Altered Coronary Vascular Responsiveness to Leukotrienes In Alloxan-Diabetic Rats

David M. Roth, Diane K. Reibel, and Allan M. Lefer

SUMMARY. Altered responsiveness to and metabolism of various eicosanoids in diabetic animals and patients has been reported by several investigators. The purpose of this investigation was to examine the coronary vascular responsiveness of alloxan-diabetic rats to the leukotrienes. Hearts from 12- to 16-week-old alloxan-diabetic rats and weight-matched controls were perfused at constant flow by the Langendorff method. Coronary vasoactivity to leukotrienes B4, C4, D4, and E4 was assessed by measuring the change in coronary perfusion pressure upon infusion of these eicosanoids. Hearts from diabetic rats showed increased responsiveness to leukotrienes C4 (4-40 nM) and D4 (10-100 nM). Both control and diabetic rat hearts were only slightly responsive to leukotriene E4, and no difference between the two groups existed in the reactivity to this leukotriene. Neither group was responsive to the chemotactic leukotriene, B4. Perfusion of the hearts with the cyclooxygenase inhibitor, ibuprofen, failed to alter the coronary vascular responses to the leukotrienes. The coronary constrictor effects of the leukotrienes are the primary effect of these agents on the rat heart, since heart rate does not change significantly, and changes in contractile force are secondary to the coronary vascular constriction. These alterations in responsiveness to leukotrienes may play a role in the cardiovascular complications associated with diabetes. (Circ Res 54: 388–395, 1984)

DIABETIC patients are known to exhibit an increased tendency to develop cardiovascular disease (Garcia et al., 1974). Blood vessels and platelets obtained from diabetic patients also exhibit altered production of eicosanoids (Halushka et al., 1981; Johnson et al., 1979). Laboratory animals with experimentally induced diabetes also exhibit alterations in both the production of and the reactivity toward various metabolites of arachidonic acid. In this regard, isolated coronary arteries of diabetic dogs have been found to be hyperresponsive to the constrictor prostaglandin, PGF2α (Palik et al., 1982), and to the endoperoxide analog, U-46619 (Sterin-Borda et al., 1982). Also, isolated perfused hearts of diabetic rats were shown to be hyperresponsive to the vasoconstrictor effects of the synthetic endoperoxide analog, U-46619 (Roth et al., 1983).

The leukotrienes are a recently discovered group of eicosanoids which are produced largely by white cells and macrophages (Samuelsson et al., 1979). More recent work has indicated that the leukotrienes are also produced by vascular and lung tissue (Piper et al., 1983). Moreover, leukotrienes possess potent cardiovascular activity in many species. The leukotrienes have been shown to constrict the coronary vasculature in cats (Roth and Lefer, 1983), dogs (Woodman and Dusting, 1983), sheep (Michelassi et al., 1982), and guinea pigs (Letts and Piper, 1981). However, there are no previously published studies examining the role of these potent coronary constrictors in the cardiovascular adaptations occurring in diabetes.

The major goals of this study were (1) to characterize the vasoactive effects of the leukotrienes on the coronary vasculature of the isolated perfused rat heart, (2) to determine whether experimentally induced diabetes alters the reactivity of the coronary vasculature to the leukotrienes, and (3) if so, to determine whether this difference is due to a direct vascular effect of leukotrienes.

Methods

Induction of Diabetes

Diabetes was produced by intravenous injection of alloxan monohydrate in ice cold saline, 45 mg/kg body weight, directly into the femoral vein of male Sprague-Dawley rats. Diabetic rats were used 12–16 weeks after induction of diabetes. Weight-matched, nondiabetic rats were used as controls. On the day of the experiment, diabetes was confirmed by detection of glucosuria using Ketodiasits (Miles Laboratory, Inc.). At the time of the experiment, blood was collected from the chest cavity of the animals, and centrifuged at 1500 g for 10 minutes. The serum was decanted and frozen until glucose determinations were carried out by the glucose oxidase method (Raabo and Terekildsen, 1960). Serum glucose averaged 9 ± 1 mM and 33 ± 1 mM for control and diabetic animals.

In another group of animals, diabetes was induced by intraperitoneal injection of streptozotocin, 75 mg/kg body weight. The vehicle for the streptozotocin was 10 mM sodium citrate buffer, at pH 5.5. Serum glucose concentrations for this group of animals was 28 ± 2 mM.

Cardiac Perfusion

The hearts were quickly excised from the rats that had been anesthetized previously with sodium pentobarbital,
35 mg/kg body weight. The hearts then were perfused by the Langendorff technique at a constant pressure of 60 mm Hg for an initial 5-minute equilibration period. The perfusate was Krebs-Henseleit (K-H) buffer containing 11 mM glucose at pH 7.35. The buffer was continuously oxygenated with 95% O₂ and 5% CO₂ and maintained at 37°C. Throughout the experiment, coronary perfusion pressure was continuously monitored from a side-arm in the aortic inflow tract which was connected to a Statham P23 pressure transducer and a Grass model 7 oscillographic recorder.

The next step in the experimental protocol following the 5-minute constant pressure perfusion consisted of a 10-minute constant flow perfusion at a rate of 9–10 ml/min. The hearts were perfused at a constant flow of 9–10 ml/min without recirculation of the buffer for the remainder of the experiment. After this 10-minute constant flow perfusion period, leukotrienes were infused by a Buchler peristaltic pump via a polyethylene catheter placed in the aortic inflow tract of the hearts. The leukotrienes were infused at a rate of 1 ml/min for 1 minute and then were flushed through the system by 10-minute infusion of K-H buffer at a rate of 1 ml/min. After this 10-minute wash of the leukotriene infusion line, the hearts were allowed to equilibrate for 5 minutes until infusion of the next dose began. Each heart received three doses of either leukotriene B₄, C₄, D₄, and E₄. Only one leukotriene was used in a given heart preparation. Until the time of the experiment, LTC₄, LTD₄, and LTE₄ were stored at −78°C in distilled water; LTB₄ was stored in methanol. Before each experiment, the leukotrienes were thawed and diluted in freshly prepared K-H buffer to the proper concentration to achieve final concentrations at the hearts of 4–100 nM.

In another group of experiments, the cyclooxygenase inhibitor ibuprofen (Upjohn Co.) was added to the perfusate reservoir to achieve a concentration of 100 μM in K-H solution. This solution then was perfused through the heart for a 10-minute equilibration period until leukotriene C₄ at a dose of 40 nM was infused into the hearts, as previously described.

In some experiments, 5-ml samples of the cardiac perfusate were collected during the rise in coronary perfusion pressure observed upon infusion of the leukotrienes. These samples were frozen and later subjected to specific radioimmunoassay for thromboxane B₂, the stable breakdown product of thromboxane A₂, according to the method of Lewy et al. (1981).

In additional experiments, control and diabetic hearts were perfused at constant flow, as previously described, except that a 3-0 silk ligature was sutured into the apex of the heart, positioned around a pulley wheel, and connected to a Grass FT-03 force-displacement transducer. A resting force of 0.2 g was placed on the heart, and contractile force was recorded on a Grass model 7 oscillograph recorder, along with coronary perfusion pressure. In some experiments, hearts were perfused at constant pressure (i.e., 60 mm Hg), and contractile force was recorded and coronary flow measured by collecting the coronary effluent in a calibrated vessel over timed intervals. Heart rate also was measured by recording the contractile force at a fast paper speed. In additional experiments, sodium pentobarbital or U-46619 (9,11-methano-epoxy-PGF₂α) (Upjohn Company) was added to the coronary perfusate.

In addition, rat papillary muscles isolated from the left ventricle were removed and placed in 10-ml chambers containing Krebs-Henseleit solution. These muscles were electrically stimulated at 1 Hz and at a voltage 2 V above threshold (i.e., abut 4–5 V). The muscles were stretched to a resting force just below that which yielded maximum developed force. Developed force was recorded by use of Grass FT-03 force-displacement transducers on a Grass oscillographic recorder. Volumes of drugs added to the bath were 10–50 μl.

The data were evaluated statistically by Student's t-test for unpaired comparisons (control vs. diabetic).

**Results**

Table 1 represents the heart weight-to-body weight ratios of the rats in the diabetic and control groups employed in these studies. There was no significant difference between the body weights, heart weights, and the heart weight-to-body weight ratios in these two groups of animals. Thus, the hearts from control and diabetic rats were comparable in size and overall dimensions.

Figure 1 illustrates a typical tracing showing the increase in coronary perfusion pressure observed when LTC₄ (8 nM) was infused into the hearts of diabetic and control animals. As shown, there was a biphasic increase in perfusion pressure in hearts from the diabetic animals. The early phase (phase a) appeared to be due to active constriction of the vasculature in the presence of a normal cardiac rhythm. The later phase (phase b), however, was associated with a reduction and, finally, a cessation of the natural heart rhythm and an apparent increase in cardiac muscle tension which was reversible upon washout of the leukotriene. The degree to which active coronary constriction contributed to phase b cannot be ascertained; therefore, the values employed for changes in coronary perfusion pressure contain only the phase a response. Phase b occurred in 50% of the hearts from diabetic animals at doses of LTC₄ (8 nM), and in 100% of hearts from diabetic animals at 40 nM. This phase also was observed in 60% of the hearts from diabetic rats given LTD₄ (100 nM), but not at lower doses of this leukotriene. Phase b was not observed in control hearts at any of the doses of LTC₄ or LTD₄ employed.

Figure 2 represents the changes in coronary perfusion pressure associated with the administration of LTC₄ in hearts of both diabetic and control animals. Compared with control hearts, those from the diabetic animals were significantly more responsive to this leukotriene at all three doses employed in the study. The hearts from the diabetic animals underwent changes in coronary perfusion pressure of 47 ± 8, 70 ± 6, and 86 ± 5 mm Hg for doses of LTC₄.

### Table 1

<table>
<thead>
<tr>
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<th>Body wt</th>
<th>Heart wt</th>
<th>Heart wt/body wt</th>
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<tbody>
<tr>
<td><strong>Control</strong></td>
<td>251 ± 7</td>
<td>1.01 ± 0.03</td>
<td>4.12 ± 0.10</td>
</tr>
<tr>
<td><strong>Diabetic</strong></td>
<td>253 ± 15</td>
<td>1.08 ± 0.06</td>
<td>4.35 ± 0.14</td>
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</table>

All values are means ± SEM for 13 hearts in each group.
Figure 1. Typical tracing of response to LTC₄ (8 nM) by hearts from diabetic and control rats. The response of the diabetic is divided into phase a, representative of the measured constriction, and phase b, representative of the later phase of the response.

The hearts from the diabetic group showed changes in coronary perfusion pressure of 50 ± 5, 70 ± 5, and 81 ± 6 mm Hg for the three concentrations of LTC₄ used. The control hearts elicited significantly lower changes in coronary perfusion pressure, 28 ± 5, 38 ± 5, and 42 ± 4 mm Hg for 10, 50, and 100 nM, respectively.

Both groups of hearts were less responsive to LTE₄ than to LTC₄ or LTD₄ at 8, 40, and 80 nM. Figure 4 shows the changes in coronary perfusion pressure associated with the infusion of LTE₄ in both groups of hearts. The changes in coronary perfusion pressure were small and did not change with increasing doses of LTE₄. Moreover, there were no significant differences between the changes, in hearts from the diabetic and control rats. The actual values were 16 ± 2, 16 ± 2, and 17 ± 2 mm Hg for the diabetic group and 12 ± 2, 19 ± 4, and 17 ± 2 mm Hg for the control group at LTE₄ at 8, 40, and 80 nM, respectively. Thus, rat coronary vessels respond only slightly to LTE₄.

Leukotriene B₄ at 10–100 nM was relatively inac-
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LEUKOTRIENE E 4 CONCENTRATION (nM)

FIGURE 4. Represents the change in coronary perfusion pressure associated with infusion of leukotriene E, (8, 40, and 80 nM) into hearts from diabetic and control rats. All values are means ± SEM for five hearts in each group.

In order to examine the possibility that the increased coronary reactivity of the diabetic rats was due to alloxan toxicity rather than diabetes, we examined a group of streptozotocin-induced diabetic rats for their reactivity to leukotriene D4 at 10, 50, and 100 nM. As shown in the results, summarized in Table 3, there were no significant differences between the LTD4 responsiveness that occurred in the alloxan- and streptozotocin-induced diabetic rats, at any dose employed. Thus, both methods of induction of diabetes yielded the same difference in leukotriene sensitivity, suggesting that the differences are, in fact, due to the diabetes, and not to the diabetogenic agent, directly.

The role that cyclooxygenase products of arachidonic acid metabolism may play in the responsiveness of the coronary vasculature to the leukotrienes was examined in another set of experiments. The cyclooxygenase inhibitor, ibuprofen (100 μM), was perfused through the coronary circulation for 10 minutes before infusion of leukotriene C4 (40 nM). This concentration of ibuprofen previously has been shown to inhibit cyclooxygenase activity totally (Reibel et al., 1983). Ibuprofen alone produced no detectable change in coronary perfusion pressure. Moreover, the change in coronary perfusion pressure associated with the LTC4 infusion was not altered significantly in the presence of ibuprofen (Fig. 5). Control hearts produced an increase in perfusion pressure of 61 ± 7 mm Hg for the untreated hearts and 51 ± 5 mm Hg for the ibuprofen-treated hearts. The hearts from diabetic rats produced an increase in perfusion pressure of 86 ± 5 mm Hg for the untreated hearts and 75 ± 5 mm Hg for the ibuprofen-treated hearts. Although there was no significant difference between the ibuprofen-treated hearts and the untreated hearts within either group, a significant difference (P < 0.02) still existed between the diabetic and control responses for both the ibuprofen-treated and the untreated hearts. In a few experiments, indomethacin (50 μM) was used as the cyclooxygenase inhibitor, with essentially the same results as with ibuprofen.

Throughout the course of the experiments, sam-

TABLE 2

<table>
<thead>
<tr>
<th>LTD4</th>
<th>Δ Coronary perfusion pressure (nm Hg)</th>
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<tr>
<td></td>
<td>10 nM</td>
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<tr>
<td>Weight-matched control</td>
<td>29 ± 5</td>
</tr>
<tr>
<td>Age-matched control</td>
<td>34 ± 2</td>
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All values are means ± SEM for five hearts in each group.

Therefore, it is unlikely that age, per se, accounts for the enhanced responsiveness of diabetic rat hearts to leukotrienes.

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TABLE 3

Comparison of LTD4 Responses of Alloxan- and Streptozotocin-Diabetic Groups

<table>
<thead>
<tr>
<th>LTD4</th>
<th>Δ Coronary perfusion pressure (nm Hg)</th>
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<tr>
<td></td>
<td>10 nM</td>
</tr>
<tr>
<td>Alloxan-induced diabetes</td>
<td>50 ± 5</td>
</tr>
<tr>
<td>Streptozotocin-induced diabetes</td>
<td>47 ± 6</td>
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Throughout the course of the experiments, sam-
FIGURE 5. Represents the change in coronary perfusion pressure associated with infusion of leukotriene C₄ (40 nM) into hearts from both diabetic and control rats before and after treatment of the hearts with ibuprofen (100 nM). Values are means ± SEM for five hearts in the control group and six hearts in the diabetic group.

Further verifying the lack of a direct negative inotropic effect in rat hearts is the absence of a decrease in developed force in the isolated rat papillary muscle. In five papillary muscles isolated from control rats, 80 nM LTC₄ increased developed force 3 ± 4% (mean ± SEM), and 100 nM LTD₄ decreased developed force by 2 ± 3%. In four papillary muscles isolated from diabetic rat hearts, 80 nM LTC₄ decreased developed force 1 ± 3%, and 100 nM LTD₄ increased developed force 2 ± 4%. In contrast, at 80 nM LTC₄ increased coronary vascular resistance 85 ± 8% and decreased contractile force 42 ± 5% in control rat hearts. LTD₄, at 100 nM, increased coronary vascular resistance 91 ± 10% and decreased contractile force 46 ± 7%. In these experiments, heart rate did not change by more than 5–10 beats/min at any time during these responses. Similar correlations between increased coronary vascular resistance and decreased contractile force were observed in three diabetic rat hearts.

In two constant pressure-perfused hearts, contractile force decreased 30% in response to 80 nM LTC₄, and coronary flow decreased 70% at the peak of the response. Thus, changes in contractile force are clearly dependent on the coronary vascular effects of LTC₄ and LTD₄ in the control or diabetic rat heart.

Figure 7 illustrates an experiment that clearly shows the cardiodepressant effect of LTC₄ and LTD₄ to be dependent upon their coronary constrictor effect. We employed a potent coronary vasoconstrictor having no inotropic effect, a synthetic endoperoxide analog (i.e., 9,ll-methano-epoxy-PGH₂, U-46619), along with an agent known to have a
direct negative inotropic effect (i.e., sodium pentobarbital). The endoperoxide produced a profile virtually identical to LTC₄ and LTD₄, shown in Figure 6—namely, no change in developed force in the rat papillary muscle and a potent coronary vasoconstriction concomitant with a marked decrease in contractile force in the perfused rat heart. One may argue that the decrease in contractile force results from the constriction or vice versa. However, the effect of sodium pentobarbital suggests that decreased force results from coronary constriction, since pentobarbital, a direct negative inotropic agent in both the papillary muscle and the isolated heart, did not significantly alter coronary vascular resistance. These results also show the rat papillary muscle to be responsive to negative inotropic agents.

**Discussion**

This investigation is the first to compare the vasoactive effects of all four major leukotrienes (i.e., LTB₄, LTC₄, LTD₄, and LTE₄) when the intact coronary vasculature is employed. Our findings demonstrate that the leukotrienes that contain a sulfhydryl-linked peptide moiety (i.e., LTC₄, LTD₄, and LTE₄) are potent constrictors of the coronary vasculature of the rat. In contrast, the chemotactic leukotriene (LTB₄), which is a dihydroxy acid derivative and does not contain a peptide group, is inactive in this preparation. These studies also show that the coronary vasculature of the diabetic rat is hyperresponsive to the constrictor effects of the two most potent leukotrienes in this preparation, leukotriene C₄ and D₄.

Our studies also point out that cyclooxygenase products (i.e., classical prostaglandins, thromboxanes) play a minor role, if any, in mediating the leukotriene-induced coronary constriction, since pretreatment of the heart with ibuprofen or indomethacin did not significantly alter the change in coronary perfusion pressure. Moreover, radioimmunoassay of coronary effluents also failed to detect significant amounts of thromboxane B₂ released during the leukotriene-induced constriction. These conclusions are in agreement with the work of Michelassi et al. (1982), who showed that coronary artery constriction induced by intracoronary injection of LTD₄ was unaffected by prior treatment with ibuprofen.

The relative potencies of the leukotrienes employed in this study on the coronary vasculature are in basic agreement with earlier reports. Woodman and Dusting (1983) found that LTC₄ and LTD₄ were equipotent in decreasing coronary blood flow in the anesthetized dog, whereas LTE₄ produced little or no constrictor effect. Burke et al. (1982) found that LTC₄, LTD₄, and LTE₄ all reduced coronary flow in isolated guinea pig hearts, LTE₄ being less potent than LTC₄ or LTD₄. Previous work in our laboratory showed the relative potencies of the leukotrienes in
the isolated perfused rat coronary artery preparation to be LTD₄ > LTC₄ > LTE₄, whereas leukotriene B₄ displayed no activity in this preparation (Roth and Lefer, 1983).

The coronary vasculature of the diabetic rats tested in this study was hyperresponsive to leukotrienes C₄ and D₄ compared with control hearts. Hyperresponsiveness of vascular tissue from diabetic animals to other constrictor eicosanoids has been documented previously. Isolated coronary arteries from diabetic dogs were hyperreactive to PGF₂α and PGE₂ (Palik et al., 1981 and 1982, respectively), and to the synthetic endoperoxide analog, U-46619, (Sterin-Borda et al., 1982). Isolated perfused hearts from alloxan diabetic rats also have been shown previously to be hyperreactive to the synthetic endoperoxide analog, U-46619 (Roth et al., 1983), and to constrictor eicosanoids generated from blood platelets (Reibel et al., 1983). One explanation for this hyperresponsiveness may be decreased release of the vasodilator prostacyclin (PGI₂) by the diabetic animals. It has previously been shown that aortas from diabetic rats (Carreras et al., 1980; Rogers and Larkins, 1981; Wey and Subbiah, 1982; Roth et al., 1983) and arteries from diabetic patients (Harrison and Johnson, 1981) exhibit reduced synthesis of PGI₂. Furthermore, Terashita et al. (1981) found that prostacyclin was released during LTC₄- and LTD₄-induced reduction in coronary flow in the guinea pig heart. However, pretreatment of the hearts with ibuprofen did not significantly alter the response to leukotriene C₄ or reduce the difference in reactivity between the two groups. It therefore seems unlikely that decreased prostacyclin production by the heart of the diabetic animal is solely responsible for the alteration in reactivity. In this regard, perfused hearts of diabetic rats have actually been found to produce more prostacyclin than control hearts when challenged with arachidonic acid, its fatty acid precursor (Rosen and Schrör, 1980; Roth et al., 1983).

Another possible explanation for the enhanced vascular reactivity observed in the coronary vasculature of the diabetic animal may be a phenomenon recently described by Broderick and Tulenko (1983). These workers found that cholesterol can increase the sensitivity of rabbit femoral arteries to constrictor actions of norepinephrine. It has previously been shown that serum cholesterol is elevated in streptozotocin-induced diabetic rats (Redgrave and Snibson, 1977) and in diabetic patients (Garcia et al., 1974). Left ventricular tissue of alloxan-diabetic dogs has also been shown to contain increased amounts of cholesterol, compared with that of controls (Regan et al., 1974). With regard to our experiment, this would not explain why the coronary vasculature of the diabetic animals was not hypersensitive to the actions of LTE₄, but further investigation into cholesterol levels in our diabetic rat model and the possible ramifications thereof, may prove to be fruitful.

The second phase (phase b) of the response to the leukotrienes observed in the diabetic group may be related to the impairment of ventricular contraction associated with coronary constriction observed by Michelassi et al. (1982) with leukotriene D₄ in the sheep. Since leukotrienes C₄ and D₄ are known to increase the permeability of the guinea pig skin microvasculature (Peck et al., 1981), this phase may also be due to increased permeability of the coronary microvasculature, which would tend to induce extravascular compression and increase perfusion pressure. Finally, phase b may be due to coronary artery vasospasm. We cannot distinguish among these mechanisms at the present time.

Animal studies in which diabetes is induced by the use of β-cell cytotoxic agents, such as alloxan (Malaisse, 1982), carry the risk of the results being influenced by the general toxicity of the agent. In this investigation, another agent commonly used to induce diabetes, streptozotocin (Argarwal, 1980), was used to test the possibility that this increased reactivity was due specifically to alloxan toxicity. The relative activities of the alloxan and streptozotocin groups were comparable, supporting the concept that the alteration in reactivity observed is due to diabetes, rather than toxicity to the diabetogenic agent.

The leukotrienes originally were thought to be produced mainly by various types of white blood cells, but recent work by Piper et al. (1983) shows that these eicosanoids are produced by vascular tissue, with coronary arteries being a prime producer of these substances. Thus, the leukotrienes may be of importance in situations such as myocardial ischemia, where white blood cells can become trapped in the vasculature and release leukotrienes, which, along with the leukotrienes formed by the coronary vessels, can promote further vasoconstriction and further cellular trapping in a positive feedback manner. The finding of an increased reactivity of the coronary vasculature in alloxan diabetic rats is consistent with the hypothesis that this alteration may play a role in increased propensity toward cardiovascular complications known to occur with diabetes.

LTC₄ and LTD₄ did not exert any direct negative inotropic effects in control or diabetic rat hearts, or in control or diabetic rat papillary muscles. In contrast, the negative inotropic agent, sodium pentobarbital, depressed the heart directly in both preparations. Thus, the coronary vascular effects of the peptide leukotrienes predominate in the rat heart, in contrast to reports of direct negative inotropic effects of these leukotrienes in the guinea pig heart (Burke et al., 1982). Thus, the rat heart may not be sensitive to the alleged direct negative inotropic effect of leukotrienes.

We wish to acknowledge the technical assistance of Judith Komlosh and Jean Edmonds during the course of these studies. We also wish...
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for the generous supply of leukotienes B±
and
from the National Heart, Lung, and Blood Institute of the National
Institutes of Health and the Trud Trust of Dr. Ralph and Marian
Falk.

Dr. Roth is a Foerderer Fellow of Thomas Jefferson University.
Address for reprints: Dr. Allan M. Lefer, Department of Physiology,
Jefferson Medical College, Thomas Jefferson University, 1020 Locust
Street, Philadelphia, Pennsylvania 19107

Received August 19, 1983; accepted for publication January 25,
1984.

References

Agarwal MK (1980) Streptozotocin: Mechanism of action, FEBs
Lett 120: 1–3

Brederseck R, Tuleno K (1983) Cholesterol increases (NE) sensivity
in perfused rabbit femoral arteries (abstr.) Fed Proc 42:
1387

Burke J, Levin R, Guo Z, Corey EJ (1982) Leukotrienes C±, D±,
and E±. Effects on human and guinea pig cardiac preparations

Carreras LO, Chamone DAF, Kierck P, Vernetten J (1980) De-
creased vascular prostacyclin (PGI2) in diabetic rats. Stimulation
of PGI2 release in normal and diabetic rats by the antithrom-

Garcia MJ, McNamara PM, Gordon T, Kannel WB (1974) Mor-
bidity and mortality in diabetics in the Framingham population.
Diabetes 23: 105–111

Halushka PV, Rogers RC, Loadholt CB, Colwell JA (1981) In-
creased platelet thromboxane synthesis in diabetics. J Lab Clin
Med 97: 87–96

Harrison HE, Johnson M (1981) Vascular prostacyclin release and
metabolic derangement in diabetes. Horm Metab Res (suppl
1): 43–49

Johnson M, Harroson HE, Raftery AT, Elder JB (1979) Vascular
prostacyclin may be reduced in diabetics in man. Lancet 1: 325–
326

Letts LG, Piper PJ (1981) Action of LTC and LTD in guinea pig
isolated hearts and the effect of indomethacin and FPL 55712.
J Pharmacol 86: 125–128

Leicht RL, Wiener L, Walinsky P, Lefer AM, Silver MJ, Smith JB
(1981) Thromboxane release during pacg-induced angina
pectoris: Possible vasoconstrictor influence on the coronary
vasculature. Circulation 61: 1165–1171

Malassez WI (1982) Allertoxicity to the pancreatic β cell: A

Michelassi F, Landi L, Hill RD, Lowenstein E, Watkins WD,
artery constrictor associated with impaired ventricular contrac-
tions. Science 217: 841–843

Palik I, Hadhazy P, Magyar K, Malomvolgyi B, Wagner M,
Pogatsa G (1981) Effects of prostaglandins F2 and 1, and indome-
thacin on isolated coronary arteries from healthy and alloox-

Palik I, Kotai MZ, Hadhazy P, Magyar K, Malomvolgyi B, Wagner
M, Pogatsa G (1982) Effects of prostaglandins E±, 1, and F± on
the tone of isolated coronary arteries from alloox-diabetic

C± and D± on the microvasculature of guinea-pig skin. Prosta-
glandins 21: 315–321

Piper PJ, Letts LG, Galton SA (1983) Generation of a leukotriene-
lke substance from porcine vascular and other tissues. Prosta-
glandins 25: 591–599

Raafo E, Terkildsen TC (1960) On the enzymatic determination

Redgrave TG, Snibson DA (1977) Clearance of chylomicron tria-
cyl-glycerol and cholesterol ether from the plasma of strepto-
zotocin-induced diabetic and hypercholesterolemic hypothy-
roid rats. Metabolism 26: 493–503

Regan TI, Ettinger PO, Khan MI, Jesrani MV, Lyons MM, Olde-
wurtel HA, Weber M (1974) Altered myocardial function and
metabolism in chronic diabetes mellitus without ischemia in
dogs. Circ Res 35: 222–237

Reibel DK, Roth DM, Lefer BL, Lefer AM (1983) Hyperreactivity
of the coronary vasculature in platelet-perfused hearts from

Rogers SP, Larkings RG (1981) Production of 6-oxo-prostaglandin
F± by rat aorta. Diabetes 30: 935–939

Rosen P, Schrôr K (1980) Increased prostacyclin release from
perfused hearts of acutely diabetic rats. Diabetologia 18: 391–
394

Roth DM, Lefer AM (1983) Studies on the mechanism of leuko-
triene induced coronary artery constriction. Prostaglandins
26: 573–581

Roth DM, Reibel DK, Lefer AM (1983) Vascular responsiveness and
eicosanoid production in diabetic rats. Diabetologia 24:
372–376

Samuelsson B, Borgeat P, Hammarstrom S, Murphy RC (1979)
Introduction of a nomenclature: Leukotrienes. Prostaglandins
17: 785–787

Stenn-Borda L, Borda ES, Gimeno MF, Hazpan MA, del Castillo
E, Gimeno AL (1982) Contractile activity and prostacyclin
generation in isolated coronary arteries from diabetic dogs.
Diabetologia 22: 56–59

Terasaka Z, Fukui H, Hirata M, Terao S, Ohkawa S, Nishikawa
by leukotrienes in isolated perfused guinea pig hearts. Eur J
Pharmacol 73: 357–361

Wehr H, Subbav MTR (1982) 6-keto-PGF±, synthetis in diabetic
rat aorta: Effect of substrate concentration and cholesterol

Woodman OL, Dusting DJ (1983) Coronary vasoconstriction
induced by leukotrienes in the anaesthetized dog. Eur J Pharm-
col 86: 125–128

INDEX TERMS: Leukotriene C± • Leukotriene D± • Diabetic rats
• Coronary vasocoactivity • Cyclooxygenase inhibition
Altered coronary vascular responsiveness to leukotrienes in alloxan-diabetic rats.

D M Roth, D K Reibel and A M Lefer

Circ Res. 1984;54:388-395
doi: 10.1161/01.RES.54.4.388

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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