Exercise training does not alter acetylcholine-induced responses in isolated pulmonary artery from rat

Y. Mitani, J. Maruyama, K. Maruyama, M. Sakurai


ABSTRACT: In chronic exercise-trained animals, acetylcholine (ACh)-stimulated endothelial nitric oxide (NO) release is enhanced in the systemic circulation. The purpose of the present study was to determine whether chronic exercise training also enhances NO-mediated relaxation in rat pulmonary artery.

Sprague–Dawley rats were randomly divided into groups of exercise-trained and sedentary control rats. The exercise-trained rats ran on a motor-driven treadmill at 30 m–min−1 up an 15° incline 10–60 min-day−1, 5 days per week for 10 weeks, and had less body weight, lower serum total cholesterol and triglyceride levels than sedentary rats.

Contraction induced by potassium chloride and prostaglandin (PG)F2α were similar between isolated conduit pulmonary arterial rings from sedentary and exercise-trained rats. There were no differences between PGF2α-precontracted rings from sedentary and exercise-trained rats in both ACh and sodium nitroprusside-induced relaxations. The NO synthase inhibitor, nitro-L-arginine, suppressed ACh-induced relaxation in both sedentary and exercise-trained rats.

These results suggested chronic exercise training did not alter the acetylcholine-induced endothelial NO production and release and the sensitivity of vascular smooth muscle cell to NO in isolated conduit pulmonary artery of rat.


Exercise increases cardiac output and pulmonary blood flow, which elicit additional shear stress on the systemic and pulmonary vascular endothelial cells. In highly trained athletes, acetylcholine (ACh)-induced nitric oxide-mediated vasodilatation in the forearm is enhanced compared to age-matched sedentary subjects [1]. In chronic exercise-trained animals, agonist-stimulated endothelial NO release is enhanced in the thoracic [2] and abdominal aorta [3] of rats, the thoracic aorta and pulmonary artery of New Zealand white rabbits [4], the epicardial coronary artery of dogs [5], and the coronary resistance arteries of pigs [6]. Several studies have shown that NO synthase gene expression increases in aortic endothelial cells from dogs after chronic exercise [7] and rats with chronic high flow via an arteriovenous fistula [8]. On the other hand, chronic exercise did not alter endothelium-dependent relaxation in the carotid artery of Wistar Kyoto rats [2] and the coronary artery of dogs [9]. Thus, the effect of exercise training on endothelial NO release might be different between the species or vascular bed examined.

In the thoracic aorta of spontaneously hypertensive rats, the endothelium-dependent response is impaired and chronic exercise increased endothelial NO release [2]. Thus, in the systemic circulation, exercise training might enhance endothelial NO release in structurally and functionally abnormal vascular beds as well as in the normal vascular beds. These observations partly suggest the benefit of chronic exercise in prevention of cardiovascular disorders such as hypertension and coronary artery disease. If exercise training enhances the endothelial release of NO in the pulmonary vasculature, it might be of benefit not only in the systemic and coronary circulation but also in the pulmonary circulation, since endothelium-dependent relaxation is impaired in hypertensive pulmonary vascular disease [10, 11]. Since the rat is widely used as an experimental model of chronic pulmonary hypertension, it is interesting to study the effect of chronic exercise on rat pulmonary artery. Therefore, the ACh (endothelium-dependent relaxant) and sodium nitroprusside (SNP; endothelium-independent relaxant)-induced relaxation were investigated in isolated pulmonary arteries of rats after chronic exercise to determine whether chronic exercise alters the NO-mediated response in rat pulmonary artery.

Materials and methods

Animals and training

Male Sprague–Dawley rats (Clea, Japan) weighing 170–200 g (7-week-old) were used. Rats were randomly assigned to one of two groups after a 1-week familiarization period: exercise-trained (ET) rats (n=9), and age-matched sedentary (SED) rats (n=7). Rats were housed under a 12-h light–dark cycle and were fed standard rat food and water ad libitum. The exercise training was performed according to the protocol of Delp et al. [3]. The ET rats ran on a
motor-driven treadmill (Columbus Instruments, OH, USA) at 30 m-min⁻¹ up a 15° incline 5 days per week. The length of time on the treadmill started at 10 min-day⁻¹ for the first week, increasing to 20 min-day⁻¹ for the second week, 30 min-day⁻¹ for the third week, 40 min-day⁻¹ for the fourth week, 50 min-day⁻¹ for the fifth week, and progressed to a maximum of 60 min-day⁻¹ by the sixth week. The length of time on the treadmill was then maintained at 60 min-day⁻¹ for an additional 4 weeks. Thus, the ET rats underwent exercise training for 10 weeks in total. All rats were weighed at the beginning of training and once a week until they were sacrificed for the isolation of the pulmonary arteries. Institutional approval of animal investigation was obtained. Table 1 lists the mean body weight (BW) for each group.

Preparation of pulmonary arteries

Rats were anaesthetized with intraperitoneal pentobarbital (50 mg·kg⁻¹). The lungs and heart were removed en bloc and placed in modified Krebs–Henseleit solution at room temperature. The composition of this solution was NaCl 115.0 mM; KCl 4.7 mM; CaCl₂ 2.5 mM; MgCl₂ 1.2 mM; NaHCO₃ 25.0 mM; KH₂PO₄ 1.2 mM; and dextrose 10.0 mM. The right ventricle (RV) of the heart was dissected from the left ventricle plus septum (LV+S) and these preparations were weighed separately. Values for RV/(LV+S) and heart weight/body weight were then calculated to determine whether ventricular hypertrophy had developed. Two pulmonary artery segments, a left pulmonary artery (EPA, 1.4–1.6 mm external diameter) and an intrapulmonary artery (IPA, 0.7–1.1 mm external diameter), were isolated and gently freed from fat and connective tissue. Ring segments (2 mm) were cut (1–2 rings from the EPA and 2–4 rings from the IPA) and suspended vertically between hooks in organ baths (20 mL) containing modified Krebs–Henseleit solution; the solution was maintained at 37°C and bubbled with a mixture of 95% air and 5% CO₂.

In preliminary experiments, active and resting tension relationships were obtained by increasing the resting tension, using a range of forces between 0.25 and 2.5 g, according to the method of Toda et al. [12]. The optimal resting tension for vasodilation studies was 0.75 g for EPA and IPA rings from both ET and SED rats. At this resting tension, the peak contraction was obtained in response to 70 mM KCl. In all experiments, changes in isometric force were measured with a force displacement transducer (TB 612; Nihon Kohden, Tokyo, Japan) connected to a carrier amplifier (AP600G; Nihon Kohden) and were recorded on a pen recorder (MC 6622; Watanabe, Tokyo, Japan).

Vasodilation studies

Pulmonary artery rings under the optimal resting tension were washed every 15–20 min and allowed to equilibrate for 2 h. After the equilibration period, 70 mM KCl contraction curves were routinely recorded twice as a measure of maximal contractility with the contraction shown by the second curve used as the maximal contraction. A cumulative concentration–response curve to prostaglandin (PG)F₂α (10⁻⁸–10⁻⁵ M) was obtained, and the approximate concentration of PGF₂α needed to produce 50% of the maximal contraction induced by 70 mM KCl (EC50) was determined. The rings were then washed every 15 min, equilibrated for 1 h in total, and precontracted with PGF₂α (10⁻⁶–10⁻⁵ M) to obtain 50–70% of the maximal contraction induced by 70 mM KCl. Following PGF₂α-induced precontraction with or without a NO synthase inhibitor, nitro-L-arginine (L-NA, 10⁻⁴ M), a cumulative concentration–response curve was obtained for Ach (10⁻⁸–10⁻⁵ M) by producing a stepwise increase in the Ach concentration as soon as a stable response to each preceding level was reached. Finally, 10⁻⁶ M papaverine was added to produce maximal relaxation. The papaverine-induced maximal relaxation and these percentages were plotted against the negative logarithmic values of the drug doses. The response to Ach was examined in the presence of 10⁻⁶ M indomethacin to eliminate vasodilation due to prostacyclin release.

Reagents

The following drugs were used: Ach hydrochloride, (Wako Pure Chemical Industries, Osaka, Japan); SNP, indomethacin, and L-NA (Sigma); and PGF₂α (Ono Pharmaceutical Co., Osaka, Japan). The concentrations of the drugs were expressed as the final molar concentrations in the organ bath.

Data analysis

Results were expressed as mean±SEM. Numbers in parentheses (n) are the number of rings from seven SED or six ET rats. Differences between SED and ET rats were determined by the unpaired Student’s t-test. When more than two means were compared, one-way analysis of variance was used. If a significant difference was found, Scheffe’s
Body weight, right ventricular hypertrophy and serum total cholesterol and triglyceride levels

SED rats gained weight throughout the experiment. The ET rats gained less body weight than SED rats at each time point during the exercise training (table 1). The heart weight/body weight ratio was greater in ET rats probably owing to lower body weight in ET rats. There was no difference in the RV/LV+S ratio between ET rats and SED rats (table 1). Serum total cholesterol (sChol) and triglyceride (sTrig) levels were significantly lower in ET rats (table 1). Serum total cholesterol (sChol) and triglyceride (sTrig) levels were significantly lower in ET rats (table 1).

Response to 70 mM potassium chloride and prostaglandin F\(_{2\alpha}\)

Mean absolute values of contraction induced by 70 mM KCl were similar between rings from SED rats and ET rats: \(387 \pm 13\) (n=12) versus \(387 \pm 13\) (n=12) mg in EPA; \(367 \pm 12\) (n=21) versus \(394 \pm 30\) (n=16) mg in IPA. PGF\(_{2\alpha}\) induced dose-dependent contractions in EPA and IPA rings, which were similar between SED rats and ET rats.

Discussion

ACh-induced relaxation was not altered by chronic exercise. Relaxation to SNP was not altered in isolated conduit pulmonary artery from ET rats, suggesting that sensitivity of guanylate cyclase to NO was not altered in the pulmonary vascular smooth muscle of ET rats and that relaxation due to ACh in this vessel could be the measure of the pulmonary endothelial NO production and/or release in ET rats. Thus, exercise training did not alter ACh-induced endothelial NO production and/or its release in isolated rat pulmonary artery. Since l-NA suppressed, but did not eliminate, the relaxation effects of ACh, it is probable that a vasodilatory substance other than NO might also be produced in response to ACh.
with the present results in rats. There might be species differences to account for this.

ET induced lower sChol and sTrig levels and higher heart weight/body weight ratios than in SED rats, consistent with earlier studies [1, 15, 16]. These results partly showed the efficacy of the training protocol in the present study. As the study progressed, the ET rats showed a much lower body weight gain compared to SED rats. It is notable that the exercise routine caused a marked stunting in normal growth, suggesting that energy demands have been excessive and that rats had been undergoing a degree of metabolic starvation. Metabolic starvation might affect free radical production. In the present study, SED rats without food restriction might not have been a true control. Possibly owing to this concern, food management is different among studies dealing with exercise. In the study by Delp et al. [3], ET rats continued to be fed ad libitum during the training period while food was restricted for SED rats to ensure matched body weight between the two groups. In other studies both ET and SED rats (4–7 week-old rats) were fed ad libitum, resulting in a lower body weight in ET rats [2, 16, 17]. The results were similar in the studies comparing exercise training enhanced endothelium-dependent relaxation of aortic rings from ET rats and SED rats with and without food restriction.

In summary, chronic exercise training did not alter the acetylcholine (endothelium-dependent relaxant) and somatostatin nitroprusside (endothelium-independent relaxant)-induced pharmacological relaxation, at least in conduit pulmonary arteries, in rats. The effect of exercise training on smaller pulmonary vasculature and/or shear stress-induced functional relaxation in the in vivo situation remains to be determined.

References