Effects of Therapy on Maximal Walking Time Following Femoral Ligation in the Rat

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SUMMARY Maximal walking times at a constant rate of 10 m/min were compared in normal rats, normal rats that had been exercised daily for 6 weeks, and rats that had undergone bilateral femoral artery ligation followed by 6 weeks of either an oral vasodilator, absence of sympathetic impulses to the extremities, cold exposure, or daily exercise. Rats maintained on the vasodilator could walk no farther than untreated, ligated rats. Both chronic cold exposure and sympathectomy significantly increased maximal walking distances of ligated rats and daily exercise increased maximal walking distances 7-fold. Rats that had received 6 weeks of exercise training following femoral artery ligation could walk longer than normal untrained rats; therefore, the increased exercise tolerance could not be explained solely by the restoration of normal large artery conductance by enlargement of collaterals bypassing the femoral occlusion.

IN PATIENTS with diffuse arterial occlusive disease, surgical repair may not be practical, and available therapeutic measures are considered of questionable value. In all but the most severe stages of the disease, when skin blood flow falls below the level adequate to fulfill nutritional requirements, the principal objective of therapy is to increase the circulatory reserve and, thereby, the ability of the limb circulation to supply the metabolic requirements of exercise. Anatomic compensation for the occlusion could involve an increase in size and/or number of resistance vessels (lowering net resistance) or enlargement of interconnections between occluded and nonoccluded arterial systems (lowering large artery resistance). Beneficial effects of chronic therapy cannot be quantified accurately in patients because of inability to control experimental variables such as progression of the obstructive process and environment and activity during the period of a chronic study. Also, in order to judge the effects of therapy, it is necessary to compare the hemodynamic alterations in the experimental subjects to changes in patients with the same lesion but not receiving therapy, since collateral growth and increases in walking ability occur without therapeutic intervention.

In the last 100 years, many investigators have sought to identify the stimuli responsible for the growth of collateral vessels (arterial interconnections between occluded and unoccluded arterial systems). Four main theories have been proposed, namely, an increase in pressure gradient around the block,1-3 an increase in blood flow around the block,4-7 a release of vasoconstrictor tone in collateral vessels,8-11 and accumulation of metabolites in tissues distal to the block.12 Studies in this laboratory have failed to reveal a correlation between flow velocity via collateral vessels or pressure drop across the arterial collateral bed and the rate of growth of arterial collaterals in experimental animals.13 If these hemodynamic factors act as stimuli for collateral development, then any factor that consistently or intermittently increases extremity flow should increase velocity of flow through and the pressure drop across the collateral bed, thereby stimulating collateral development. Treatment with vasodilators or sympathectomy should provide a small continuous increase in collateral flow and regular exercise should provide a maximal intermittent increase in collateral flow.

In order to quantify the effects of factors that chronically alter extremity flow or the ability to exercise, rats were studied following a standard arterial occlusion and maintenance for 6 weeks under controlled conditions of environment, exercise opportunity, and diet. The effect of a vasodilator, a cold environment, lumbar sympathectomy, and regular exercise (to the point of inability to walk) on the maximal ability to exercise following femoral artery ligation were evaluated in these rats.

Methods
Seventy Sprague-Dawley rats with an initial body weight of approximately 300-350 g were utilized for the study. Twenty rats were maintained double-caged in the animal quarters for 6 weeks as normal controls and were divided into the following groups: group 1, maintained for 6 weeks without intervention; and group 2, maintained for 6 weeks and exercised daily 5 days/week for 0.5 hour/day.

The control, exercised rats (group 2) were allowed to walk 0.5 hour per day, 5 days per week, on a specially constructed series of motor-driven exercise wheels. The speed of rotation was set so that the rats walked at a constant rate of 10 m/minute.

Fifty rats were subjected to bilateral femoral artery ligation and were maintained for 6 weeks to provide time for collateral development. The ligated rats were divided into the following experimental groups: group 3, no further intervention; group 4, given isoxsuprine hydrochloride orally; group 5, maintained at an environmental temperature of 14°C; group 6, subjected to bilateral lumbar sympathectomy at the time of femoral ligation;
and group 7, exercised to the point of claudication 5 days per week.

The experimental rats were anesthetized with ether and the femoral arteries were exposed bilaterally, isolated, and double-ligated with silk sutures just proximal to the origin of the pelvic artery. In 10 rats (group 6), the lumbar sympathetic chain was exposed through a midline abdominal incision. The chain was cut at the level of the renal arteries and the distal sympathetic chain was stripped bilaterally. The incisions were closed and the rats were returned to quarters. All except those rats in the cold exposure group (group 5) were maintained at a comfortable environmental temperature, with two housed in a cage, and were fed Purina Laboratory Chow and water ad libitum. Ten of the rats (group 4) received oral isoxsuprine hydrochloride, 0.2 mg/day (Vasodilan; Mead-Johnson) in their drinking water. Cold-exposed rats (group 5) were fed the same diet but were kept single-caged to prevent huddling in 5 × 10-inch cages in a ventilated refrigerator maintained at 14°C.

The ligated, exercised rats were allowed to walk at 10 m/min, 5 days/week to the point of inability to walk. The end point selected was the time at which the rats refused to walk and slid or rolled as the wheel turned. This point always was preceded by a period in which the rat intermittently rode backwards with the wheel and then leaped forward to catch up, i.e., a short period of rest followed by several jumps.

At the end of the 6-week period, maximal exercise ability was measured in all animals as the number of minutes the rat could walk on the exercise wheel driven at 10 m/min. Again, the end point chosen was the time at which the rat refused to walk, which always was preceded by short periods of intermittent resting and jumping.

**Results**

Following bilateral femoral artery ligation and 3 days of recovery, maximal walking time was reduced by 59% from the normal control, preligation value (Fig. 1). Maintenance for 6 weeks in a 7 × 10-inch cage which provided little opportunity for exercise (ligated control) increased exercise capacity over that 3 days postligation by only 28%, but the change was significant. Treatment for 6 weeks following ligation with an oral vasodilator did not increase walking distance above that of ligated control rats. However, rats that were maintained in a 14°C environment for 6 weeks following bilateral femoral ligation increased their walking distance by 36% above that of the untreated, ligated control rats. Although these rats were housed single-caged to prevent huddling for warmth, the opportunity for exercise was not increased.

Exercise tolerance was enhanced even further by lower body sympathectomy performed at the time of femoral artery ligation (Fig. 1). The average walking time was increased by almost 70% of the exercise time of ligated control rats. The exercise tolerance of the ligated sympathectomized rats that had been maintained for 6 weeks for collateral growth was not significantly different from that of the nonligated, normal control rats, although the walking time averaged somewhat less.

Regular exercise to the point of refusal to run resulted in a marked increase in the tolerance to exercise (Fig. 1). Repeated exercise increased maximal walking time in the chronically ligated rats approximately 7-fold, as compared to ligated control rats, and maximal walking time was almost 3 times that of the normal control rats. The increase in walking ability during the 6 weeks of exercise training was most rapid during the first 2 weeks following ligation (Fig. 2), but continued to increase in a somewhat linear fashion throughout the next 4 weeks. There was no...
indication that the increase in exercise tolerance had attained a plateau at the end of the 6-week period of training.

Since exercise produced alterations in walking tolerance over and above that explained by reduction in large vessel resistance to that of normal untrained rats, a group of normal rats was exercised daily for 6 weeks and the change in exercise tolerance measured. Normal rats also increased their maximal walking distance nearly 7-fold in response to sympathectomy. Longland found a 59% increase in the number of interar-terial collaterals above 0.4 mm in diameter in response to sympathectomy at 6 months but no differences from nonsympathectomized beds at 1 year. He attributed the increase in collateral growth to a direct response to the denervation rather than to an indirect response to the persistent functional decrease in peripheral resistance, and the present study supports this conclusion. Although both sympathectomy and treatment with oral vasodilators would result in a continuous vasodilation, only sympathectomy increased exercise tolerance.

It was anticipated that cold exposure would have a detrimental effect on exercise tolerance by introducing a chronic increase in arteriolar resistance and, thereby, a chronic reduction in collateral velocity and pressure drop. However, cold exposure resulted in a small but significant increase in exercise tolerance, which suggested that an increase in metabolic rate or shivering induced by the cold may have had an overriding effect on the circulation. Exercise training was by far the most potent stimulus to increased exercise tolerance, and the stimulus appeared to act on factors other than restoration of normal large vessel conductance. In view of the significant clinical results that have been obtained with exercise training, it was not surprising that walking time was increased significantly in rats that had regularly been exercised maximally. It was the magnitude of the response that was unexpected. The rats that had been exercised for 6 weeks following femoral artery ligation could walk almost 3 times longer than rats with normal vessels which had not been allowed to exercise. The exercise training, therefore, had a beneficial effect on walking time over and above that of restoration of normal large vessel conductance.

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Effects of Potassium on Isolated Canine Coronary Arteries
Modulation of Adrenergic Responsiveness and Release of Norepinephrine

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SUMMARY We studied effects of changes in the extracellular potassium concentration ([K⁺]o) on the mechanical responsiveness of canine coronary artery preparations. Four different contractile behaviors were delineated between [K⁺]o = 0 and 40 mM: (1) at [K⁺]o < 1 mM, a contracture developed which was augmented by β-adrenergic stimulation and inhibited by propranolol, 1 × 10⁻⁶ M; (2) at [K⁺]o between 1 and 2.5 mM, arterial tone was minimum and propranolol acted as a potent constrictor. Within this range of [K⁺]o, arteries exhibited calcium-induced relaxations and verapamil-induced contractions; and (3) at [K⁺]o between 5 and 15 mM there was a steep rise in force which was increased by isoproterenol (β-adrenergic contraction), almost completely blocked by β-propranolol, but not attenuated by α-adrenergic blockade with phentolamine; and (4) at [K⁺]o > 30 mM, effects of β-adrenergic stimulation and blockade became very small. Steady increases in force elicited by isoproterenol and by sudden increases in [K⁺]o, were preceded by transient relaxations. Effects of exogenous and endogenous (tyramine, nerve stimulation) norepinephrine paralleled those of isoproterenol and were blocked by propranolol but not attenuated by phentolamine. In contrast, modulation of the effects of phenylephrine by potassium consisted of monotonically increasing constrictor responses over the whole range of [K⁺]o tested. Arteries labeled with ³H-­norepinephrine showed substantial changes in (³H)-efferent with relatively small changes in [K⁺]o. Maximum releases were observed with [K⁺]o ranging between 10 and 25 mM. The smallest releases were observed at the highest [K⁺]o (40 mM). Thus, changes in [K⁺]o influence arterial tone by modulating α- and β-adrenergic effects and by regulating the release of neurotransmitter from the coronary nerves.

IT IS generally accepted that acute myocardial ischemia and hypoxia result in acute coronary vasodilation. However, effects of prolonged ischemia on coronary vascular tone have been less well defined. In conscious dogs subjected to acute coronary ligation, collateral flow may increase substantially after a few hours of ischemia, suggesting that dynamic factors may limit effective collateral perfusion temporarily. Among the vasoactive substances that are released from the ischemic myocardium and may potentially increase arterial tone are potassium and norepinephrine. In the present study, we examined the effects of potassium and adrenergic agents on the tone of isolated coronary arteries. Results demonstrate that small changes in the extracellular potassium concentration influence arterial tone by two interacting mechanisms: modulation of the responsiveness of the artery to adrenergic stimuli and regulation of the release of norepinephrine from the vascular nerves.

Methods

ANIMALS

Coronary arteries were obtained from mongrel dogs weighing between 16 and 22 kg. Dogs were anesthetized with pentobarbital (25-30 mg/kg, iv) and the beating heart was rapidly removed from the chest. In some experiments dogs were chemically sympathicotomized with 6-hydroxydopamine by the method of Gauthier et al. and studied 4-5 days after treatment. Effectiveness of the
Effects of therapy on maximal walking time following femoral ligation in the rat.

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