0.5 \cdot 10^{-7}$ M in muscles from both groups of rats. Thus, affinity changes cannot account for the observed effect of training on [^{3}H]ouabain binding. It should be noted that when EO_{max} values are corrected as in Fig. 2, they denote the [^{3}H]ouabain-binding-site concentration with complete recovery. Separate control experiments showed that the increase is not the result of changes in unspecific uptake or retention of [^{3}H]ouabain during incubations.

Immobilisation of one hindlimb by denervation has earlier in mice and rats been shown to cause a decrease in [^{3}H]ouabain-binding-site concentration in extensor digitorum longus and soleus

TABLE I

THE EFFECT OF ENDURANCE TRAINING FOR 6 WEEKS ON THE [^{3}H]OUABAIN-BINDING-SITE CONCENTRATION IN VARIOUS RAT SKELETAL MUSCLES

Rats, training and [^{3}H]ouabain binding determination were as described in the legend to Fig. 1. The results are given as means ± S.E. with the number of animals in parentheses. Statistical significance of differences was determined using the two-tailed *t*-test for non-paired observations and expressed by *P*.

	[^{3}H]Ouabain-binding-site concentration (pmol/g wet wt.)		Increase (%)	*P*
	control	trained 6 weeks		
Extensor digitorum longus	288 ± 23 (5)	413 ± 14 (5)	43	< 0.005
Gastrocnemius	265 ± 32 (5)	331 ± 8 (5)	25	< 0.05
Spine muscle	215 ± 14 (5)	263 ± 15 (5)	22	< 0.05
Diaphragm	315 ± 18 (5)	322 ± 26 (5)	2	< 0.40

muscles by up to 30% ($P < 0.001$). The decrease was fully reversible with reinnervation [22,23]. In the present study, the immobilisation of one hindlimb in rats by plaster or tenotomy for 1 week reduced the concentration of [^{3}H]ouabain-binding sites in soleus by 20 ($P < 0.02$) and 22% ($P < 0.02$), respectively. When compared to the maximum stimulating effect of training, it can be estimated that in rat soleus muscle contractile activity may increase the concentration of [^{3}H]ouabain-binding sites by up to 83%. On the basis of the observation that during detraining, the [^{3}H]ouabain-binding-site concentration in skeletal muscles shows a slow return to the control level, a more pronounced effect of immobilisation could be expected to be seen using techniques allowing immobilisation of rat hindlimbs for more than 1 week.

The results show that endurance training leads to a significant and reversible rise in the total concentration of $(Na^+ + K^+)$-ATPase in rat skeletal muscle. This confirms the observation of an increase obtained using purified membrane fractions from dog gracilis muscle, although the relative rise is smaller. This may be related to differences in recovery of $(Na^+ + K^+)$-ATPase or possibly between species and training procedure. Taken together, the observations indicate that training may induce a widespread increase in the capacity to clear K^+ from the extracellular space and that this effect may last for a few weeks after the return to normal activity.

It has repeatedly been suggested that an increase in plasma K^+ interferes with physical performance [4,24–26]. The training-induced rise in $(Na^+ + K^+)$-ATPase concentration may limit the net K^+ loss from exercising muscles and thereby dampen the rise in plasma K^+ [8,9]. This may be of importance for the general beneficial effects of training and at the same time reduce the risk of possible cardiotoxic effects of exercise-induced hyperkalemia.

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