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Effect of captopril on skeletal muscle angiogenic growth factor responses to exercise

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Gavin, Timothy P., David A. Spector, Harrieth Wagner, Ellen C. Breen, and Peter D. Wagner. Effect of captopril on skeletal muscle angiogenic growth factor responses to exercise. *J Appl Physiol* 88: 1690–1697, 2000.—Acute exercise increases vascular endothelial growth factor (VEGF), transforming growth factor-β_1 (TGF-β_1), and basic fibroblast growth factor (bFGF) mRNA levels in skeletal muscle, with the greatest increase in VEGF mRNA. VEGF functions via binding to the VEGF receptors Flk-1 and Flt-1. Captopril, an angiotensin-converting enzyme inhibitor, has been suggested to reduce the microvasculature in resting and exercising skeletal muscle. However, the molecular mechanisms responsible for this reduction have not been investigated. We hypothesized that this might occur via reduced VEGF, TGF-β_1, bFGF, Flk-1, and Flt-1 gene expression at rest and after exercise. To investigate this, 10-wk-old female Wistar rats were placed into four groups (n = 6 each): *1*) saline + rest; *2*) saline + exercise; *3*) 100 mg/kg ip captopril + rest; and *4*) 100 mg/kg ip captopril + exercise. Exercise consisted of 1 h of running at 20 m/min on a 10° incline. VEGF, TGF-β_1, bFGF, Flk-1, and Flt-1 mRNA were analyzed from the left gastrocnemius by quantitative Northern blot. Exercise increased VEGF mRNA 4.8-fold, TGF-β_1 mRNA 1.6-fold, and Flt-1 mRNA 1.7-fold but did not alter bFGF or Flk-1 mRNA measured 1 h after exercise. Captopril did not affect the rest or exercise levels of VEGF, TGF-β_1, bFGF, and Flt-1 mRNA. Captopril did reduce Flk-1 mRNA 30–40%, independently of exercise. This is partially consistent with the suggestion that captopril may inhibit capillary growth.

vascular endothelial growth factor; basic fibroblast growth factor; transforming growth factor-β_1; Flk-1; Flt-1

REGULARLY PERFORMED ENDURANCE EXERCISE induces major adaptations in skeletal muscle. These include training-induced changes in muscle substrate utilization, mitochondrial content, biochemical enzyme/protein activities, and capillarization (see Refs. 1, 4, 16, 18 for review). Despite extensive characterization of these training-induced changes, very little is known about the molecular events responsible for initiating and maintaining these adaptations.

Acute exercise induces a greater expression of genes known to promote angiogenesis. Submaximal, systemic exercise increases vascular endothelial growth factor (VEGF) mRNA levels three- to fourfold and transforming growth factor-β_1 (TGF-β_1) and basic fibroblast growth factor (bFGF) mRNA levels to a lesser extent in the rat gastrocnemius (5, 13). VEGF is a 45-kDa heparin-binding homodimeric protein and is an important regulator of angiogenesis during embryonic development, wound healing, reproductive functions, and tumor growth (11). The physiological actions of VEGF include increases in vascular permeability, endothelial cell proliferation, and angiogenesis (22, 36, 50). The angiogenesis-promoting action of VEGF is produced primarily through VEGF binding to its two receptors, Flk-1 and Flt-1 (see Ref. 11 for review). TGF-β_1 is a 25-kDa homodimeric protein that can function in autocrine and paracrine manners in processes such as embryogenesis, cell proliferation, and wound healing (8), whereas bFGF is an 18-kDa protein known to stimulate smooth muscle cell growth, wound healing, and tissue repair (3). The mechanisms regulating these angiogenic growth factors in skeletal muscle are not well understood.

Captopril is a well-known angiotensin-converting enzyme (ACE) inhibitor taken by 5–10 million people worldwide for the treatment of hypertension and heart failure (47). ACE converts the inactive peptide angiotensin I (ANG I) to the active vasoconstrictor angiotensin II (ANG II) while inactivating the vasodilator bradykinin. It is well established that captopril administration reduces plasma ANG II and increases plasma bradykinin (2, 25, 38, 39).

In addition to its hypotensive effect, captopril can also alter the skeletal muscle microvasculature (10, 30, 48). Administration of captopril at a dose of 100 $mg \cdot kg^{-1} \cdot day^{-1}$ reduces the skeletal muscle microvasculature in hypertensive and normotensive rats (48). It has been reported (in abstract) that captopril (100 $mg \cdot kg^{-1} \cdot day^{-1}$) inhibits exercise-induced angiogenesis in skeletal muscle (30). Similarly, captopril fails to promote angiogenesis in an animal model of hindlimb ischemia, whereas quinaprilat, an ACE inhibitor similar to captopril in that it also is orally active but lacks the sulfhydryl group of captopril, promotes angiogenesis (10). Despite these morphological findings, the molecular mechanisms responsible for the antiangiogenic action of captopril in skeletal muscle have not been investigated. Thus the primary purpose of this study was to test the hypothesis that acute administration of 100 mg/kg captopril would reduce skeletal muscle VEGF, TGF-β_1, bFGF, Flk-1, and Flt-1 gene

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expression both at rest and after exercise. In this report, we demonstrate that acute administration of captopril reduces Flk-1 mRNA independently of exercise. This is consistent with reports that captopril reduces the skeletal muscle vasculature at rest and inhibits the skeletal muscle angiogenic response to exercise training. In contrast, acute administration of captopril does not affect VEGF, TGF-β_1, bFGF, or Flt-1 gene expression.

METHODS

This study was approved by the University of California, San Diego, Animal Subjects Committee. Female Wistar rats were used throughout the study. Mean age was 69 ± 5 (SD) days and weight was 221 ± 17 (SD) g. All rats were first familiarized with a rodent treadmill (Omnipacer model LC-4, Omnitech, Columbus, OH) and taught to run at 20 m/min on an incline of 10° for 5 min, 48 h before the experimental bout. Animals were housed in their cages and allowed standard rat food and water ad libitum before undertaking the study. The exercise bout consisted of 1 h of treadmill running at 20 m/min, 10° incline. This speed represents 50% of the speed required to attain maximal O_2 consumption (5). Four treatment groups were defined with six rats in each group: *1*) saline + rest; *2*) saline + exercise; *3*) 100 mg/kg captopril (Sigma Chemical, St. Louis, MO) + rest; and *4*) 100 mg/kg captopril + exercise. Animals were injected intraperitoneally with either saline or captopril 20 min before the start of rest or exercise. After completing the 1 h of rest or exercise, animals were anesthetized with pentobarbital sodium (50 mg/kg ip), breathing 100% O_2 to avoid hypoxemia, which has been shown to stimulate skeletal muscle VEGF mRNA (5). Within 30 min of the completion of exercise and after topical administration of lidocaine, the left carotid artery was catheterized for the measurement of mean arterial pressure (MAP) to determine the effect of captopril on the vasculature. Previous work suggests that the largest increase in growth factor response to exercise is produced immediately after exercise (5); therefore, only a single measurement of MAP was made to promote the expedient removal of the muscle. After the measurement of MAP, the left gastrocnemius muscles were removed, frozen in liquid nitrogen, and stored at −80°C until further RNA analysis. Thus the RNA data reported herein reflect samples taken ~1 h after the completion of exercise.

Efficacy of ACE inhibition. To determine the efficacy of captopril in inhibiting ACE after the completion of exercise, six additional female Wistar rats were divided into two groups ($n = 3$): saline + exercise and 100 mg/kg captopril + exercise. Drug administration and exercise protocol were as described above. After the completion of exercise, animals were anesthetized with pentobarbital sodium (50 mg/kg ip) and mechanically ventilated (Harvard rodent ventilator, model 683) to maintain P_{O_2}, P_{CO_2}, and pH in the normal range. Maintenance doses of pentobarbital sodium were given to maintain a steady level of anesthesia, and temperature was held constant by use of a heating pad. The left carotid artery was catheterized for continuous measurement of blood pressure, and the jugular vein was catheterized for bolus injection of ANG I (Sigma Chemical). One animal from each group was used to determine the appropriate dosages of ANG I and to ensure that the P_{O_2}, P_{CO_2}, and pH were well maintained (P_{O_2} 85 ± 3 Torr, P_{CO_2} 27 ± 1 Torr, and pH 7.41 ± 0.03; means ± SE). The initial values of MAP for these animals are included in the comparison of MAP between the animal protocols but not in the comparisons on the effect of ANG I. The effect of bolus intravenous injections of ANG I (5, 10, 20, 50, 100, and 200 pM/kg) on MAP was measured in saline- and captopril-treated rats ($n = 2$).

RNA isolation and Northern analysis. The left gastrocnemius muscles were removed, and total cellular RNA was isolated from each sample by the method of Chomczynski and Sacchi (7). RNA preparations were quantitated by absorbance at 260 nm, and RNA intactness was assessed by ethidium bromide staining after separation by electrophoresis in a 6.6% formaldehyde-1% agarose gel. Fractionated RNA was transferred by Northern blot to Zeta-probe membrane (Bio-Rad, Hercules, CA). RNA was cross-linked to the membrane by ultraviolet irradiation for 1 min and stored at 4°C. The blots were then probed with oligolabeled [α-^{32}P]deoxycytidine triphosphate cDNA probes specific for rat VEGF (23), rat TGF-β_1 (31), human bFGF (20), rat Flk-1 (51), and rat Flt-1 cDNA (51). Prehybridization and hybridization were performed in 50% formamide, 5× saline sodium citrate (SSC; 20 × SSC is 0.3 M sodium chloride, 0.3 M sodium citrate), 10× Denhardt's solution (100× Denhardt's solution is 2% Ficoll, 2% polyvinylpyrrolidone, 2% BSA factor V), 50 mM sodium phosphate (pH 7.0), 1% SDS, and 250 μg/ml salmon sperm DNA at 42°C. Blots were washed with 2× SSC and 0.1% SDS at room temperature and 0.1× SSC and 0.1% SDS at 55°C (bFGF, TGF-β_1, Flk-1, and Flt-1) or 65°C (VEGF). Blots were exposed to XAR-5 X-ray film (Eastman Kodak, New Haven, CT) by use of a Cronex Lightning Plus screen at −80°. Autoradiographs were quantitated by densitometry within the linear range of signals and normalized to ribosomal 18S RNA levels.

Statistical treatment. A two-way ANOVA (drug × exercise level) was used to determine differences in postexercise MAP and mRNA. Bonferroni's test was used to determine significance between conditions. Student's *t*-test was used to determine differences in the MAP response to ANG I. Significance was established at $P \leq 0.05$ for all statistical sets, and data reported are means ± SE.

RESULTS

Injection of 100 mg/kg captopril resulted in significant systemic hypotension (saline: rest 120 ± 6 and exercise 121 ± 3; captopril: rest 95 ± 6 and exercise 82 ± 7 mmHg, $P \leq 0.05$), as measured under anesthesia, ~1 h 50 min after injection of captopril (Fig. 1*A*). All animals completed the 1 h of exercise without incident. There was no observable difference in performance between the saline- and captopril-treated rats.

There was no difference in resting MAP between the experiments used in the molecular analysis (Fig. 1*A*) and the experiments determining the efficacy of ACE inhibition (Fig. 1*B*): saline + exercise = 121 ± 3 vs. 131 ± 5 mmHg and captopril + exercise = 82 ± 7 vs. 78 ± 12 mmHg for Fig. 1*A* vs. Fig. 1*B*, respectively ($P \geq 0.05$). The effect of intravenous administration of ANG I on MAP is illustrated in Fig. 1*B*. It is evident that effective ACE inhibition had occurred at all concentrations of ANG I (5–200 pM/kg). To ensure that ACE was still "active" in the captopril-treated rats, a single dosage of 200,000 pM/kg ANG I was given. This resulted in a 25 ± 5 mmHg rise in MAP, demonstrating that large dosages of ANG I could overcome the ACE inhibition produced by captopril (data not shown).

Figure 2 shows representative Northern blots in

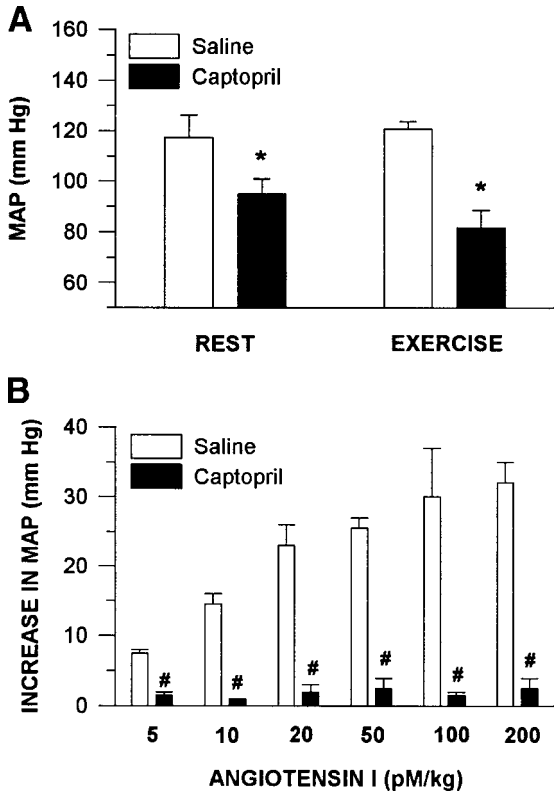

Fig. 1. Effect of 100 mg/kg captopril on mean arterial pressure (MAP) for saline + rest, saline + exercise, captopril + rest, and captopril + exercise (*A*) and response to bolus injections of ANG I after exercise (*B*). Error bars represent SE. *Significantly different from saline + rest; #significantly different from saline ($P \leq 0.05$).

which VEGF (*A*), TGF-β_1 (*B*), and bFGF (*C*) mRNA levels were examined after the single 1-h submaximal exercise run. Exercise increased VEGF and TGF-β_1 mRNA levels. Captopril did not affect the rest or exercise levels of VEGF, TGF-β_1, or bFGF mRNA.

Figure 3 portrays the quantitative densitometry for VEGF (*A*), TGF-β_1 (*B*), and bFGF (*C*) mRNA, normalized to 18S ribosomal RNA, with the saline + rest value set to 1.0 for each factor. Figure 3 demonstrates that exercise induced an ~4.8-fold increase in VEGF mRNA and 1.6-fold increase in TGF-β_1 mRNA ($P \leq 0.05$). These responses were unaffected by captopril. Neither exercise nor captopril affected bFGF mRNA levels.

Figure 4 presents representative Northern blots in which Flk-1 (*A*) and Flt-1 (*B*) mRNA levels were examined ~1 h after the completion of a single 1-h submaximal exercise run. Exercise increased Flt-1 mRNA levels, whereas Flk-1 mRNA was reduced with captopril independently of exercise.

Figure 5 displays the quantitative densitometry values for Flk-1 (*A*) and Flt-1 (*B*) mRNA. Flk-1 and Flt-1 mRNA were normalized to 18S ribosomal RNA, with the saline + rest value set to 1.0 for each factor. Figure 5 demonstrates that exercise induced an ~1.7-fold increase in Flt-1 mRNA ($P \leq 0.05$). This response was unaffected by captopril. However, captopril reduced Flk-1 mRNA ~30–40% independently of exercise ($P \leq 0.05$).

DISCUSSION

The principal findings of the present study are *1*) captopril does not affect the exercise-induced increases in skeletal muscle VEGF, TGF-β_1, or Flt-1 mRNA levels and *2*) captopril decreases skeletal muscle Flk-1 mRNA ~30–40%. In rats, treadmill running increases the skeletal muscle VEGF mRNA levels and to a lesser extent TGF-β_1 and bFGF mRNA (5, 13). Our findings here suggest that the potential antiangiogenic effects of

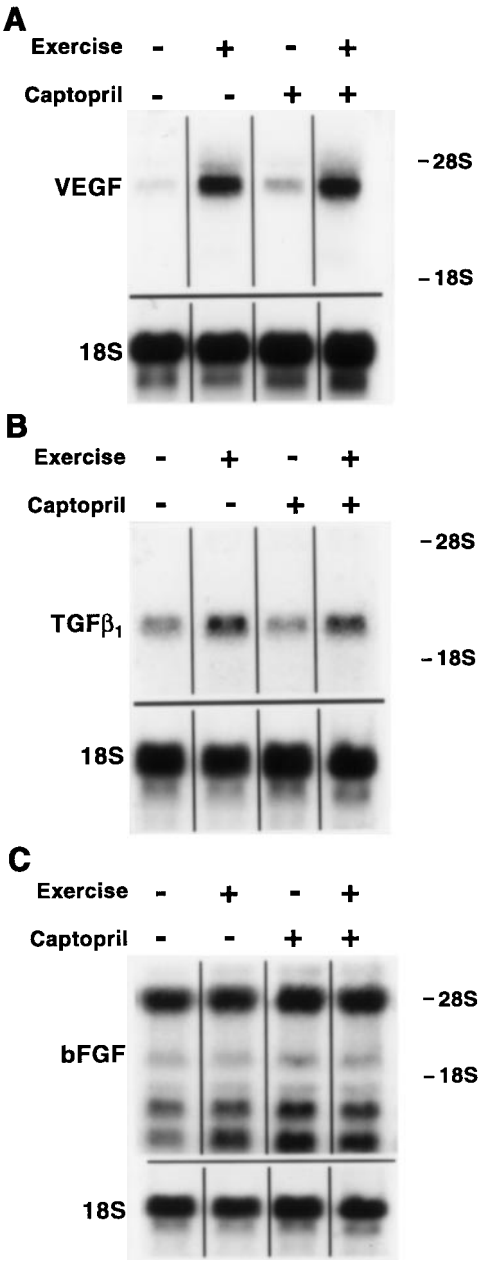

Fig. 2. Representative Northern blots showing mRNA signals for vascular endothelial growth factor (VEGF; *A*), transforming growth factor-β_1 (TGF-β_1; *B*), and basic fibroblast growth factor (bFGF; *C*) in rats that had either exercised for 1 h at 20 m/min, 10° incline, or rested and had been injected with either saline or 100 mg/kg captopril. Lane loading was controlled for by 18S rRNA. Exercise increased VEGF and TGF-β_1 mRNA. Captopril did not affect VEGF, TGF-β_1, or bFGF mRNA.

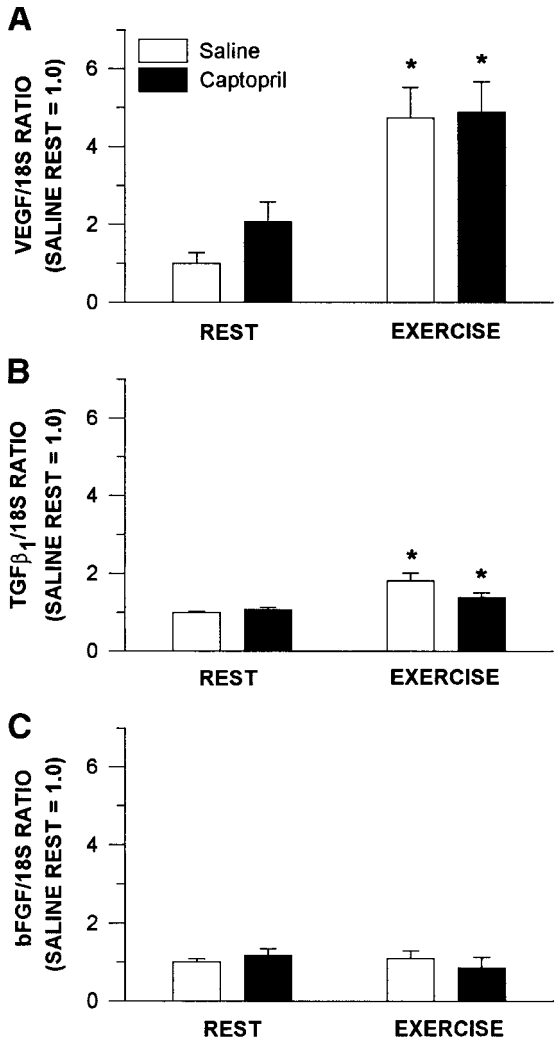

Fig. 3. Effect of 100 mg/kg captopril on the exercise-induced mRNA response ~1 h after the completion of rest or exercise. Saline + rest data were normalized to 1.0. All other data were normalized to saline + rest to allow for comparisons. Quantitative densitometry of Northern blots for the ratio of VEGF mRNA to ribosomal 18S rRNA (*A*), TGF-β_1 mRNA to ribosomal 18S rRNA (*B*), and bFGF mRNA to ribosomal 18S rRNA (*C*) is shown. VEGF was increased 4.8-fold and TGF-β_1 was increased 1.6-fold by exercise. Captopril did not affect VEGF, TGF-β_1, or bFGF mRNA. Error bars represent SE. *Significantly different from saline + rest ($P \leq 0.05$).

captopril on the skeletal muscle vasculature are manifested not via alterations in growth factor gene expression but more likely at the VEGF receptor level through regulation of Flk-1 gene expression. The finding that captopril reduces Flk-1 gene expression independently of exercise is partially consistent with previous findings that captopril can reduce or inhibit skeletal muscle vessel growth at rest and in response to exercise (10, 30, 48).

Captopril and Flk-1. Captopril not only lowers the circulating levels of ANG II but also alters the circulating levels of other peptides and hormones. Within the renin-angiotensin system (RAS), captopril increases the levels of ANG I and renin (2, 25, 38, 39). In addition, because ACE is the same enzyme as kinase II, the enzyme that inactivates the vasodilator bradykinin, captopril increases bradykinin (25). Among oral ACE inhibitors, captopril is unique in that, in addition to its effects on blood pressure and peptide levels, it contains a sulfhydryl group that can inhibit zinc-dependent metalloproteinases, which are active in angiogenesis in the remodeling of the extracellular matrix (49). Therefore, the antiangiogenic effects of captopril on gene expression may result from alterations in peptide levels, reductions in blood pressure, or interactions with metalloproteinases.

We found a reduction in Flk-1 mRNA with captopril (Figs. 4 and 5). Our results are consistent with the work of Otani et al. (29), in which administration of ANG II to retinal microcapillary endothelial cells increased Flk-1 mRNA by increasing both the rate of Flk-1 transcription and mRNA half-life. This increase in Flk-1 mRNA was found to be inhibited by angiotensin II type 1 (AT_1) receptor antagonism (29). This is in agreement with previous reports that the angiogenic activity of ANG II

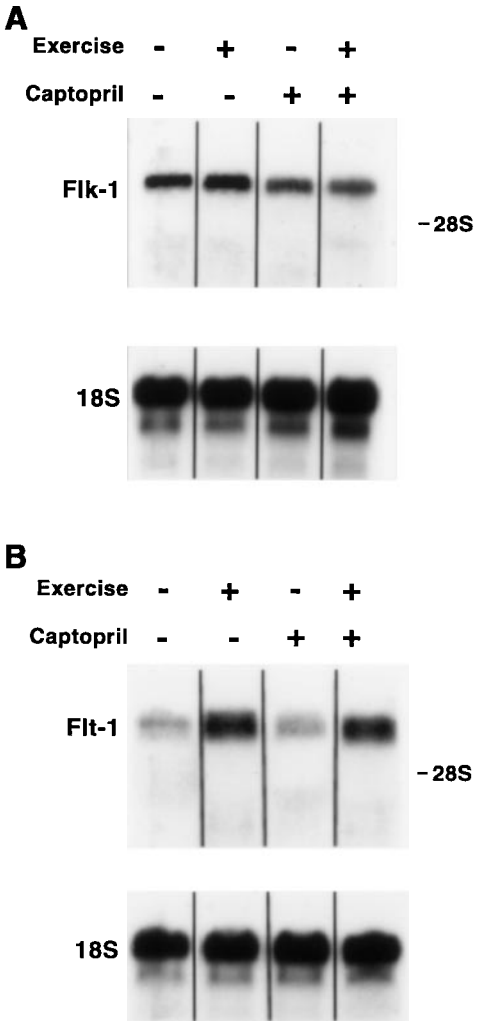

Fig. 4. Representative Northern blots showing mRNA signals for Flk-1 (*A*) and Flt-1 (*B*) in rats that had either exercised for 1 h at 20 m/min, 10° incline, or rested and had been injected with either saline or 100 mg/kg captopril. Lane loading was controlled for by 18S rRNA. Exercise increased Flt-1 mRNA. Captopril reduced Flk-1 mRNA independently of exercise.

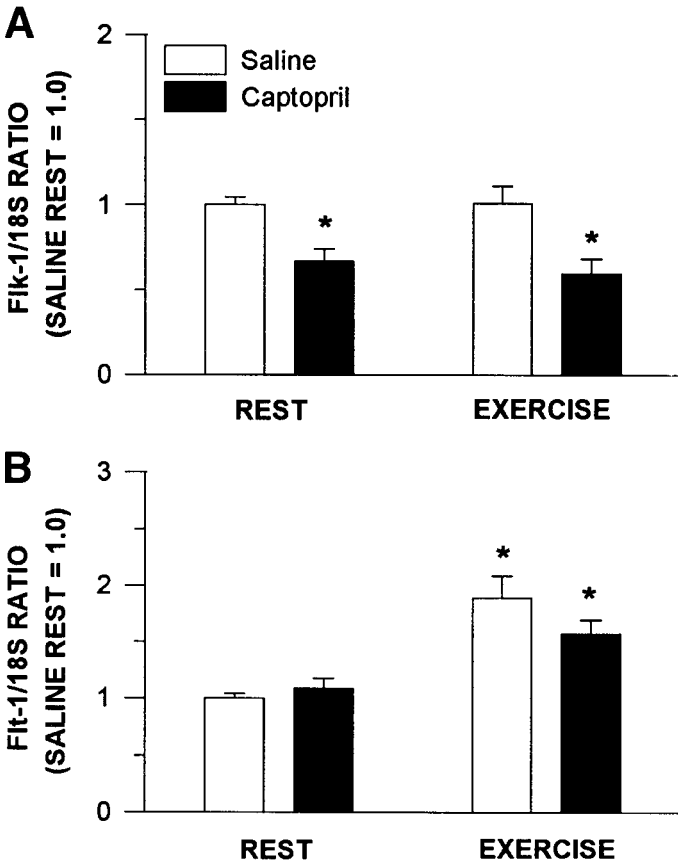

Fig. 5. Effect of 100 mg/kg captopril and exercise on Flk-1 and Flt-1 mRNA ~1 h after the completion of rest or exercise. Saline + rest data were normalized to 1.0. All other data were normalized to saline + rest to allow for comparisons. Quantitative densitometry of Northern blots for the ratio of Flk-1 mRNA to ribosomal 18S rRNA (*A*) or Flt-1 mRNA to ribosomal 18S rRNA (*B*) is shown. Exercise increased Flt-1 mRNA 1.7-fold. Captopril reduced Flk-1 mRNA 30–40% independently of exercise but did not affect Flt-1 mRNA. Error bars represent SE. *Significantly different from saline + rest ($P \leq 0.05$).

is promoted via the AT_1 receptor in skeletal muscle (27). The hypothesis that Flk-1 mRNA is regulated by ANG II is consistent with our findings that captopril reduces Flk-1 mRNA.

The exogenous administration of bradykinin induces neovascular growth in subcutaneous rat sponges (17). In postcapillary venules, bradykinin increases DNA synthesis and promotes the growth of endothelial cells (26). On the basis of these reports, it would be predicted that, if bradykinin promotes angiogenesis through Flk-1 regulation, captopril should increase Flk-1 mRNA. However, our results demonstrating that Flk-1 mRNA is reduced after captopril administration do not support this hypothesis.

In recent work, ACE inhibition by quinaprilat promoted angiogenesis in a hindlimb model of ischemia similar in magnitude to the administration of recombinant VEGF, whereas captopril-treated animals improved no better than controls (10). Fabre et al. (10) suggest that the differing outcomes for the ACE inhibitors quinaprilat and captopril may result from two factors: *1*) greater tissue ACE inhibition with quinaprilat or *2*) non-ACE-related activity of the sulfhydryl group on captopril. Captopril inhibits neovascularization in the rat cornea not by reduced ACE activity but apparently by inhibition of zinc-dependent metalloproteinase activity, which is required for endothelial cells to respond to angiogenic stimulus (47). The matrix metalloproteinases are thought to be important in angiogenesis, not as regulators of growth factors, but rather as enzymes active in the remodeling of the extracellular matrix that are regulated by growth factors such as VEGF (49). It is possible that matrix metalloproteinases could function in a negative feedback manner on VEGF via Flk-1 regulation. However, the VEGF regulation of matrix metalloproteinases expression appears to be regulated by Flt-1 and not Flk-1 (49). These results suggest that, although it is possible that captopril could interact with metalloproteinases in regulating Flk-1, given the short time between the administration of captopril and the removal of the muscles it does not appear probable.

The reduction we observed in Flk-1 mRNA may have been in response to the overall reduction in blood pressure. There is no direct evidence that changes in shear stress can regulate Flk-1 gene expression. In bovine aortic endothelial cells, increases in shear stress induce rapid and transient tyrosine phosphorylation of Flk-1 (6). In rats running at 20 m/min, 10° incline, AT_1 receptor antagonism results in nonsignificant increases in hindlimb blood flow and conductance that would be expected to increase shear stress (42). Increases in vasodilation and thus blood flow promote increases in capillarization in skeletal muscle (18); therefore, the potential increase in blood flow with captopril would be expected to increase Flk-1 mRNA not decrease it as we observed here.

VEGF receptors and angiogenesis. Captopril reduced Flk-1 but not Flt-1 gene expression, consistent with previous work demonstrating that ANG II increases Flk-1 but not Flt-1 mRNA (29). In addition, the early gene expression of Flt-1 but not Flk-1 in response to exercise suggests that distinct differences may exist in their respective roles. Homozygous mutations of either the Flk-1 or Flt-1 gene in mice result in embryonic lethality as a result of profound deficits in vasculogenesis. Flk-1 is essential for embryonic endothelial cell differentiation and vasculogenesis, whereas Flt-1 is crucial in the organization of the developing vasculature (12, 37). Both Flk-1 and Flt-1 gene expression can be increased by hypoxia, suggesting that both receptors may be crucial for angiogenesis (33, 43, 45). In addition, both receptors have been coupled to various intracellular signal transduction systems (11). Clearly, further investigations are needed to elucidate the function of each receptor in adult angiogenesis.

Captopril and exercise-induced increases in VEGF and TGF-β_1. The exercise-induced increase in plasma ANG II is both exercise intensity and hypoxia dependent (19, 24). Similarly, the exercise-induced increases in VEGF and TGF-β_1 mRNA are exercise intensity and hypoxia dependent (5). In rats, exercise at 20 m/min, 10° incline, activates RAS (42). This finding logically leads to the hypothesis that increases in skeletal

muscle VEGF and TGF-β_1 mRNA are a function of increases in ANG II. If this hypothesis were true, then captopril should reduce the exercise-induced increases in VEGF and TGF-β_1 gene expression by reducing circulating levels of ANG II. Contrary to this hypothesis, captopril did not affect the exercise-induced increases in VEGF or TGF-β_1 mRNA.

It has been suggested that skeletal muscle may produce ANG I and II de novo, thereby implying that skeletal muscle contains a complete RAS (9, 34). Despite significant systemic ACE inhibition in our model (Fig. 1), if local skeletal muscle RAS does exist, it could still be responsible for the exercise-induced increases in VEGF and TGF-β_1 mRNA (Figs. 2 and 3). Although a positive venoarterial concentration difference in ANG II and an elevation in this concentration difference with 2-Hz electrical stimulation has been shown in perfused canine gracilis (34), a more recent report from these authors concludes that skeletal muscle does not contain a complete RAS and that the net outflow of ANG II observed in their protocol is caused by local tissue conversion of ANG I artificially generated in their arterial catheter system (35). In addition, there are no reports demonstrating that skeletal muscle contains the necessary precursor mRNA for either renin or kallikrein, which would be required for local ANG II production. Therefore, the exercise-induced increases in VEGF and TGF-β_1 mRNA reported here are unlikely to have resulted from de novo ANG II produced in the skeletal muscle.

AT_1 receptor-mediated endocytosis of ANG II is an important mechanism by which the in vivo activity of RAS is regulated (46). Plasma membrane localization is thought to be essential for ANG II receptor function, whereas the internalization of the receptor is important for signal transduction (46). Recent evidence has shown that ANG II can be sequestered in tissues via AT_1 receptor-mediated internalization (46). Although this is true in heart, kidney, and adrenal tissue, receptor-mediated ANG II accumulation in skeletal muscle does not occur (46). In addition, the half-life of sequestered ANG II is 15 min (46). If skeletal muscle did sequester ANG II from circulating ANG II, the half-life of sequestered ANG II would severely limit its bioavailability during exercise in our protocol. Thus local sequestering and release of ANG II by skeletal muscle would not be expected to regulate the exercise-induced increases in VEGF and TGF-β_1 (Figs. 2 and 3).

On the basis of the work of Symons et al. (42), it is possible that captopril could increase hindlimb blood flow and thus shear stress. However, we found no difference in the exercise-induced increases in VEGF or TGF-β_1 mRNA between saline and captopril-treated rats. These results are consistent with those of Roca et al. (32), who demonstrated that passive hyperperfusion does not increase VEGF, TGF-β_1, or bFGF mRNA.

Exercise and angiogenesis. It is now well established that endurance exercise training increases skeletal muscle capillarization (see Refs. 1 and 18 for review). In this report, we have employed a systemic exercise model of treadmill running that produces significant increases in growth factor gene expression (Figs. 2 and 3 and Refs. 5 and 13). In rats, treadmill running of similar intensity and duration produces exercise-induced angiogenesis (14, 21, 41). Although dependent on the specific exercise protocol and skeletal muscle analyzed, treadmill running in rats at similar intensities and durations as those employed in this study produces 10–28% increases in capillarization (14, 21, 41). Although our exercise protocol represents only the initial bout of an exercise training program, it would be expected that training of animals at this intensity and duration would produce exercise-induced angiogenesis.

Captopril, ACE inhibition, and blood pressure. Captopril was the first orally active ACE inhibitor designed for the treatment of hypertension. In this study, acute administration of captopril produced significant hypotension in initially normotensive rats (Fig. 1*A*). This is in agreement with previous reports on administering captopril acutely in normotensive humans and rats (2, 38, 39). In rats, low doses of acute administration of captopril produce significant hypotension, whereas in humans single doses of 25 and 50 mg of captopril produce small but significant reductions in MAP (2, 38, 39). In addition to the hypotensive effect, acute captopril administration lowers plasma ACE activity and plasma levels of ANG II (2, 38, 39). Our findings of a significant reduction in MAP (Fig. 1*A*) and a near ablation of the response to ANG I in captopril-treated rats (Fig. 1*B*) demonstrate that effective ACE inhibition had occurred in our protocol.

Clinical implications. It is estimated that over 50 million Americans have arterial pressures that would classify them as hypertensive, with over 90% of these being classified as primary or essential hypertension (44). In 80–90% of the essential hypertension cases, the responsible mechanisms are unknown (44). In recent years, regular exercise has been advocated as an effective tool in the nonpharmacological treatment of hypertension and as an adjunct to the pharmacological treatment of hypertension (44). Exercise training is associated with a 5 to 25-mmHg reduction in systolic blood pressure and a 3 to 15-mmHg decline in diastolic blood pressure when the training intensity is between 40 and 70% of maximal O_2 consumption (44). The exercise intensity utilized in this study is within this range and would be expected to produce a significant reduction in the resting blood pressures of hypertensive animals.

It has been hypothesized that a diminished growth of the microvascular bed is an early and important pathogenic mechanism in essential hypertension (40). Structural abnormalities in hypertensive patients include reductions in vessel density, known as rarefaction, which occur predominantly in the smallest vessels (15, 40). Henrich et al. (15) demonstrated a 37% rarefaction in quadriceps muscle capillaries and a 51% rarefaction in the pectoralis major muscle of hypertensive patients (15). Recent evidence further suggests that young adults with only a predisposition to high blood pressure demonstrate impaired microvascular dilation and capillary rarefaction (28). Volpert et al. (47) raise the possibility that captopril may provide hidden benefits

via its antiangiogenic activity, decreasing the incidence and severity of a variety of angiogenesis-dependent diseases including neoplasia. If capillary rarefaction is an important pathogenic mechanism in hypertension, the use of captopril for the treatment of hypertension may promote further capillary rarefaction. However, it is difficult to argue that capillary rarefaction would improve exercise tolerance, and in fact the opposite effect would be anticipated.

In summary, we have demonstrated that captopril, in doses sufficient to reduce systemic blood pressure by 40 mmHg, does not alter the expression of VEGF, TGF-β_1, bFGF, or Flt-1 mRNA at rest or in response to exercise. Captopril does, however, reduce Flk-1 mRNA by ~30–40% independently of exercise. This is partially consistent with previous reports that captopril reduces the skeletal muscle vasculature at rest and in response to exercise and with the hypothesis that ANG II can regulate Flk-1 mRNA.

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