Effect of swimming on vascular reactivity to phenylephrine and KCl in male rats

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- 1 The present study aimed to examine whether there is any change in vascular responsiveness to phenylephrine and KCl during exercise, and whether the vascular endothelium plays a role in these changes.
- 2 Adult male rats were subjected to a swimming schedule every day for 5–6 weeks. Studies were performed *in vitro* on thoracic aortae.
- 3 Maximum contractile response to phenylephrine of endothelium-intact thoracic aortic rings (passive tension 1.0 g) obtained from swimming rats (1.2 ± 0.2 g, $n=8$) was lower than that of sedentary control rats (2.1 ± 0.2 g, $n=8$). When the endothelium was removed, however, the dose-response curves of both groups of rats were shifted to the left with an increase in maximum responses and they were no longer significantly different (max. tension, swimming rats: 3.2 ± 0.3 g, $n=6$, control rats: 3.4 ± 0.4 g, $n=5$).
- 4 Indomethacin did not significantly alter the dose-response curves. A similar effect to that obtained by removal of the endothelium was observed when methylene blue and indomethacin were both added.
- 5 Passive tension in the range of 2.5–3.0 g, caused a significant increase in active tension developed to phenylephrine ($1 \mu\text{M}$ for endothelium-intact and $0.1 \mu\text{M}$ for endothelium-denuded) of thoracic aortic rings of both swimming and sedentary control rats compared to their corresponding groups when using passive tension of 1.0–1.5 g.
- 6 The reduction in responses to phenylephrine of endothelium-intact thoracic aortic rings of swimming rats persisted with the use of a passive tension of 3.0 g. The presence of $300 \mu\text{M}$ N^{G} -nitro-L-arginine (L-NOARG) caused a significant leftward shift of the curve with an increase in maximum responses when a passive tension of either 1.0 or 3.0 g was applied to the rings. However, for the rings with a passive tension of 1.0 g, L-NOARG caused a smaller increase in maximal contractile responses to phenylephrine of the rings of sedentary controls than those of swimming rats.
- 7 There was no difference in the dose-response curves to depolarizing concentrations of KCl (20, 40, 80 and 120 mM) of endothelium-intact thoracic aortic rings from swimming and sedentary control rats. When the endothelium was removed, however, the dose-response curves of both groups of animals were shifted to the left with an increase in maximum responses. Moreover, the responses to KCl of endothelium-denuded thoracic aortic rings of swimming rats were greater than those of sedentary control rats.
- 8 These results suggest that there were changes in vascular responsiveness to phenylephrine and KCl during exercise. The fall in sensitivity to phenylephrine with no change in KCl responses, and the increase in maximum responses to phenylephrine in the presence of L-NOARG in endothelium-intact aortae (passive tension 1.0 g) from swimming rats, were due to an increase in spontaneous release and upregulation of phenylephrine-stimulated release of EDRF/NO, and may not be a consequence of an increase in prostaglandins or a decrease in the production of endothelial constrictors by vascular endothelium. EDRF/NO may play an important role in modulating local vasodilatation.

Keywords: EDRF; endothelium; exercise; KCl; phenylephrine; swimming

Introduction

It has long been known that exercise training causes beneficial changes in the cardiovascular system, one effect being to lower resting blood pressure, both in laboratory animals (Lutgemeier *et al.*, 1987; Noma *et al.*, 1987; Overton *et al.*, 1988) and in man (Meredith *et al.*, 1991; Seals & Reiling, 1991). A decrease in resting peripheral resistance in spontaneously hypertensive rats (SHR) (Tipton *et al.*, 1979) and an increase in iliac and coronary blood flow in rats (Buttrick *et al.*, 1986; Yancey & Overton, 1993) have also been found. In conscious dogs, acute treadmill exercise causes dilatation of the circumflex coronary artery, decreased coronary vascular resistance and increase in coronary blood flow (Berdeaux *et al.*, 1991; Wang *et al.*, 1993). An increase in human leg vasodilator capacity was also found in the elderly following exercise by jogging (Martin III *et al.*, 1990). However, the mechanism responsible for these changes is not yet understood.

In *in vivo* studies, Harri (1979) reported that long term swimming of the rat caused a decrease in sensitivity of blood

pressure responses to phenylephrine, and increased vasodilator response to isoprenaline, a β -adrenoceptor agonist. Similar results were also reported by Pavlik *et al.* (1976) and Wiegman *et al.* (1981), using noradrenaline injected via the jugular vein. In the *in vitro* preparations, however, conflicting results have been obtained. Edwards *et al.* (1985) found no changes in vascular responsiveness to noradrenaline of thoracic aortic strips between exercise trained and sedentary control rats, while Delp *et al.* (1993) found diminished sensitivity to noradrenaline with no changes in maximum responses of endothelium-intact aortic rings of exercise training rats. Ohkubo *et al.* (1992) demonstrated that exercise training by swimming altered the vascular fatty acid composition by increasing the potential for prostacyclin production which may be involved in the increase in vasodilator capacity in exercise-trained rats.

Since 1980, it has become apparent that endothelium derived relaxing factor (EDRF) recently defined as nitric oxide (NO) (Palmer *et al.*, 1987), generated by vascular endothelium,

plays an important role in vasodilatation in many circumstances. It is released from the endothelial cells under both basal conditions and during stimulation with acetylcholine (Griffith *et al.*, 1984; Rubanyi *et al.*, 1985; Busse *et al.*, 1993). Physical stimuli, such as elevation shear stress due to increased blood flow, can also induce EDRF release and promote vasodilatation (Gerova *et al.*, 1983; Smiesko *et al.*, 1985). Thus, it is possible that the hyperaemia associated with strenuous exercise, such as swimming, may cause an increase in spontaneous release of EDRF/NO from the endothelial cell, which may contribute to a dilatation of the vascular smooth muscle, in order to achieve enough blood flow to the skeletal muscle during exercise (Armstrong & Laughlin, 1984; Sinoway *et al.*, 1987). Therefore, the present study aimed to examine (1) whether there are any changes in vascular responsiveness to the α_1 -adrenoceptor agonist, phenylephrine and to KCl during exercise, and (2) whether the vascular endothelium plays a role in these changes. Studies were performed *in vitro* on thoracic aortae obtained from adult male rats, which were subjected to a swimming schedule every day for 5–6 weeks. Dose-responses curves to phenylephrine, and depolarizing concentration of KCl were studied in rings of endothelium-intact or denuded thoracic aortae. The effects of passive tension, indomethacin, methylene blue, and N^G -nitro-L-arginine (L-NOARG) on the dose-response curves to phenylephrine were also investigated.

Methods

Adult male Wistar rats, initial weight 350–420 g, were used in the study. The animals were housed at 25°C on a 10 h dark and 14 h light cycle. All rats were allowed free access to food and drinking water. Animal weights were recorded on the first and the last day of swimming schedule.

Exercise was performed by swimming in a round fibre tank (100 cm in diameter and 70 cm height) containing tap water approximately 45 cm deep and maintained at room temperature (28–29°C). A maximum of only ten rats was allowed to swim each time. The swimming schedule followed the protocol of Ohkubo *et al.* (1992). Briefly, the swimming time on the 1st day was 10 min, and then increased in 10 min steps everyday. At 100 min per day, swimming was carried out in two routines of 50 min each. The twice-daily swimming was further increased 50 min each, in steps of 10 min each day until the maximum swimming time of 90 min was achieved. Rats were then allowed to swim at this maximum swimming time every day for another two weeks. Animals were used for the study of vascular reactivity changes during the following week.

Blood pressure and heart rate measurement

Five animals of each group were selected randomly after completion of the swimming schedule for measurement of blood pressure and heart rate. Animals were anaesthetized with Nembutal (60 mg kg⁻¹, i.p.). A tracheal tube was inserted into the trachea. A polyethylene catheter was cannulated through the right common carotid artery and connected to a pressure transducer and polygraph for monitoring blood pressure and heart rate. The data for basal mean arterial blood pressure and heart rate were collected at 20 min after equilibration.

Organ bath studies

Animals were killed by cervical dislocation, and the descending thoracic aorta was removed, rinsed with oxygenated Krebs solution (37°C), and placed in a Petri dish containing the same solution. All fat and connective tissue were removed. Two adjacent rings were cut, each approximately 7 mm long. From one, the endothelial layer was removed by mechanical disruption, by the method modified from Furchgott & Zawadzki (1980) as previously described (Jansakul *et al.*, 1989).

Each ring was placed in a 20 ml organ bath by use of two vertically placed stainless steel stirrup hooks passed through its lumen, the lower being fixed, and the other connected to an isometric force transducer (Grass FT03C). Connections from both were recorded continuously on a Grass polygraph (7DWU). Each organ bath contained Krebs-Henseleit solution of the following composition (mM): NaCl 118.3, KCl 4.7, CaCl₂ 1.9, MgSO₄ 7H₂O 0.45, KH₂PO₄ 1.18, NaHCO₃ 25.0, glucose 11.66, Na₂ EDTA 0.024 and ascorbic acid 0.09 at 37°C, continuously bubbled with 95% O₂ and 5% CO₂. Prior to addition of drugs, rings were equilibrated for 60 min under a resting tension of 1.0 g, and the Krebs solution replaced every 10 min.

After equilibration, the presence of a functional endothelium was tested as follows. Each aortic ring was pre-constricted with 3×10^{-7} M phenylephrine (PE) for 5–8 min (by which time the response had plateaued), and dilator responses to 10^{-6} M acetylcholine recorded. Vasodilatation (80–90%) to acetylcholine occurred with endothelium-intact, but no dilatation was observed for endothelium-denuded rings. After this test, 45 min was allowed (with washing every 10 min) for re-equilibration prior to determination of dose-response relationship to phenylephrine or KCl.

Effects of swimming, indomethacin and methylene blue on vasoconstrictor responses to phenylephrine

After testing for the presence of functional endothelium as above, tissues were incubated for 45 min. A cumulative dose-response curve to phenylephrine was obtained. Following multiple washing to remove phenylephrine, the tissues were then incubated for a further 45 min in the presence of indomethacin (10^{-6} M). The second dose-response curve to phenylephrine was then obtained. Using the same procedure, the third dose-response curve to phenylephrine was obtained in the presence of both indomethacin (10^{-6} M) and methylene blue (10^{-5} M).

Passive-active tension relationship

After testing for the presence of functional endothelium as above, tissues were incubated for 30 min. The aortic rings were stretched to increase their passive tension by 0.5 g for each stage from 1.0 to 3.0 g. At each stage, the tissues were further equilibrated for another 10 min, the tissues were then exposed to phenylephrine (10^{-6} M for endothelium-intact and 10^{-7} M for endothelium-denuded rings) for 5–8 min, at which time the maximum responses were reached. This was followed by multiple washing to remove the drug and re-equilibration for 20–30 min; the rings were then adjusted to the next passive tension and the above procedure repeated.

Effects of swimming, passive tension and L-NOARG on vasoconstrictor response to phenylephrine

Only rings with intact-endothelium were studied. Passive tension of 1 g was applied to one of the aortic rings. After testing for the presence of a functional endothelium, a test for complete blockade of EDRF release by a specific nitric oxide synthase inhibitor, L-NOARG, was made as follows. The tissues were incubated with 3×10^{-4} M L-NOARG for 30 min and were then exposed to 3×10^{-6} M phenylephrine in the presence of L-NOARG for 5 min and dilatation in response to 3×10^{-6} M acetylcholine recorded. In the present study, no dilatation to acetylcholine was found. After multiple washing to remove the drugs, the aortic rings were re-equilibrated for 30 min in the presence of 3×10^{-4} M L-NOARG. A cumulative dose-response curve to phenylephrine was obtained.

Another aortic ring with intact endothelium obtained from the same animal as above, was equilibrated under a resting tension of 3.0 g. After testing for the presence of a functional endothelium as described above, the tissues were re-equilibrated for 45 min. A cumulative dose-response curve to

phenylephrine was obtained. This was followed by multiple washing to remove the drug and the tissues were re-equilibrated for 20 min, at which time the tissue tension had returned to normal (3.0 g). The tissues were then incubated with 3×10^{-4} M L-NOARG for 30 min. The second dose-response curve to phenylephrine was obtained in the presence of L-NOARG.

Effects of swimming on vasoconstrictor responses to KCl

Discrete responses to KCl were obtained with depolarizing concentrations of KCl (20, 40, 80, 120 mM). In these experi-

Figure 1 Constrictor responses to phenylephrine of endothelium-intact and denuded thoracic aortic rings obtained from swimming and sedentary control rats. Each point represents the mean $\pm$ s.e. mean from 5–8 experiments. ($\square$) Swimming, endothelium intact; ($\blacksquare$) swimming, endothelium-denuded; ($\circ$) sedentary control, endothelium intact; ($\bullet$) sedentary control, endothelium-denuded rings. *Significantly different from the means obtained from endothelium-intact aortic rings of sedentary control rats, $P < 0.05$.

ments, amounts of KCl (mM) were substituted for NaCl in the Krebs-Henseleit solution so that the tonicity of the solution remained the same.

Drugs

All drug solutions were prepared daily and kept on ice until used. (–)-Phenylephrine HCl (Sigma, U.S.A.) was dissolved in a solution containing NaCl 9.0 g l^{-1} , NaH_2PO_4 0.19 g l^{-1} and ascorbic acid 0.03 g l^{-1} . Acetylcholine chloride (Sigma, U.S.A.), methylene blue (Sigma, U.S.A.) and N^G -nitro-L-arginine, (Sigma, U.S.A.) were dissolved in distilled water. Indomethacin was dissolved in 0.1% sodium carbonate (Na_2CO_3) solution.

Statistical analysis

Absolute tension developed was measured throughout so that comparisons could be made of both sensitivity and the maximal responsiveness of endothelium-intact and denuded thoracic aortic rings. The drug concentration which produced 50% of the maximal response for the drug (EC_{50}) was derived from regression analysis over the linear portion of the dose-response curve (Diem & Leutner, 1970). Other data are expressed as mean $\pm$ s.e. mean of 5–15 experiments ($n = 5–15$), and tests of significance made with ANOVA and Fisher PLSD or Student's unpaired t test. In all cases, a P value of 0.05 or less was considered statistically significant.

Results

The weights of the swimming and sedentary control rats at the beginning of the studies were not different (swimming, $369.9 \pm 9.6 \text{ g}$, $n = 15$; sedentary control, $361.6 \pm 3.5 \text{ g}$, $n = 15$). Swimming rats lost weight during the studies ($324.9 \pm 6.9 \text{ g}$, $n = 15$, $P > 0.05$), while sedentary control rats gained weight ($381.6 \pm 4.2 \text{ g}$, $n = 15$, $P > 0.05$). There were no differences in

Table 1 EC_{50} and maximum contractile responses to phenylephrine (Phe) and KCl of thoracic aortic rings obtained from swimming and sedentary control rats using passive tension of either 1.0 or 3.0 g.

Endothelium	EC_{50} (95% c.i.) (nM)		Maximum responses ($\pm$ s.e. mean) (Increase in tension, g)	
	Present	Removed	Present	Removed
<i>Passive tension 1.0 g</i>				
<i>Swimming</i>				
Phe	35.0(21.0–59.0)	1.8(0.01–33.0)	1.2 ± 0.1	$3.2 \pm 0.3^*$
Phe + Indo	45.0(35.0–56.0)	0.9(0.002–45.0)	0.9 ± 0.2	$3.1 \pm 0.4^*$
Phe + Indo + MB	12.1(8.3–17.8) ^a		3.4 ± 0.3^a	
Phe + L-NOARG	4.4(3.1–6.2) ^a		4.7 ± 0.5^a	
KCl	32.1(28.0–36.9)	17.7(11.1–27.6) [*]	1.7 ± 0.1	$2.8 \pm 0.2^*$
<i>Sedentary control</i>				
Phe	25.0(9.5–65.0)	4.0(1.3–13.0)	2.1 ± 0.2	$3.4 \pm 0.4^*$
Phe + Indo	20.0(6.4–63.0)	5.4(1.8–16.0)	2.5 ± 0.2	$3.5 \pm 0.4^*$
Phe + Indo + MB	17.7(11.5–27.2)		3.4 ± 0.2^a	
Phe + L-NOARG	4.2(3.0–6.0) ^a		3.6 ± 0.6^a	
KCl	36.5(31.8–42.0)	18.7(11.8–29.7) [*]	1.6 ± 0.2	$2.1 \pm 0.2^*$
<i>Passive tension 3 g</i>				
<i>Swimming</i>				
Phe	22.0(11.0–45.0)		1.6 ± 0.3	
Phe + L-NOARG	4.5(2.7–7.7) ^a		5.3 ± 0.8^a	
<i>Sedentary control</i>				
Phe	9.7(3.1–30.0)		2.5 ± 0.3	
Phe + L-NOARG	1.6(1.0–2.5) ^a		5.0 ± 0.3^a	

Values were obtained in the presence or after removal of endothelium, in the presence of indomethacin (Indo, $1 \mu\text{M}$), indomethacin ($1 \mu\text{M}$) plus methylene blue (MB, $10 \mu\text{M}$), and N^G -nitro-L-arginine (L-NOARG, $300 \mu\text{M}$). *Significantly different from results obtained in the presence of endothelium of their corresponding group ($P < 0.05$). ^aValues were significantly different from the values obtained with phenylephrine alone within each activity group, $P < 0.05$.

Figure 2 Effects of indomethacin ($1 \mu\text{M}$) on constrictor responses to phenylephrine of endothelium-intact and denuded thoracic aortic rings of (a) swimming rats and (b) sedentary control rats. Each point represents the mean $\pm$ s.e. mean from 5–8 experiments: (○) endothelium intact; (●) endothelium-denuded; (△) endothelium intact, in the presence of indomethacin; (▲) endothelium-denuded, in the presence of indomethacin.

Figure 3 Effects of indomethacin ($1 \mu\text{M}$) and methylene blue ($10 \mu\text{M}$) on constrictor responses to phenylephrine of endothelium-intact thoracic aortic rings obtained from swimming and sedentary control rats. Each point represents the mean $\pm$ s.e. mean from 6–7 experiments: (■) swimming, endothelium-denuded; (●) sedentary control, endothelium-denuded; (□) swimming, endothelium intact in the presence of indomethacin and methylene blue; (○) sedentary control, endothelium intact in the presence of indomethacin and methylene blue.

mean arterial blood pressure between these two groups (swimming, 167.9 ± 12 mmHg; sedentary control, 186.0 ± 16.0 mmHg, $n=5$). Swimming animals, however, had a significantly decreased heart rate compared to sedentary controls (swimming, 371 ± 6.4 beats min^{-1} , $n=5$; sedentary control, 404 ± 5.1 beats min^{-1} , $n=5$, $P>0.05$). The weight of thoracic aortic rings (7 mm long) obtained from swimming and sedentary control rats were not significantly different (swimming, 9.2 ± 0.5 mg, $n=15$; sedentary control, 8.4 ± 0.7 mg, $n=15$).

When using passive tension of 1.0 g, both phenylephrine and KCl produced concentration-related constriction of the rat thoracic aortic rings. Figure 1 shows vasoconstrictor responses to phenylephrine of endothelium-intact and -denuded thoracic aortic rings obtained from swimming and sedentary control rats. The responses to phenylephrine of endothelium-intact thoracic aortic rings obtained from swimming rats were lower than those of sedentary control rats at almost all concentrations (10^{-8} – 3×10^{-6} M) studied, with a decrease in maximum contractile responses (max.tension, swimming: 1.2 ± 0.1 g, $n=8$, control: 2.1 ± 0.2 g, $n=8$, $P>0.05$). When

Figure 4 Constrictor responses to KCl of endothelium-intact or denuded thoracic aortic rings obtained from swimming or sedentary control rats. Each point represents the mean $\pm$ s.e. mean from 6–8 experiments: (□) swimming, endothelium intact; (■) swimming, endothelium-denuded; (○) sedentary control, endothelium intact; (●) sedentary control, endothelium-denuded. *Significantly different from the mean obtained from endothelium-denuded aortic rings of sedentary control rats, $P<0.05$.

the endothelium was removed, however, the dose-response curves of both groups of rats were shifted to the left with an increase in maximum responses which were no longer significantly different between swimming and control animals (max.tension, swimming rats: 3.2 ± 0.3 g, $n=6$, control rats: 3.4 ± 0.4 g, $n=5$). However, the EC_{50} values of phenylephrine in responses of the aortic rings from swimming or sedentary control rats either with or without endothelium were not significantly different (Table 1).

The effects of indomethacin on the vasoresponsiveness to phenylephrine are shown in Figure 2. Indomethacin (10^{-6} M) did not alter the dose-response curves to phenylephrine of the endothelium-intact or -denuded thoracic aortic rings obtained from either swimming (a) or sedentary control (b) rats. However, when methylene blue (10^{-5} M) was also added to the preparation of endothelium-intact thoracic aortic rings of both swimming and sedentary control rats, the dose-response curves to phenylephrine were shifted to the left with increase in

maximal responses and were no longer different from those obtained from endothelium-denuded rings of both groups of animals (Figure 3 and Table 1).

Vasoconstrictor responses to depolarizing concentrations of KCl (20–120 mM) of endothelium-intact and denuded thoracic aortic rings obtained from swimming and sedentary control rats are shown in Figure 4. There was no difference in dose-response curves to KCl of endothelium-intact thoracic aortic rings obtained from swimming and sedentary control rats. When the endothelium was removed, however, the vasoconstrictor responses to KCl of both groups of animals were significantly shifted to the left with an increase in maximum contractile responses. In addition, the contractile responses to

Figure 5 Effects of passive tension on constrictor responses to phenylephrine ($1 \mu\text{M}$ for endothelium-intact and $0.1 \mu\text{M}$ for endothelium-denuded rings) of endothelium-intact and denuded thoracic aortic rings obtained from swimming or sedentary control rats. Each point represents the mean $\pm$ s.e. mean from 5 experiments: (■) swimming, endothelium intact; (▨) swimming, endothelium denuded; (▤) sedentary control, endothelium intact; (▥) sedentary control, endothelium denuded rings. a* Significantly different from a; b* significantly different from b; c* significantly different from c; d* significantly different from d ($P < 0.05$). Values of (a) were significantly different from those of (b) and (c) at all levels of passive tension; values of (b) were significantly different from those of (d) at all levels of passive tension ($P < 0.05$).

Figure 6 Effects of N^G -nitro-L-arginine (L-NOARG) on constrictor responses to phenylephrine of endothelium-intact thoracic aortic rings obtained from swimming and sedentary control rats under passive tension either of 1.0 (a) or 3.0 g (b); (□) swimming; (■) swimming in the presence of L-NOARG; (○) sedentary control; (●) sedentary control in the presence of L-NOARG. *Significantly different from sedentary control in the presence of L-NOARG, $P < 0.05$.

KCl of endothelium-denuded thoracic aortic rings of swimming rats were greater than those of sedentary controls (Figure 4 and Table 1).

With differences in passive tension applied to the aortae, the active tension developed by thoracic aortic rings of both swimming and sedentary control rats to a maximum concentration of phenylephrine (10^{-6} M) were significantly different. The passive tension in the range of 1.0–2.0 g did not cause a significant difference in active tension developed compared to their corresponding groups. When the passive tension was increased up to 2.5–3.0 g, however, it did significantly increase the active tension developed compared to its corresponding group under a passive tension of 1.0–2.0 g. Nevertheless, in the case of endothelium-intact aortic rings obtained from swimming rats, the responses to phenylephrine were significantly lower than those of sedentary controls for all levels of passive tension applied. On the other hand, after removal of endothelium of the rings obtained from both swimming and sedentary control rats, the responses to phenylephrine were not different between these two groups at all levels of passive tension studied (Figure 5).

Figure 6 shows the effects of L-NOARG and passive tension (1.0 g and 3.0 g) on constrictor responses to phenylephrine of endothelium-intact aortic rings obtained from swimming and sedentary control rats. When the aortic rings were set up with a passive tension of 3.0 g, the responses to phenylephrine were lower in the rings of swimming rats than in those of sedentary controls at all concentrations studied. The presence of L-NOARG, caused a significant leftward shift of the dose-response curves and the EC_{50} values were reduced about 5 fold, with an increase in maximum responses, which were no longer significantly different. For the aortic rings with a passive tension of 1.0 g, although the response to phenylephrine of swimming rats was lower than those of sedentary control and L-NOARG caused a significant shift of the curves to the left of both swimming and sedentary control rats, the rings of swimming rats had higher maximum responses to phenylephrine than did those of sedentary control rats (Figure 6 and Table 1).

Discussion

The present study demonstrates that there is a decrease in reactivity to phenylephrine, an α -adrenoceptor agonist, but not to KCl, of endothelium-intact thoracic aortic rings obtained from swimming rats compared to those from sedentary control rats. The decrease in reactivity is defined as a lower maximum

contractile response to phenylephrine of swimming rats than that of sedentary controls. A similar finding using noradrenaline and KCl was reported in exercise training of Yucatan miniature swine (Oltman *et al.*, 1992). In the dog, however, Rogers *et al.* (1991) demonstrated that contractile responses of the endothelium-intact epicardial coronary arteries (rings) to noradrenaline and phenylephrine were not different between exercised and sedentary control dogs. In exercise trained rats (both running and swimming) Edwards *et al.* (1985) reported no changes in contractile responses to noradrenaline of endothelium-intact thoracic helical strips between those obtained from exercise trained and sedentary control rats. Delp *et al.* (1993), using abdominal aortic rings of young adult male normotensive Sprague Dawley rats, found no difference in dose-response curves to phenylephrine between exercise training and sedentary controls, but did find a significant decrease in vascular sensitivity to noradrenaline and KCl with no difference in maximum contraction between these two groups. These inconsistent results are probably due to differences in animal species and strain and/or differences in vascular tissues and the type of tissue used. Whereas the present study used thoracic aortic rings of Wistar rats, Edwards *et al.* (1985) used helical strips of aortae. It is well known that the manipulation of aortae while cutting into helical strips destroys some of the vascular endothelium (Furchgott, 1993). Delp *et al.* (1993) however, used abdominal aortic rings from the area distal to the renal arteries and proximal to the bifurcation of the iliac arteries of Sprague Dawley rats. It is possible that different sections along the dorsal aorta have differences in vascular receptors and/or reactivity. The blunting of contractile responses to phenylephrine, but not to KCl in the present study, suggests that there are some differences during exercise between responses of the thoracic aorta mediated via the α -adrenoceptor and voltage-operated channel. KCl does not stimulate endothelial NO production whereas phenylephrine does. Thus, a possible reason for this could be an up-regulation of phenylephrine-mediated NO responses in tissue that otherwise had greater smooth muscle contractile responses as a result of exercise.

Watanabe *et al.* (1991) demonstrated that there was an increase in plasma vasodilator prostaglandin E_2 after acute exercise by swimming in rats. In addition, in the *ex vivo* model studied, Ohkubo *et al.* (1992) found an increase in vascular tissue production of prostacyclin, another vasodilator substance, after exercise training in the rat. Thus, it is possible that the decrease in sensitivity to phenylephrine of endothelium-intact thoracic aortic rings of swimming rats compared to those of sedentary control may be attenuated by increasing production of vasodilator prostaglandins from vascular tissue. In order to investigate this possibility, responses to phenylephrine were obtained in the presence of indomethacin, a cyclo-oxygenase inhibitor. Indomethacin did not alter the dose-response curves to phenylephrine in either swimming or control groups. Therefore, the possibility that changes in vascular reactivity to phenylephrine during exercise training are unlikely to be due to increased production of vasodilator prostaglandins.

The decrease in vascular responsiveness to phenylephrine of endothelium-intact thoracic aortic rings of swimming rats in the present studies are unlikely to be due to changes in tissue size or smooth muscle mass, since there was no significant difference in tissue weight of endothelium-intact aortic rings between swimming and sedentary control rats. In addition, when using different passive tensions for the thoracic aortic rings (when it would be expected that at the higher passive tensions applied, the tissues which had a bigger mass would develop the higher active tensions), the smaller responses to phenylephrine of swimming rats, compared to those of sedentary control rats, persisted, and this difference was abolished after removal of endothelium. Delp *et al.* (1993) found no differences of the wall thickness and the effects of stretch on passive tension developed of thoracic aortic rings obtained from treadmill running exercise and sedentary control rats. In their experiments, the maximum stretch of the thoracic aortic rings was about 150% above the initial unstretched outer dia-

meter, and the passive tension developed was about 2.7 g, and this passive tension was used for their pharmacological responsiveness studies. They found no difference in tension developed to phenylephrine of the intact endothelium aortae between those obtained from treadmill running exercise and from sedentary control rats. Thus, it is possible that the passive tension of 1.0 g, which was used in most of the present study, may not be appropriate for the physiological adaptation to swimming. Therefore, in the present study dose-response curves to phenylephrine with a passive tension of 3.0 g were a similar range to those of Delp's group (1993). Results show that the response to phenylephrine of the aortic rings with endothelium-intact from swimming rats were still lower than those of sedentary control rats as was found with a passive tension of 1.0 g and these differences disappeared in the presence of L-NOARG. This finding confirms that the lower responsiveness to phenylephrine of aortic rings of swimming rats is not due to an increase in smooth muscle mass during exercise.

The possible mechanisms for the decrease in responsiveness to phenylephrine of endothelium-intact aortic rings of swimming rats in the present study may (1) involve an alteration in adrenoceptor number at the vascular smooth muscle or (2) be modulated by the vascular endothelium. Recently it has been documented that the vascular endothelium plays an important role in controlling vascular responsiveness by producing a variety of factors mediating relaxation (EDRF/NO), vascular smooth muscle cell hyperpolarization, and vasoconstriction (Vanhoutte, 1993).

To examine whether changes in adrenoceptor-mediated responsiveness at the vascular smooth muscle and/or the vascular endothelium plays a role in the lowering of vascular responsiveness to phenylephrine of endothelium-intact thoracic rings of exercise trained rats, dose-response curves to phenylephrine were also observed in rings of endothelium-denuded thoracic aortae. Also, the dose-response curves to phenylephrine of endothelium-denuded rings were obtained in the presence of indomethacin, a cyclo-oxygenase inhibitor, in order to confirm that prostaglandins did not play a part in these changes. Removal of the vascular endothelium caused a significant shift of the curves to the left with an increase in maximum responses to phenylephrine of both exercise-trained and sedentary control rats. Moreover, the two curves obtained were no longer different, and indomethacin did not alter dose-response curves to phenylephrine of any groups studied. This suggests that vascular endothelium plays an important role in reducing vasoconstrictor responses to phenylephrine of endothelium-intact thoracic aortic rings of exercise trained rats, but not by altering the α -adrenoceptor mediated response in the vascular smooth muscle during exercise training. These results are analogous to those reported by Oltman *et al.* (1992), who found a selective alteration at the vascular smooth muscle for noradrenaline and adenosine receptor-mediated responses in exercise training miniature swine, defined by a decrease in maximum contraction to phenylephrine and reduced vasodilatation to adenosine of proximal coronary artery of the exercise trained animals, whether in the presence or absence of vascular endothelium. The reason for this may be the difference in animal species and/or type of vascular tissues used. In young adult Sprague Dawley rats, however, Delp *et al.* (1993) reported that exercise training by treadmill running, diminished the vascular sensitivity (no changes in maximum contractile response) to noradrenaline but not to phenylephrine of endothelium-intact abdominal aortic rings and the reduced sensitivity was abolished by removal of the endothelium. In addition, removal of the vascular endothelium did not alter the maximum contractile responses to noradrenaline of either exercise-trained rats or sedentary controls. This led them to suggest that there is an endothelium-dependent mechanism involving α_2 -adrenoceptor enhancement of the abdominal aortic rings during exercise training in the rat. The anomaly between the present study and that of Delp *et al.* may be due to differences of type of exercise, animal strain and/or section along the dorsal aorta.

However, removal of the vascular endothelial layer may remove both vasodilator and vasoconstrictor substances generated by the endothelium. Thus, the role of the endothelium in attenuation of the vasoconstrictor response to phenylephrine during exercise training in the present study may involve (1) an increase in spontaneous and/or stimulated production of EDRF/NO and then attenuation of the vasoconstriction to phenylephrine and/or (2) a decrease in the production of vasoconstrictor substances e.g. endothelin (Yanagisawa *et al.*, 1988) and then diminishing the facilitation of the phenylephrine-induced vascular response. In order to examine this possibility, the thoracic aortic rings with endothelium were used and incubated with indomethacin, a cyclo-oxygenase inhibitor, and methylene blue, a presumed inhibitor of the effect of EDRF by abolishing cyclic GMP formation elicited by the EDRF (Gruetter *et al.*, 1981). The dose-response curves to phenylephrine were performed in the presence of these inhibitors, allowing the normal generation of endothelin and other substances by the vascular endothelium. The dose-response curves to phenylephrine of the endothelium-intact tissues in the presence of indomethacin and methylene blue were shifted to the left with an increase in maximum responses to the same extent as those obtained from endothelium-denuded thoracic aortic rings of both exercise training and sedentary control rats. Furthermore, L-NOARG, a specific nitric oxide synthase inhibitor, which is able to block both basal and agonist-stimulated production of endothelium nitric oxide (Frew *et al.*, 1993), also shifted the dose-response curves of phenylephrine to the left with increase in maximum responses in both groups of rats. Beside this, in the case of the aortic rings which had a passive tension of 1.0 g, the curves to phenylephrine of those obtained from swimming rats were higher in maximum contractile responses than those of sedentary controls, but the differences were not found when the tissues had a passive tension of 3.0 g. These findings suggest that the blunting of maximum contractile response to phenylephrine of endothelium-intact rings is due to an attenuation by the increase in released EDRF/NO from vascular endothelium, and not to a decrease in the production of vasoconstrictors and/or

changes in α -adrenoceptor mediated responses at the vascular smooth muscle. This possibility was also supported by Sessa *et al.* (1994) who have shown an enhanced production of EDRF/NO and nitric oxide synthase gene expression at the endothelial cell in chronically exercised dogs.

In the present study no change was found in dose-response curves to KCl of endothelium-intact thoracic aortic rings between exercised and sedentary control rats. This may be due to changes in vascular smooth muscle for voltage-operated channel-mediated responses during exercise training. The present study also tested this hypothesis, by studying the dose-response curves to KCl of endothelium-denuded rings. KCl caused an increase in both sensitivity and maximum contractile response of endothelium-denuded aortic rings of both swimming and sedentary controls. Moreover, the responses to KCl of swimming rats were higher than those of sedentary controls to all doses of KCl. This suggests that there is an increase in both sensitivity and reactivity to KCl of vascular smooth muscle during exercise in rats. Therefore, even though there is an increase in spontaneous release of EDRF/NO from vascular endothelium, the dose-response curves to KCl were restored to the same level as those of sedentary controls.

In conclusion, the present studies suggest that strenuous and long-term exercise by swimming in adult male rats causes an increase in both spontaneous and stimulated release of EDRF/NO from the vascular endothelium to attenuate the vasoconstrictor response to phenylephrine and KCl.

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