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Endothelium-dependent relaxation and hyperpolarization evoked by bradykinin in canine coronary arteries: enhancement by exercise-training

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1 Kinins, which are produced locally in arterial walls, stimulate the release of endothelium-derived vasodilator substances. Therefore, they may participate in the metabolic adaptation to chronic exercise that occurs in the coronary circulation. Experiments were designed to compare the reactivity to bradykinin in coronary arteries isolated from sedentary and exercised-trained dogs (for 8–10 weeks).

2 The organ chambers used in this study were designed for measurement of isometric tension and cell membrane potential with glass microelectrodes. Rings of canine isolated coronary arteries with endothelium were suspended in the organ chambers filled with modified Krebs-Ringer bicarbonate solution (37°C, gassed with 5% CO_2 in 95 O_2), and were all treated with indomethacin to prevent interference from prostaglandins.

3 Bradykinin evoked concentration-dependent relaxations of the coronary arteries. However, the kinin was significantly less potent in relaxing coronary arteries from the sedentary dogs than those from the trained ones.

4 In the presence of N^G-nitro-L-arginine (an inhibitor of nitric oxide synthases), concentration-relaxation curves to bradykinin were shifted to the right in both types of preparations. Nonetheless, the peptide was still significantly more potent in arteries from exercise-trained animals.

5 In the electrophysiological experiments, concentration-hyperpolarization curves to bradykinin obtained in arteries from sedentary dogs were also significantly to the right of those in vessels from exercise-trained animals. Thus, in arteries from exercised animals, bradykinin more potently evoked the release of both nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF).

7 The angiotensin converting enzyme (ACE)-inhibitor, perindoprilat, shifted to the left the concentration-relaxation curves to bradykinin obtained under control conditions and in the presence of N^G-nitro-L-arginine. The concentration-hyperpolarization curves to bradykinin were also shifted to the left by perindoprilat. The shift induced by the ACE-inhibitor in either type of preparation was not significantly different.

8 These findings demonstrate that exercise-training augments the sensitivity of the coronary artery of the dog to the endothelium-dependent effects of bradykinin. This sensitization to bradykinin may reflect an increased role of both NO and EDHF, and is not the consequence of differences in ACE activity in the receptor compartment.

Keywords: Kinins; endothelium-derived hyperpolarizing factor; nitric oxide; N^G-nitro-L-arginine; angiotensin I converting enzyme; kininase II; vascular smooth muscle

Introduction

Exercise-training has reversible beneficial effects on coronary physiology, which depend on both structural and metabolic adaptative mechanisms (Blomquist & Saltin, 1983; Stone, 1983). The endothelium is a major source of vasomotor mediators which may participate in these changes. It releases vasodilator substances in response to a variety of stimuli, including shear stress, circulating catecholamines, platelet products, kinins, and thrombin (Furchgott & Zawadzki, 1980; Lüscher & Vanhoutte, 1991). These vasodilator mediators include endothelium-derived relaxing factor (EDRF); which is either nitric oxide (Palmer *et al.*, 1987; Furchgott, 1988; Ignarro *et al.*, 1988; Moncada *et al.*, 1991) or a nitrosocompound (Myers *et al.*, 1989), vasodilator prostaglandins, and endothelium-derived hyperpolarizing factor (EDHF; Feletou & Vanhoutte, 1988; Furchgott & Vanhoutte, 1989; Beny & Weid, 1990; Lüscher & Vanhoutte, 1990; Garland *et al.*, 1995; Mombouli & Vanhoutte, 1995a). Kinins generated within the vascular wall contribute to the basal release of endothelium-derived vasodilator mediators (Mombouli & Vanhoutte, 1991; 1995b) which supports a paracrine/autocrine role for kinins in vasomotion (Carretero & Scicli, 1991; Mombouli & Vanhoutte, 1995b). Therefore, changes in coronary reactivity to kinins may participate in the metabolic adaptational changes induced by chronic exercise. The present study was designed to assess whether or not differences exist in the endothelium-dependent responses induced by bradykinin in coronary arteries of the dog following chronic exercise.

Endothelium-dependent vasodilatation evoked by acetylcholine or induced by flow in canine coronary arteries is enhanced following chronic exercise (Wang *et al.*, 1993). This enhancement is accompanied by an increase in nitric oxide synthase mRNA (Sessa *et al.*, 1994). In these coronary arteries, relaxations to bradykinin are strictly endothelium-dependent and mediated by both EDRF (Cherry *et al.*, 1982; Toda *et al.*, 1987; Mombouli *et al.*, 1991) and EDHF (Mombouli *et al.*,

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1992). However, it is not known whether or not the EDHF-mediated component of the endothelium-dependent relaxation is also affected following chronic exercise.

Angiotensin-converting enzyme (ACE), which is expressed at the surface of endothelial cells, can rapidly inactivate bradykinin (Yang *et al.*, 1970); this would affect the concentration of the peptide in its receptor compartment (Mombouli & Vanhoutte, 1992). Thus, the effects of an inhibitor of ACE, perindoprilat, were examined also in vessels from both groups of dogs to assess whether exercise-induced changes are a consequence of alteration in the degradation of bradykinin by ACE.

Methods

Experimental animals and training

Heartworm negative and antiparasitic drug-free dogs were selected for these experiments. The animals (between 1 and 5 years of age) were of either sex and all were mixed-breed dogs. No animal of Huskie or Greyhound mixture was used. The animals were first acquainted with the laboratory and with the treadmill until each was comfortable running on the treadmill at various speeds and grades. The animals then performed a submaximal exercise test based on the Tipton protocol on each of two successive days (Tipton *et al.*, 1974). Heart rates for each exercise workload were averaged. Then the animals were assigned randomly to one of two groups: a sedentary group in which the animals were maintained in their cages for a period of 8 to 10 weeks, or a group in which the animals followed an exercise programme (Tipton *et al.*, 1974). This programme included 5 days a week exercise, including speed, alternating sprint, and endurance training with weekly increments of speed and grade for the same period of 8 to 10 weeks. This period of time with the Tipton exercise protocol typically results in the trained condition (Tipton *et al.*, 1974; Stone, 1977). Utilisation of this time frame in our laboratory has resulted in the trained condition, as indicated by significant reduction in exercise heart rates and significant increases in muscle citrate synthase activity (Tate *et al.*, 1993; Hamra & McNeil, 1995). The sedentary control animals were brought to the treadmill laboratory several times a week for socialisation and to maintain their familiarity with the environment. All animals were placed on special diets of two-a-day feeding of puppy chow to meet the energy requirements of the exercised dogs. The weight of the animals was monitored and the food supply was adjusted so that weight gain in the two groups was comparable. Following the exercise/cage rest period, the animals were given a second submaximal exercise test to document the presence of training-induced bradycardia in the exercised group. The *in vitro* experiments were performed on the day the dogs were killed. Selection of the animal for study each day (sedentary versus exercise-trained) was randomized. Only one animal was studied per experimental day.

Coronary arteries

On the day of the experiments the dogs were anaesthetized by an intravenous injection of 30 mg ml^{-1} of sodium pentobarbitone. The heart was removed and placed in cold modified Krebs-Ringer bicarbonate solution [composition in mM; NaCl 118.3, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11.1, calcium disodium edetate 0.026, pH 7.4 (control solution)]. The left circumflex (for organ chamber experiments), and branches of the left anterio-descending (for electrophysiological experiments), coronary arteries were dissected and cleaned of connective tissue. The arteries were cut into rings (4 mm) or transverse strips (3 mm; for electrophysiological studies). Special care was taken not to damage the luminal surface of the preparations. All the experiments were carried out in the presence of indomethacin (10^{-5} M) to avoid possible interference of vasodilator prostanoids.

Organ chamber experiments

Coronary artery rings were suspended between two stirrups in organ chambers (20 ml) filled with control solution (gassed with 95% O_2: 5% CO_2 and maintained at 37°C). One of the stirrups was anchored to the bottom of the organ chamber and the other was connected to a force transducer (UC2, Gould Inc., Cleveland, Ohio, U.S.A.) to record changes in isometric tension. The rings were stretched to the optimal point of their length-active tension relationship (sedentary: 8.43 ± 0.17 g, for 28 rings; exercise-trained: 8.60 ± 0.29 g for 30 rings) as determined by the contraction to 60 mM KCl at progressive levels of stretch. Relaxations to bradykinin were observed in rings contracted with prostaglandin $F_{2\alpha}$ (8×10^{-6} M) in control solution or in the presence of N^G-nitro-L-arginine (10^{-4} M, to block the production of nitric oxide; Ishii *et al.*, 1990). Under the latter conditions the relaxations were transient (Mombouli *et al.*, 1992; Mombouli & Vanhoutte, 1992); therefore, the stepwise increase of the concentration of bradykinin was performed immediately following the maximal relaxation observed at each concentration of agonist. In a second set of rings the effect of the ACE-inhibitor, perindoprilat (10^{-6} M; 30 min incubation) on these relaxations was studied either under control conditions or in the presence of N^G-nitro-L-arginine.

Electrophysiological studies

Strips of coronary artery were pinned to the bottom of an organ chamber with the endothelial side upward. After 90 min of equilibration, a glass microelectrode filled with 3 M KCl (40–80 MΩ tip resistance) was inserted into a smooth muscle cell (Feletou & Vanhoutte, 1988; Mombouli *et al.*, 1992). The microelectrode was connected to an amplifier (World Precision Instruments, New Heaven, CT, U.S.A.) and the membrane potential was monitored simultaneously on an oscilloscope (Textronix 5223, Beaverton, OR, U.S.A.) and a chart recorder (Gould TA550, Cleveland, OH, U.S.A.). Successful impalements were characterized by a sudden negative shift in voltage followed by a stable negative membrane potential. Concentration-hyperpolarization curves to bradykinin were obtained in a non-cumulative fashion (30 min intervals between additions of the kinin); this procedure allowed the microelectrode to remain impaled after a challenge of the preparation with the peptide. Experiments were performed first under control conditions, and they were repeated in the presence of perindoprilat (10^{-6} M, 30 min) following a 40 min washout period.

Table 1 Submaximal exercise heart rates (b.p.m.)

Workloads (m.p.h./% grade)	*4/8*	*4/12*	*4/16*	*4/20*
Pre-training	167 ± 7	181 ± 7	196 ± 8	215 ± 8
Post-training	155 ± 10*	167 ± 10*	181 ± 11*	194 ± 12*

*Statistically significant difference between pre- and post-training values in $n = 6$ exercise-trained animals.
b.p.m.: heart beats per minute.
m.p.h.: miles per hour.

Drugs and chemicals

The following drugs were used; bradykinin acetate, indomethacin, prostaglandin $F_{2\alpha}$ (all from Sigma, St-Louis, MO, U.S.A.), N^G-nitro-L-arginine (Aldrich, Milwaukee, WI, U.S.A.), perindoprilat (S9490-3, Servier, Neuilly-sur-Seine, France). Drugs were prepared in water except for indomethacin (dissolved by sonication in water and Na_2CO_3- which had no effect at the final bath concentration of 5×10^{-6} M).

Statistical analysis

In each experimental group, n refers to the number of animals from which blood vessels were taken. Results are given as means ± s.e.mean. The potency of bradykinin is expressed as the negative logarithm of the concentration of bradykinin causing half of the maximal response to the kinin (pD_2). Statistical comparisons between results obtained in the two types of coronary arteries were performed by Student's *t* test for unpaired observations. Differences induced by perindoprilat within the same group of coronary arteries were assessed by Student's *t* test for paired observations. Differences were considered to be statistically significant when *P* was less than 0.05.

Results

There was a significant reduction in exercise heart rate in the exercised group at the four highest exercise workloads after the training programme (Table 1). Post-training data could not be obtained for two of the exercised animals, although they completed the programme at very high workload demands. Likewise, several of the sedentary dogs would not run on the treadmill, which precluded a comparison of the values of heart rate before and after the period of cage rest. However, this exercise/cage rest model in our laboratory always yields reduced exercise heart rates in the trained groups and the same or higher heart rates in the sedentary groups (Tate *et al.*, 1993; Hamra & McNeil, 1995). Weight gains in the exercise-trained and sedentary dogs were 1.9 ± 0.8 and 1.8 ± 0.5 kg, respectively.

Organ chamber experiments

Under control conditions, the contractions evoked by prostaglandin $F_{2\alpha}$ (8×10^{-6} M) in coronary arteries from exercise-trained dogs did not differ significantly from those obtained in the sedentary animals (Table 2). Treatment of the blood vessels with the ACE-inhibitor, perindoprilat (10^{-6} M) did not alter the contractions to prostaglandin $F_{2\alpha}$ significantly. In the presence of N^G-nitro-L-arginine (10^{-4} M), prostaglandin $F_{2\alpha}$ elicited contractions of comparable magnitude both in the absence and in the presence of perindoprilat (Table 2).

During the plateau of the contractions to prostaglandin $F_{2\alpha}$, cumulative additions of bradykinin induced complete relaxations in both types of coronary artery, in a concentration-dependent manner. However, the concentration-relaxation curve to bradykinin obtained in blood vessels from exercise-trained dogs was significantly to the left of the curves obtained in arteries from the sedentary animals (Figure 1). Thus, the potency of bradykinin was greater in exercise-trained (pD_2: 9.52 ± 0.12) than in sedentary animals (pD_2: 9.08 ± 0.11).

Table 2 Contractions[a] (g) to prostaglandin $F_{2\alpha}$

Treatment	*Trained*	*Sedentary*
Control	11.80 ± 2.06	12.06 ± 1.79
Perindoprilat (10^{-6} M)	16.4 ± 2.78	12.10 ± 1.60
L-NOARG (10^{-4} M)	17.40 ± 2.28	19.24 ± 2.75
L-NOARG + perindoprilat	18.42 ± 1.87	15.49 ± 2.39

[a] Data shown as means ± s.e.mean ($n = 7-8$). L-NOARG: N^G-nitro-L-arginine.

In vessels treated with the inhibitor of nitric oxide synthase, N^G-nitro-L-arginine, higher concentrations of the peptide were required to obtain relaxations (Figure 2). In arteries from both groups, the concentration-relaxation curves were shifted significantly to the right of the curves obtained under control conditions (Figure 2).

During ACE-inhibition with perindoprilat (10^{-6} M), which by itself had no effect on vascular tone, the concentration-relaxation curve to bradykinin was shifted towards lower concentrations in both types of preparations. Thus the potency values for bradykinin were enhanced significantly in the presence of the ACE-inhibitor (pD_2: 10.04 ± 0.23 for the sedentary, and 10.58 ± 0.07 for the exercise-trained). The shift induced by perindoprilat was not significantly different between the two types of coronary arteries (ΔpD_2: 0.96 ± 0.37 for the sedentary, and 1.06 ± 0.29 for the exercise-trained). As

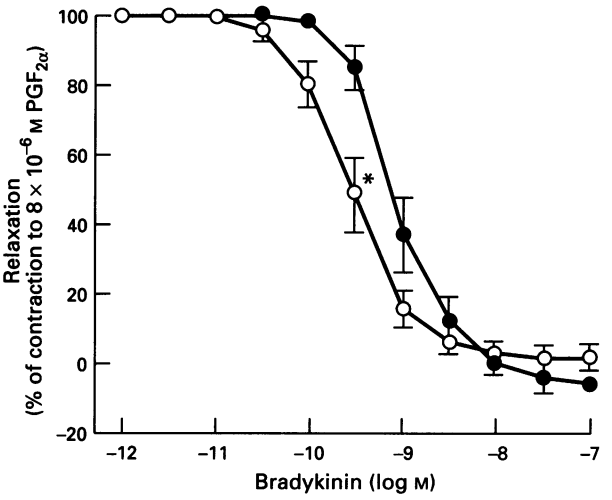

Figure 1 Concentration-relaxation curves to bradykinin obtained in coronary arteries from sedentary (●) and exercised (○) dogs. Results are expressed as a percentage of the contraction evoked by 8×10^{-6} M prostaglandin $F_{2\alpha}$, and are presented as means ± s.e.mean (for sedentary: $n = 8$, and for exercise-trained: $n = 7$). *Statistically significant difference between the two groups.

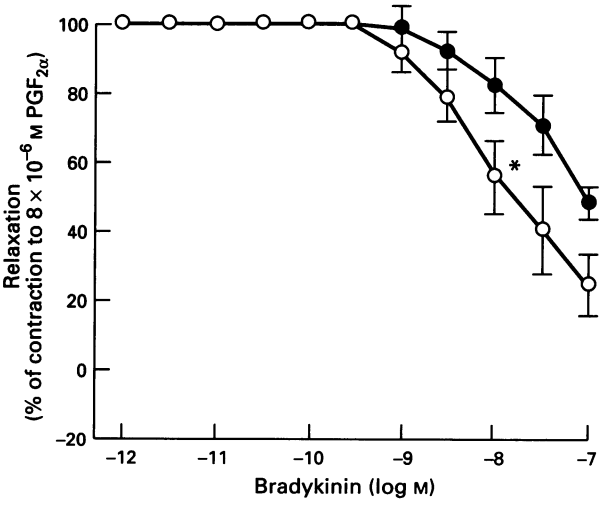

Figure 2 Concentration-relaxation curves to bradykinin obtained in coronary arteries from sedentary (●) and exercised (○) dogs in the presence of 10^{-4} M N^G-nitro-L-arginine. Results are expressed as a percentage of the contraction evoked by 8×10^{-6} M prostaglandin $F_{2\alpha}$, and are presented as means ± s.e.mean (for sedentary: $n = 8$, and for exercise-trained: $n = 8$). *Statistically significant difference between the two groups.

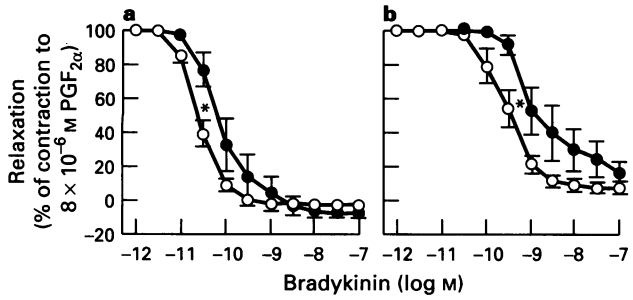

Figure 3 Concentration-relaxation curves to bradykinin obtained in coronary arteries from sedentary (●) and exercised (○) dogs treated with perindoprilat (10^{-6} M), in the absence (a) and in the presence (b) of 10^{-4} M N^G-nitro-L-arginine. Results are expressed as a percentage of the contraction evoked by 8×10^{-6} M prostaglandin $F_{2\alpha}$, and are presented as means ± s.e.mean. *Statistically significant difference between the two groups.

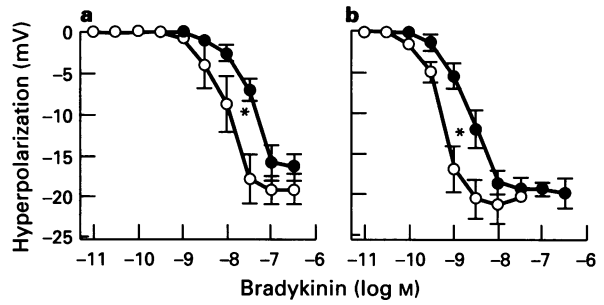

Figure 4 Concentration-hyperpolarization curves to bradykinin obtained in coronary arteries from sedentary (●) and exercise-trained (○) dogs. The experiments were performed in the presence of 10^{-4} M N^G-nitro-L-arginine and 10^{-5} M indomethacin. Variations in membrane potential were monitored in the absence (a) and in the presence of 10^{-6} M perindoprilat (b). Results are expressed in mV, and are presented as means ± s.e.mean (for sedentary: $n = 5$, and for exercise-trained: $n = 5$). *Statistically significant difference between the two groups.

under control conditions, the concentration-relaxation curves to bradykinin obtained in the presence of perindoprilat in the vessels from sedentary dogs were still significantly to the right of those obtained in tissues from the exercise-trained animals (Figure 3a). In the presence of perindoprilat, the concentration-relaxation curves to bradykinin were shifted significantly to the left, in arteries from both groups that were treated with N^G-nitro-L-arginine (Figure 3b). The curves obtained in coronary arteries from sedentary animals were still to the right of those obtained in rings from exercise-trained dogs (Figure 3b).

Electrophysiological experiments

The resting plasma membrane potential recorded in vascular smooth muscle cells was comparable in both groups of preparations (Table 3). Treatment of the blood vessels with indomethacin (10^{-5} M) and N^G-nitro-L-arginine (10^{-4} M) had no effect on the resting membrane potential (data not shown). Bradykinin, added non-cumulatively in the presence of indomethacin and N^G-nitro-L-arginine, evoked a concentration-dependent hyperpolarization of the plasma membrane potential in vascular smooth muscle (Figure 4). As shown in Figure 4a, the concentration-hyperpolarization curves to bradykinin obtained in preparations from sedentary animals (pD_2: 7.36 ± 0.08) was significantly to the right of those obtained in tissues from exercise-trained dogs (pD_2: 7.92 ± 0.20). The maximal hyperpolarization induced by bradykinin in arteries from the sedentary animals did not differ significantly from that in exercised-trained dogs (Table 3).

Treatment with perindoprilat did not affect the resting plasma membrane potential in either type of tissue. However, the ACE-inhibitor shifted significantly to the left the concentration-hyperpolarization curves to bradykinin, in both groups (Figure 4b). Under these conditions, the pD_2 values for bradykinin were greater in exercise-trained animals (9.24 ± 0.06) than in the sedentary group (8.84 ± 0.14). The maximal hyperpolarization induced by bradykinin in arteries from both the sedentary and the exercised-trained dogs was not augmented significantly by perindoprilat (Table 2). The shift in the curves induced by the ACE inhibitor was comparable in both types of blood vessels (Δpotency: 1.48 ± 0.32 in the sedentary group and 1.32 ± 0.41 in the exercised-trained dogs).

Discussion

The major finding of the present study is that bradykinin is more potent in coronary arteries isolated from exercise-trained dogs than in those from sedentary animals. This potentiation appears to affect the components of the relaxation to bradykinin, mediated by both EDRF and EDHF. Modifications in the participation of prostanoids in this enhancement were not investigated since the experiments were conducted in the continuous presence of indomethacin. The differences in the potency of bradykinin in eliciting relaxations and hyperpolarizations in the coronary arteries isolated from sedentary dogs as compared to exercise-trained animals was approximately a half-log unit.

Following exercise-training, there is also an increase in coronary artery reactivity to the direct vasodilators, vasoactive intestinal peptide (in the dog; Rogers *et al.*, 1991), and adenosine (in the pig; Oltman *et al.*, 1992). However, endothelium-dependent relaxations to α_2-adrenoceptor agonists or substance P, and endothelium-independent relaxations induced by isoprenaline are not enhanced following exercise-training in canine isolated coronary arteries (Rogers *et al.*, 1991). Since, EDRF contributes to the relaxations induced by bradykinin in the canine coronary artery, the potentiation of the response obtained under control conditions in preparations from exercised animals may reflect the enhanced expression of the constitutive form of nitric oxide synthase. However, the relaxations to bradykinin in the canine coronary artery, as in several other blood vessels, are partially resistant to the combined inhibition of nitric oxide synthase, and cyclo-oxygenase. These EDRF- and prostaglandin-independent relaxations are

Table 3 Electrophysiological effects of bradykinin (mV)[a]

	Controls		*Perindoprilat*	
	E_m	H_{max}	E_m	H_{max}
Sedentary	49.5 ± 1.4	16.6 ± 1.6	48.9 ± 0.8	19.8 ± 1.6
Trained	49.2 ± 0.9	19.4 ± 1.7	48.3 ± 0.7	21.3 ± 2.4

[a] E_m: resting membrane potential ($n = 5$, for both sedentary and trained dogs). Each value is an average of 10 to 14 impalements. H_{max}: magnitude of the maximal hyperpolarization induced by bradykinin from the resting membrane potential value.

mediated by EDHF (Mombouli *et al.*, 1992; Nagao & Vanhoutte, 1992). In some blood vessels, nitric oxide may also cause hyperpolarization and relaxation of vascular smooth muscle by activating potassium channels either directly (Bolotina *et al.*, 1994) or through the production of cyclic GMP (Archer *et al.*, 1994). However, in the latter case, these responses are inhibited by nitric oxide synthase inhibitors or by methylene blue, unlike EDHF-mediated hyperpolarization and relaxation (Mombouli *et al.*, 1992; Nagao & Vanhoutte, 1992; Garland *et al.*, 1995; Mombouli & Vanhoutte, 1995a, b).

The shift to the right induced by the inhibitor of nitric oxide synthase, N^G-nitro-L-arginine, may reflect the poor coupling of endothelial B_2-kinin receptors in the canine coronary artery to the generation of EDHF when compared to that of EDRF (Mombouli *et al.*, 1992; Mombouli & Vanhoutte, 1992; 1995a). This is in contrast to porcine coronary arteries where the release of the two mediators follows the same concentration-response relationship (Nagao & Vanhoutte, 1992). The mechanism of the lesser coupling of kinin receptors to the release of EDHF in the canine coronary arteries are not fully understood (Mombouli & Vanhoutte, 1995a). Nonetheless, the present study shows that the EDHF-mediated component of the relaxation and the hyperpolarization evoked by bradykinin are potentiated following exercise training. This indicates that in addition to an enhanced expression of nitric oxide synthase, the EDHF-dependent pathway may also be up-regulated.

Enhancement of the actions of kinins can result from a lesser inactivation by ACE, which is present at the surface of endothelial cells. Indeed, ACE activity in endothelial cells can be modulated by several endogenous substances, including fibroblast growth factor (Okabe *et al.*, 1987), endothelin (Kawaguchi *et al.*, 1991) and substances which generate platelet-activating factor (Kawaguchi *et al.*, 1990) or those which elevate cyclic AMP levels (Krulewitz & Fanburg, 1986). However, both the EDRF- and EDHF-mediated responses to bradykinin were potentiated by an order of magnitude by the ACE inhibitor, perindoprilat, in coronary arteries from both sedentary and exercise-trained dogs. The concentration of perindoprilat used in the present study was shown previously to protect bradykinin efficiently from degradation by coronary arteries *in vitro* (Mombouli *et al.*, 1992). Since the shift was similar in both types of preparations, it is unlikely that a difference in ACE activity in the kinin-receptor compartment is involved in the augmented responsiveness observed in exercise-trained animals.

A vascular kallikrein-kinin system may play an important role in arterial vasomotility (Carretero & Scicli, 1991; Mombouli & Vanhoutte, 1995b). In perfused canine arteries, the basal release of EDRF is enhanced by ACE-inhibitors and reduced by antagonists of B_2-kinin receptors (Mombouli & Vanhoutte, 1991). ACE-inhibitors increase plasma kinin levels by two or three fold only; however, the vasodilator actions of these antihypertensive agents is in part mediated by kinins (Carretero & Scicli, 1991; Mombouli & Vanhoutte, 1995b). Interestingly, in the present study, the potency of bradykinin was increased by three fold following exercise-training. Kinin levels increase during exercise (Rett *et al.*, 1990), therefore, it is possible that, in combination with shear-stress, the enhanced sensitivity of endothelial cells to kinins may contribute significantly to the adaptative mechanisms involved in the coronary circulation of trained individuals.

The enhancement of nitric oxide synthase mRNA (Sessa *et al.*, 1993), and of the relaxations mediated by EDRF (Wang *et al.*, 1993) and by EDHF, respectively, suggests that exercise exerts its influence on events downstream of receptor activation. If this were the case, there should be a generalized potentiation of endothelium-dependent vasodilator responses in canine coronary arteries. However, previous reports showed a lack of potentiation of the relaxation which is triggered by activation of endothelial receptors responding to α_2-adrenoceptor agonists or substance P (Rogers *et al.*, 1991); this indicates that certain receptors may be down-regulated or desensitized following chronic exercise. Moreover, the mechanisms which govern these phenotypic adjustments are not known, although, it has been established that chronic increases in blood flow enhances endothelium-dependent relaxations both in arteries and veins (Miller *et al.*, 1986; Miller & Vanhoutte, 1990).

In summary, the data demonstrate that exercise-training enhances the sensitivity of canine coronary arteries to bradykinin, an endogenous endothelium-dependent vasodilator. This potentiation does not result from an increased functional impairment of bradykinin by ACE. This peptide causes relaxation in that artery by releasing two factors, EDRF (nitric oxide) and an EDHF.

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