Nitroglycerin dilates coronary collateral vessels during exercise after blockade of endogenous NO production

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Klassen, Christopher L., Jay H. Traverse, and Robert J. Bache. Nitroglycerin dilates coronary collateral vessels during exercise after blockade of endogenous NO production. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H918–H923, 1999.—In a previous study nitroglycerin failed to dilate coronary collateral vessels during exercise. This study tested the hypothesis that failure of nitroglycerin to increase collateral flow occurred because endogenous nitric oxide (NO) production. Few collateral vessels could have had no additional effect. Six dogs were collateralized using intermittent 2-min occlusions of the left anterior descending coronary artery followed by permanent occlusion. One week after permanent coronary occlusion, dogs were exercised on a treadmill (heart rate 202 ± 5 beats/min), while blood flow was measured with radioselective microspheres. Blood flow to the collateral zone during control exercise was 1.90 ± 0.11 ml·min⁻¹·g⁻¹ compared with 2.28 ± 0.15 ml·min⁻¹·g⁻¹ in the normal zone (P < 0.05); systolic wall thickening was 23 ± 3% in the collateral zone compared with 27 ± 2% in the normal zone. When NG-nitro-L-arginine (L-NNA; 20 mg/kg iv) was administered to block NO production, collateral zone flow during exercise decreased to 1.43 ± 0.20 ml·min⁻¹·g⁻¹ (P < 0.05), and systolic wall thickening decreased to 12 ± 4% (P < 0.05). A subsequent infusion of nitroglycerin (2 µg·kg⁻¹·min⁻¹ iv) increased collateral zone blood flow to 1.65 ± 0.16 ml·min⁻¹·g⁻¹ (P < 0.05) and increased systolic wall thickening to 22 ± 5% (P < 0.05). These findings demonstrate that endogenous NO contributes to collateral zone blood flow during exercise. If endogenous NO synthesis is blocked, then nitroglycerin is effective in improving collateral zone blood flow and contractile function during exercise.

angiogenesis; blood flow; collateral circulation; endothelium-derived factors

GRADUAL CORONARY ARTERY occlusion stimulates growth of collateral vessels that provide alternate pathways for blood flow into the dependent myocardium. These vessels can protect the myocardium from infarction, but the balance between blood flow and myocardial demand can be precarious in the collateral-dependent zone. Thus collateral vessels that conduct sufficient blood flow to meet myocardial demands during resting conditions can become flow limiting during exercise (4, 8, 18). These vessels possess a functionally competent muscular media, so that they are capable of active vasomotion that can affect blood flow to the dependent myocardium (17). Nitroglycerin causes dilation of collateral vessels in open-chest dogs (1, 7, 17), but in a previous study nitroglycerin failed to increase collateral zone blood flow in chronically instrumented awake dogs (23). In that study, collateral zone blood flow measured with radioactive microspheres during treadmill exercise was significantly less than normal zone flow. Despite this hypoperfusion, nitroglycerin did not increase blood flow to the collateral-dependent myocardium. The failure of nitroglycerin to improve collateral blood flow in that study could be explained by the endogenous nitric oxide (NO) production. Endogenous NO and NO derived from nitroglycerin both act through guanylate cyclase. If the guanylate cyclase pathway for vasodilation is activated by endogenous NO, then nitroglycerin might have no additional effect. This possibility is suggested by reports demonstrating that coronary collateral vessels do possess endothelium-dependent, NO-mediated vasodilator mechanisms (9, 27).

This study was performed to test the hypothesis that failure of nitroglycerin to increase collateral zone blood flow during exercise is the result of endogenous NO production. In this study, the effect of nitroglycerin on coronary collateral flow was examined after the endogenous NO system had been blocked with NG-nitro-L-arginine (L-NNA). This sequence of interventions was used to model the clinical situation in which endothelial dysfunction and impaired vascular NO production might alter the response to nitroglycerin.

METHODS

All experiments were approved by the Animal Care Committee of the University of Minnesota and conducted according to the position of the American Heart Association on research animal use.

Surgical instrumentation. Six adult mongrel dogs weighing 25–30 kg were trained to run on a motorized treadmill. The dogs were premedicated with acepromazine (10 mg im) and anesthetized with pentobarbital sodium (30–35 µg/kg iv). They were intubated and ventilated with a respirator (Harvard Apparatus, South Natick, MA) using room air supplemented with oxygen. Using sterile technique, we performed a left thoracotomy through the fifth intercostal space. A heparin-filled polyvinyl chloride catheter (3.0 mm ID) was inserted into the left internal thoracic artery and advanced into the ascending aorta. The heart was then suspended in a pericardial cradle. A fluid-filled catheter was inserted into the left atrium through the atrial appendage. A fluid-filled catheter and solid-state pressure transducer (Konigsberg Instruments) were inserted into the left ventricle at the region of the apical dimple. The proximal left anterior descending coronary artery (LAD) was dissected free. A 5-MHz Doppler velocity probe (Craig Hartley, Houston, TX) was secured around the artery followed by a snare occluder, and a hydraulic occluder was placed immediately distal to the Doppler probe. Finally, distal to the occluders, a silicone catheter (0.3 mm ID) was inserted into the LAD by the method of Gwirtz (14). Ultra-
sonic crystals (Craig Hartley) were sutured to the epicardial surface of the heart in the distribution of the left circumflex and LAD arteries for measurement of systolic wall thickening using the Doppler displacement technique. The pericardium was loosely closed, and the catheters were tunneled subcutaneously to exit at the base of the neck. The thoracotomy incision was then closed in layers. Catheters were flushed daily with heparinized saline and protected with a nylon vest.

Induction of collateral vessel growth. Collateral vessel development was produced using a modification of the Franklin intermittent coronary artery occlusion protocol (10). Beginning 5–7 days postoperatively, we returned the dogs to the laboratory and 2-min coronary occlusions were performed every 15 min for 3 h, 5 days each week. Occlusions were produced by injecting saline into the hydraulic occluder and verified by observing the flow signal from the coronary Doppler probe. The degree of collateral development was assessed by monitoring distal coronary artery pressure during occlusion. During the initial occlusions, distal coronary pressure was 10–15 mmHg. As collateral development occurred, distal pressure during occlusion gradually increased and the postocclusion reactive hyperemia decreased. When the LAD distal pressure during occlusion reached 40 mmHg or when the reactive hyperemia was nearly abolished, the artery was permanently occluded with the snare occluder. We have previously observed that this degree of collateralization is sufficient to allow permanent coronary occlusion without producing infarct (27). This occurred 20 ± 6 days after beginning the occlusion protocol. Animals were returned to the laboratory for study 8 ± 1 days after permanent occlusion.

Experimental protocol. Data were obtained with the dogs standing on a motor-driven treadmill (Quinton model 18-72). Aortic and coronary pressures were measured with fluid-filled pressure transducers (Spectramed model TNF-R, Oxnard, CA) maintained at midchest level and recorded on an eight-channel recorder (Coulbourn Instruments, Lehigh Valley, PA) or recorded in digital form on magnetic disk using CODAS data acquisition software (Dataq Instruments) at a rate of 250 Hz. Myocardial systolic wall thickening was measured using a Doppler displacement system (Craig Hartley). Studies were performed during exercise on the treadmill at 6.4 km/h with a 10% grade. Hemodynamic measurements were recorded and microspheres injected when steady-state hemodynamic conditions had been present for at least 2 min. Myocardial blood flow was measured with 15-μm diameter microspheres labeled with 141Ce, 51Cr, 85Sr, 95Nb, or 46Sc (NEN, Boston, MA). For each measurement, 2 × 106 microspheres were injected into the left atrium and flushed in with 10 ml of isotonic saline. Beginning at the time of microsphere injection and continuing for 90 s, a reference blood sample was withdrawn from the aortic catheter at a rate of 15 ml/min using a peristaltic pump. After completion of the control exercise, the dogs were allowed to rest for 1 h. L-NNA was then administered in a dose of 20 mg/kg iv over 30 min to block endogenous NO production. In a separate group of dogs without coronary occlusion, this dose of L-NNA caused 62 ± 4% inhibition of the endothelium-dependent vasodilation elicited by acetylcholine (15 μg/min infused into the LAD). Thirty minutes after we completed the L-NNA infusion, exercise of the dogs was begun at the identical speed and grade as during control exercise. After steady-state exercise conditions had been present for 2 min, hemodynamic measurements were recorded and microspheres injected. The dogs were again allowed to rest for 1 h. An infusion of nitroglycerin was then begun at a rate of 2 μg·kg⁻¹·min⁻¹ iv. Ten minutes after the nitroglycerin infusion was begun, the treadmill was restarted and all measurements were repeated as described above.

Shadow technique. After the exercise protocol was completed, the shadow technique of Patterson and Kirk (21) was used to identify the collateral perfused myocardium. The animals were premedicated with morphine sulfate (2 mg/kg im), anesthetized with α-chloralose (100 mg/kg iv followed by 10 mg·kg⁻¹·h⁻¹), intubated, and then ventilated with a respirator. The thoracotomy was reopened and the LAD dissected free. A 4-mm diameter thin-wall stainless steel cannula was inserted into the LAD immediately distal to the snare occluder. A 26-gauge tube incorporated into the wall of the cannula measured pressure at the cannula tip, whereas the larger 4-mm lumen was used for blood infusion. Radioactive microspheres were injected into the left atrium, whereas nonradioactive arterial blood was perfused through the cannula from a pressurized reservoir. Cannula tip pressure was maintained 10–15 mmHg above aortic pressure so that the LAD-perfused myocardium received nonradioactive blood, while the normal myocardium was marked with microsphere containing blood. Immediately after this procedure, the animals were euthanized with an overdose of pentobarbital, and the heart from each dog was fixed in 10% buffered Formalin.

Myocardial tissue processing. After the aorta and right ventricle were removed, the left atrioventricular valve was sectioned into five transverse rings from base to apex. The central three rings were sectioned into 16 radial specimens, and the apex was sectioned into 4 radial specimens. Each specimen was then further subdivided into subendocardial and subepicardial samples. Tissue samples were weighed and placed in glass vials. Myocardial and blood reference samples were counted in a gamma counter (model 5912, Packard Instrument, Downers Grove, IL) with window settings corresponding to the peak emissions of each radionuclide. Counts were corrected for background and overlap between isotopes, and myocardial blood flow was calculated using the formula $Q_m = (Q_b \times C_m/C_b)$, where $Q_b$ is the blood withdrawal rate (15 ml/min), $C_m$ is the tissue sample counts, and $C_b$ is the blood sample counts. Collateral-dependent specimens were identified using the shadow radionuclide as those having blood flow more than two standard deviations below the mean blood flow to the noncollaterally perfused zone. For all animals the shadow procedure was technically impossible because scarring around the LAD prevented cannulation of the vessel; in these cases the collateral region was identified anatomically as the anterior portion of myocardium in the distribution of the LAD. Mean collateral zone blood flow was calculated as the average flow rate of all collateral-dependent myocardial specimens.

Data analysis. Heart rate, aortic pressure, left ventricular pressure, and the first derivative of left ventricular pressure (dp/dt) were measured directly from the strip charts or from digitized data. Myocardial function expressed as the percent systolic wall thickening was calculated as the difference in wall thickness from end diastole to end systole divided by the depth of the measurement. End-systolic thickness was measured 20 ms before peak negative dp/dt, and end-diastolic thickness was measured immediately preceding the upstroke of positive dp/dt. Maximum systolic excursion (SE) in two myocardial layers [subendocardium (Endo) and subepicardium (Epi)] were recorded at depths (D) of ~10 and 5 mm, respectively. Percent systolic wall thickening (%WT) in each layer was calculated using the following equations: transmural %WT = $SE_{Endo}/D_{Endo} \times 100$; subendocardial %WT = $(SE_{Endo} - SE_{Epi})/(D_{Endo} - D_{Epi}) \times 100$, and subepicardial %WT = $SE_{Epi}/D_{Epi} \times 100$. Total collateral zone vascular resistance was calculated as aortic pressure minus left ven-
tricular end-diastolic pressure divided by mean collateral zone flow. Data are expressed as means ± SE. Statistical analysis was performed using analysis of variance for repeated measures with P < 0.05 regarded as significant. When a statistically significant result between interventions was found, individual comparisons were made using the Wilcoxon signed rank test. Comparisons between normal and collateral zones were performed using the independent t-test (two-tailed) with a Bonferroni correction.

RESULTS

Hemodynamics. Hemodynamic data are shown in Table 1. During control exercise mean heart rate was 202 ± 5 beats/min. Heart rate was not affected by L-NNA or by nitroglycerin in the presence of L-NNA. L-NNA caused an 18 ± 4% increase in mean aortic pressure during exercise (P < 0.05). After L-NNA, nitroglycerin infusion significantly decreased aortic pressure, although aortic pressure remained significantly higher than during control exercise (P < 0.05). Left ventricular end-diastolic pressure was 13 ± 2 mmHg during control exercise and did not change significantly in response to any of the interventions. The rate-pressure product tended to increase after L-NNA, although this was not statistically significant relative to control exercise. Left ventricular dP/dt during control exercise was 3,810 ± 460 mmHg/s and did not change significantly with L-NNA or nitroglycerin.

Myocardial blood flow. Myocardial blood flow is shown in Table 2. Collateral zone blood flow was 16 ± 2% less than normal zone blood flow during control exercise in the subendocardium and 16 ± 5% less than normal zone flow in the subepicardium (each P < 0.05). After L-NNA, mean collateral zone blood flow decreased 26 ± 7%, with a 36 ± 11% decrease in subendocardial flow and a 16 ± 4% decrease in subepicardial flow (each P < 0.05). The collateral zone subendocardial-to-subepicardial (Endo/Epi) blood flow ratio decreased from 1.09 ± 0.05 during control exercise to 0.81 ± 0.15 after L-NNA (P < 0.05) (Fig. 1). Blood flow in the normal zone also decreased with L-NNA by 8 ± 3% (P < 0.05) with no change in Endo/Epi. After L-NNA, nitroglycerin increased mean blood flow to the collateral zone by 22 ± 15%, with a 68 ± 47% (P < 0.05) increase in subendocardial flow and a 5 ± 7% (not significant) increase in the subepicardial flow. The Endo/Epi blood flow ratio increased from 0.81 ± 0.15 with L-NNA to 1.20 ± 0.10 after the addition of nitroglycerin (P < 0.05). After nitroglycerin, blood flow in the collateral zone was not significantly different from values obtained during control exercise. Nitroglycerin had no significant effect on blood flow in the normal zone.

Vascular resistance data are shown in Table 3. L-NNA increased total collateral zone resistance by 74 ± 3% and normal zone resistance by 28 ± 6% (each P < 0.05). After L-NNA, nitroglycerin decreased total collateral zone vascular resistance by 28 ± 11% (P < 0.05), although resistance remained elevated relative to control exercise, in part because of the increased aortic pressure following L-NNA. Measurement of pressure in the collateral-dependent artery allows separate calculation of transcollateral resistance and small vessel resistance in the collateral zone. Unfortunately, at the time of the study reliable coronary pressures could be obtained from only two of the animals. In those two animals transcollateral resistance increased from 25.8 ± 6% (each P < 0.05). The subsequent addition of nitroglycerin reduced transcollateral resistance to the pre-L-NNA level (24.2 ± 6% (each P < 0.05). L-NNA also caused an increase in small vessel resistance in the collateral zone from 28.5 ± 7% (each P < 0.05) and nitroglycerin caused little change in small vessel resistance (40.6 ± 6% (each P < 0.05). Although statistical testing could not be done because data were available from only two animals, the findings indicate that after
blockade of endogenous NO synthesis, nitroglycerin exerted its vasodilator effect principally at the level of the collateral vessels.

Myocardial function. Systolic wall thickening measurements are shown in Table 4. During control exercise, systolic thickening for the full wall tended to be less in the collateral zone (23 ± 3%) than in the normal zone (27 ± 2%), but this difference was not significant. In the collateral zone L-NNA caused a decrease in systolic wall thickening during exercise for the full wall from 23 ± 3% to 12 ± 4% with significant decreases in both the subendocardial and the subepicardial layers (each P < 0.05). In the normal zone, systolic thickening for the full wall decreased to 21 ± 2% after L-NNA (P < 0.05). Nitroglycerin improved collateral zone systolic wall thickening for the full wall to 22 ± 5% (P < 0.05), with significant increases in thickening in both the subendocardium and the subepicardium (each P < 0.05). Normal zone thickening did not change significantly in response to nitroglycerin.

Discussion

This study resolves previous conflicting reports that nitroglycerin increased coronary collateral blood flow in open-chest animals (1, 7, 17) but did not increase blood flow to collateral-dependent myocardium in awake dogs during exercise (23). The present findings demonstrate that endogenous NO normally contributes to maintenance of coronary collateral blood flow during exercise. When endogenous NO production was blocked, nitroglycerin then improved blood flow and contractile function in the collateral-dependent myocardium. The results indicate that the effect of nitroglycerin is dependent on the activity of the endogenous NO system.

Endothelial NO production. Increased endothelial shear resulting from the increase in coronary blood flow during exercise would be expected to activate endothelial NO synthase (eNOS) to generate NO. Schwartz et al. (24) found that treadmill exercise caused a significant increase in left circumflex coronary artery diameter in dogs, which was abolished when the normal increase in coronary flow during exercise was prevented by partial inflation of a hydraulic occluder. Wang et al. (28) showed that this exercise-induced coronary artery dilation was reversed to constriction by L-NNA, indicating that the flow-mediated dilation during exercise depends on NO. In addition, cyclic compression of the intramyocardial vessels is implicated in stimulating NO production by in vitro studies in which cyclic strain increased guanylate cyclase activity in coronary microvessels (19). In cultured endothelial cells, pulsatile flow increased NO production (20). These data indicate that the increased blood flow and pulsatility during exercise provide signals for increased endothelial NO production.

The present study confirms previous reports demonstrating the importance of endogenous NO in maintaining blood flow to collateral-dependent myocardial regions. Frank et al. (9) used gradual followed by total permanent occlusion of the left circumflex coronary artery to create a collateral-dependent zone in dogs. With the dogs standing quietly in a sling, they found that L-NNA caused a 17% decrease in blood flow in the left circumflex region, indicating that NO contributed to maintenance of blood flow in the collateral zone during resting conditions. Similarly, Traverse et al. (27) found that L-NNA caused a 20% decrease in collateral zone blood flow during treadmill exercise in chronically instrumented dogs. In that study L-NNA caused a marked increase in collateral resistance from 19.9 ± 2.2 to 42.4 ± 7.3 mmHg·ml⁻¹·min⁻¹·g as well as a 30% increase in small vessel resistance in the collateral zone. Although distal coronary pressure could be mea-
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In the present study, a similar response to L-NNA was observed with increases of both transcollateral resistance and small vessel resistance. The subsequent addition of nitroglycerin caused a decrease of transcollateral resistance, with little change in small vessel resistance. Although these observations were made in only two animals, they suggest that the principal site of action of nitroglycerin after blockade of endogenous NO synthesis is at the collateral vessels.

Previous studies have failed to show an effect of endogenous NO in maintaining myocardial blood flow in normal dogs. Thus Düncker and Bache (6) and Altman et al. (2) found that blockade of NO synthesis with L-NNA did not impair the increase in blood flow that occurred in response to treadmill exercise in normal dogs. Because exercise has been shown to cause increased coronary NO production (3), failure of L-NNA to decrease myocardial blood flow suggests that in the normal heart other vasodilator mechanisms can compensate for decreased NO production. In contrast to the normal heart, in the present study L-NNA caused a decrease in blood flow in the remote normally perfused myocardial region. This finding suggests that the presence of a coronary occlusion or a collateral-dependent myocardial region affects the control of vascular resistance in the normally perfused region so that NO assumes a more important role in determining blood flow.

Effect of nitroglycerin. Nitroglycerin dilates coronary vessels as it is transformed to NO at the vascular smooth muscle cell membrane through an enzymatic process that requires glutathione as a cofactor. Glutathione is the principal source of cellular thiols (sulfhydryl groups) that serve as reducing agents for nitroglycerin (16). Nitroglycerin has a gradient of vasodilator activity in the coronary arterial vasculature with greater potency in large than in small vessels. Using an in vitro video-imaging apparatus to study isolated preconstricted porcine coronary vessels, Selke et al. (25) reported that large arterial microvessels (191–300 µm in diameter) relaxed by 90% of the preconstricted diameter in response to nitroglycerin, whereas smaller microvessels (60–100 µm diameter) relaxed by only 20%. Harrison and Bates (16) proposed that oxidative stress causes depletion of thiols in microvessels <100 µm in diameter that may account for the diminished response of these vessels. Interestingly, several investigators have reported that vessels perfused by collaterals acquire greater sensitivity to nitroglycerin. Selke et al. (25) reported that isolated collateral perfused canine arterial microvessels (100–220 µm in diameter) showed enhanced relaxation to nitroglycerin relative to control coronary microvessels. Similarly, in patients with occlusive coronary artery disease, Fujita et al. (12) found enhanced vasodilator responses to nitroglycerin in collateral perfused arteries. The mechanism for this increased responsiveness to nitroglycerin has not been studied but might be the result of decreased endogenous NO production.

Studies in open-chest dogs with well-developed coronary collateral vessels have consistently demonstrated collateral vessel dilation in response to nitroglycerin. Using measurements of retrograde blood flow from the cannulated collateral-dependent left circumflex artery 5–6 mo after coronary occlusion with Ameroid constrictors in dogs, Fam and McGregor (7) found that nitroglycerin (0.6 or 0.9 mg iv or sublingually) significantly decreased collateral resistance. In studies performed 4–16 wk after occlusion of the anterior descending coronary artery, Hautamaa et al. (17) observed that nitroglycerin (2 µg·kg⁻¹·min⁻¹ iv) increased retrograde blood flow from the cannulated collateralized coronary artery by 63 ± 27%. In dogs studied 4–6 mo after occlusion of the anterior descending coronary artery, Altman et al. (1) observed that nitroglycerin (150 µg/min iv bolus dose) increased retrograde blood flow from 43 ± 5 to 55 ± 9 ml/min. Despite this evidence that nitroglycerin dilates coronary collateral vessels in anesthetized open-chest animals, we previously found that nitroglycerin did not increase coronary collateral blood flow in chronically instrumented awake dogs during treadmill exercise (23). The failure of nitroglycerin to increase coronary collateral blood flow in that study is explained by the present results in which nitroglycerin was effective after endogenous NO production had been blocked. Because both NO produced by eNOS and that from nitroglycerin biotransformation converge on the guanylate cyclase pathway, the effect of nitroglycerin will depend on the state of the endogenous NO system. The present findings suggest that endogenous NO caused collateral vessel dilation during control exercise, so that supplying additional exogenous NO from nitroglycerin did not cause further vasodilation.

Endothelial dysfunction. In the present study collateral vessel growth was stimulated by intermittent followed by permanent coronary artery occlusion. This results in development of collateral vessels that have intact endothelium-dependent vasodilator mechanisms (26). In contrast, in patients with ischemic heart disease, hyperlipidemia, atherosclerosis, or hypertension can cause endothelial dysfunction with impaired NO-dependent vasodilation (5, 15). If the collateral vessels had endothelial dysfunction, then nitroglycerin would be expected to improve blood flow. This is in agreement with reports that sublingual nitroglycerin is effective in reducing effort-induced angina in patients in which atherosclerosis had resulted in a totally occluded coronary artery and a collateral-dependent region of viable myocardium (3, 11, 22). Fujita et al. (13) observed that intracoronary nitroglycerin (50 µg) significantly reduced S-T segment depression during pacing-induced angina in patients with a totally occluded coronary artery and chronic effort angina but no previous myocardial infarction. Because intracoronary nitroglycerin caused no change in systemic hemodynamics, alleviation of ischemia was likely caused by improved collateral blood flow. The results suggest that endothelial dysfunction in patients with coronary atherosclerosis...
likely involves the collateral vessels, so that nitroglycerin can exert a beneficial vasodilator effect.

In conclusion, this study demonstrates that NO can cause vasodilation of coronary collateral vessels. The failure of nitroglycerin to increase collateral blood flow during exercise in this canine model of coronary occlusion in which endothelial function is intact occurred because the endogenous NO system had already activated the guanylate cyclase-mediated vasodilator pathway. When endogenous NO production was blocked, then nitroglycerin did increase blood flow to the collateral zone during exercise. This latter situation is analogous to the clinical circumstance of endothelial dysfunction with impaired endogenous NO production in patients with ischemic heart disease and supports the effectiveness of nitroglycerin in this situation.

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REFERENCES