the integrity of the phototransduction process. The relation between the two processes, however, remains to be elucidated. Our results with mutants, nevertheless, support the idea that the light-dependent modifications of polypeptides reflect molecular events occurring in the visual processes of *Drosophila*.

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6. The compound eye is composed of about 800 ommatidia each of which consists of the image-forming apparatus and a group of eight photoreceptor cells. Both the image-forming apparatus and the photoreceptive system are surrounded by a group of pigment cells. In the photoreceptor-layer preparation, the corneal lenses, which form a major portion of the image-forming apparatus, were dissected away from the compound eye. The preparation still contained most of the pigment cells in addition to the photoreceptors.
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10. Under these conditions all the proteins are presumably in a denatured state. For this reason we refer to the spots as "polypeptides" rather than "proteins." It may be noted that all three classes of light-modifiable polypeptides show varying degrees of charge heterogeneity in both light and dark states (Fig. 1, a and b). It is not clear whether the multiple spots in each cluster are due to heterogeneous polypeptides that cannot be separated by our experimental procedures or to multiple modification events occurring on a single polypeptide.
11. If two gels—one containing a dark-adapted sample and the other a light-treated sample (Fig. 1, a and b)—are treated identically and processed at the same time, they produce polypeptide patterns that are superimposable upon each other except for the 80 K, 49 K, and 39 K spots. However, because of a slight difference in photographic reproduction and in staining and destaining, some of the minor spots show up more clearly in Fig. 1b than in Fig. 1a. In Fig. 2 the gel patterns are slightly streaky along the first dimension, but all spots can be unequivocally assigned to the corresponding ones in Fig. 1.
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Leukotriene D_4: A Potent Coronary Artery Vasoconstrictor Associated with Impaired Ventricular Contraction

Abstract. *Leukotriene D_4 (2×10^{-9} mole), injected into the left circumflex coronary artery of anesthetized sheep, produced profound coronary vasoconstriction and impaired regional ventricular wall motion. This cardiac effect was neither inhibited by prior treatment of the sheep with a cyclooxygenase inhibitor nor associated with thromboxane B_2 release into the coronary sinus. Intravenous FPL 55712 completely abolished the coronary vasoconstriction of leukotriene D_4, but a significant reduction of regional wall shortening persisted.*

Hypotensive shock and cardiac failure are clinical manifestations of systemic anaphylaxis (*1*). Electrocardiographic changes characteristic of coronary artery spasm have been reported during human anaphylaxis (*2*). Leukotrienes C_4 and D_4 (LTC_4 and LTD_4) have recently been identified as the major active constituents of slow-reacting substance of anaphylaxis (SRS-A) (*3–5*). Both are produced in vitro by the combined activation of human mast cells and pulmonary interstitial mononuclear cells (*6*). Human polymorphonuclear cells convert synthetic leukotriene A_4 to LTC_4 (*7*), which can then be transformed to LTD_4. To seek clues to the precise mechanisms of cardiovascular depression, we evaluated the effects of intracoronary injections of synthetic LTD_4 on sheep coronary artery blood flow and myocardial performance in vivo before and after the systemic administration of an inhibitor of cyclooxygenase and an SRS-A antagonist, and observed the concentrations of coronary sinus thromboxane B_2 (TxB_2) and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) before and after intracoronary injection of synthetic LTD_4.

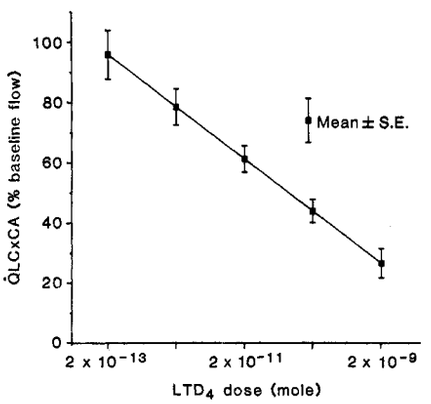

Fig. 1. Inverse log dose response relationship between increasing doses of LTD_4 and LCx coronary artery flow. Left circumflex coronary artery (*LCxCA*) flow is indicated as a percentage of the baseline value.

Eight Suffolk sheep of both sexes weighing 30 to 35 kg were anesthetized and ventilated with 0.5 percent halothane in oxygen. Pulmonary artery and wedge pressure and right ventricular filling pressure were recorded with a flow-directed catheter. Cardiac output was intermittently determined by thermodilution. Systemic arterial pressure, airway pressure, and lead II of the electrocardiogram were continuously recorded. Through a left thoracotomy an electromagnetic flow probe was placed around the main pulmonary artery. An electromagnetic flow probe was positioned around the left circumflex (LCx) coronary artery proximal to the first diagonal branch. The first diagonal branch was cannulated in retrograde fashion with an 0.7-mm (outer diameter) polyethylene catheter and the tip advanced to the confluence of that branch with the LCx coronary artery. A catheter was introduced into the coronary sinus for blood sampling. Left ventricular pressure was measured with a Millar micromanometer tip pressure catheter. The first derivative of left ventricular pressure ($LVdP/dt$) was obtained by electronic differentiation.

In the first group of five sheep, 1 ml of normal saline at 39°C was initially inject-

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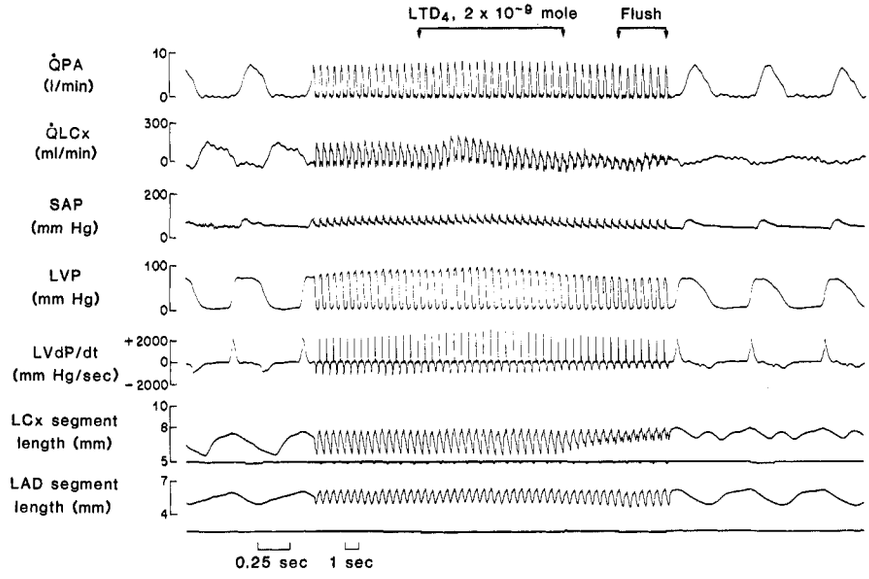

Fig. 2. Effects of LCx intracoronary injection of 2×10^{-9} mole of LTD_4 on pulmonary artery flow (*QPA*), left circumflex coronary artery flow (*QLCx*), systemic arterial pressure (*SAP*), left ventricular pressure (*LVP*) and its first derivative (LV*dP/dt*), ventricular segment length in the area supplied by the LCx coronary artery (LCx segment length), and by the LAD coronary artery (LAD segment length). Note the decrease of *QLCx* and *LCx* region systolic shortening after the injection of LTD_4.

ed over 10 seconds through the polyethylene catheter in the LCx coronary artery to document any effects of the saline injection. Subsequently, increasing doses of LTD_4 (2×10^{-13} to 2×10^{-9} mole) diluted in 1 ml of normal saline at 39°C were injected. We allowed 5 to 10 minutes between injections for all hemodynamic parameters to return toward baseline conditions. A similar sequence was repeated 30 minutes after the intravenous injection of a cyclooxygenase inhibitor, ibuprofen (12 mg/kg) (*8*). Blood samples (5 ml) for radioimmunoassay measurement (*9*) of plasma 6-keto-$PGF_{1\alpha}$ and TxB_2 were drawn from both the coronary sinus and the left ventricle (three sheep, 12 assays) immediately preceding and for 90 seconds after the intracoronary injection of 2×10^{-9} mole of LTD_4.

In three additional sheep, two pairs of 5-MHz ultrasonic piezoelectric crystals were inserted in the subendocardial myocardium for continuous measurements of ventricular segment length (*10*). One crystal pair was implanted in the region supplied by the LCx coronary artery while the other pair was implanted in the area supplied by the left anterior descending (LAD) coronary artery so that simultaneous measurements of regional ventricular function could be obtained in the territory affected by the LTD_4 injection and in a control area. In these animals, 2×10^{-9} mole of LTD_4 was injected in the LCx coronary artery before the intravenous administration of FPL 55712 (1 mg/kg) and 2 to 5 minutes afterwards. FPL 55712 is an effector site antagonist of SRS-A (*11*). All data represented are means ± standard error. A probability of $< .05$ was considered significant (*12*).

Injection into the LCx coronary artery of 2×10^{-11} mole or more of LTD_4 induced a significant decrease of LCx coronary artery flow compared with the effects of 1 ml of normal saline at 39°C ($P < .001$). There was an inverse log dose response relationship between increasing doses of LTD_4 and the LCx coronary artery flow (Fig. 1).

Maximum vasoconstriction after LCx intracoronary injection of 2×10^{-9} mole of LTD_4 occurred within 20 seconds from the start of the injection, with full recovery by 3 minutes. Within the first 60 seconds after LCx intracoronary injection of 2×10^{-9} mole of LTD_4, pulmonary artery flow, right ventricular stroke volume, and LV*dP/dt* decreased 20 to 25 percent from the baseline value, whereas heart rate was unchanged. Systolic shortening decreased 70.2 ± 6.2 percent from the control value in the LCx area, whereas shortening remained unchanged in the territory supplied by the LAD (Fig. 2). End diastolic length was consistently increased only in the LCx area. Reversible T-wave inversion consistent with myocardial ischemia occurred between 20 and 80 seconds from the start of the injection.

Intravenous administration of ibuprofen did not inhibit the LCx coronary vasoconstriction induced by subsequent injections of LTD_4. The concentration of TxB_2 and 6-keto-$PGF_{1\alpha}$ in blood samples from the left ventricle and coronary sinus did not increase during the first 90 seconds after LCx intracoronary injection of 2×10^{-9} mole of LTD_4. In contrast to ibuprofen, FPL 55712 (1 mg/kg) had a profound effect on the cardiac response to LTD_4. Injection of 2×10^{-9} mole of LTD_4 into the LCx coronary artery from 2 to 5 minutes after intravenous injection of FPL 55712 did not produce significant reduction of LCx coronary flow but caused a 14.7 ± 5 percent decrease of LCx area systolic shortening from baseline ($P = .04$). Shortening in the LAD area was unaffected by LCx injection of LTD_4.

Previous workers (*13*) have shown that the addition of either LTC_4 (1.6×10^{-13} to 1.6×10^{-10} mole) or LTD_4 (2×10^{-13} to 2×10^{-10} mole) to the isolated perfused guinea pig heart decreased the coronary flow rate, left ventricular systolic pressure, and LV*dP/dt* in a dose-dependent manner without altering the heart rate. In perfused working guinea pig hearts (Langendorff preparation), addition of either LTC_4 or LTD_4 produced a decrease in coronary flow followed by small and variable decreases in the force of contraction and the heart rate (*14*). Addition of indomethacin to the perfusing solution significantly decreased the peak reduction of coronary flow induced by LTC_4 and inhibited the decrease in contractility caused by LTD_4. Adding FPL 55712 significantly blocked the LTC_4-induced decrease of coronary flow and also the initial phase of LTD_4-reduced coronary flow. Both LTC_4- and LTD_4-induced reductions of contractility were completely antagonized by FPL 55712.

In our study, as little as 2×10^{-11} mole of intracoronary LTD_4 induced statistically significant LCx coronary vasoconstriction in the sheep. Larger doses were associated with ischemic electrocardiographic changes, mechanical contraction abnormalities, and impairment of overall cardiac function as demonstrated by a decrease in cardiac output, stroke volume, and LV*dP/dt*. An inverse log dose relationship was observed between the dose of LTD_4 administered and the LCx coronary flow obtained.

Coronary vasoconstriction was induced by LTD_4 without the release of TxB_2 or 6-keto-$PGF_{1\alpha}$ into coronary sinus blood. Intravenous administration of ibuprofen did not inhibit the coronary vasoconstriction induced by LTD_4.

In contrast, FPL 55712 blocked the coronary vasoconstricting effect of

2×10^{-9} mole of LTD_4, but only partially blocked (79.1 percent) the decrease of systolic shortening of the myocardial region supplied by the LCx coronary artery. There is a major statistically significant difference between the average response to LTD_4 of both LCx coronary flow and regional shortening before and after FPL administration ($P < .005$), but no such difference for LAD regional shortening.

In the sheep, intracoronary injection of LTD_4 causes local coronary vasoconstriction and myocardial dysfunction. Our observations suggest that ischemia is in part responsible for the impairment of ventricular contraction after LTD_4 injection since the onset of dysfunction occurs concomitantly with decreased coronary artery flow and the contraction pattern then appears characteristic of myocardial ischemia (*15*). The magnitude of coronary flow reduction produced by 2×10^{-9} mole of intracoronary LTD_4 is greater than that required to produce contraction abnormalities on an ischemic basis. Waters *et al.* (*16*) have demonstrated in dogs that a 48 ± 4 percent or greater reduction of coronary flow is associated with both metabolic and mechanical contraction abnormalities. Our studies suggest that LTD_4 causes a negative inotropic effect as well, independent of its effect on coronary blood flow, since intracoronary injection of 2×10^{-9} mole of LTD_4 induced a 14.7 ± 5 percent decrease of systolic shortening in the LCx territory after the administration of FPL 55712 despite lack of significant reduction of coronary flow.

Although the heart and lungs are closely mechanically coupled in the thorax, investigators have long suspected a humoral connection between cardiac and pulmonary function. The recent chemical identification of LTC_4 and LTD_4 produced by lung mast cells and macrophages may provide such a link in selected disease states. Our studies support this possible role for LTD_4. New and sensitive analytical methodology should elucidate the role played by the leukotrienes.

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High-Affinity Choline Transport in Proteoliposomes Derived from Rat Cortical Synaptosomes

Abstract. *Functional high- and low-affinity choline transport processes from rat cortical plasma membranes were reconstituted in phosphatidylcholine bilayer liposomes. The high-affinity choline transporter demonstrated a pharmacological profile and ion dependency that were identical to those of intact synaptosomes. This preparation may be used to further characterize choline transport and, with appropriate supplementation, to investigate the release of acetylcholine in the absence of synaptic vesicles.*

Cholinergic nerve terminals have a high-affinity transporter for choline that subserves acetylcholine synthesis and that is not found in other neurons (*1*, *2*). This transporter appears to be both rate-limiting and regulatory for transmission in the central nervous system under various conditions in vivo (*3*, *4*). High-affinity choline uptake, like other transport systems in the brain, is poorly understood because of the biochemical and morphological complexity of the preparations currently available to study it. This is in contrast to uptake systems in other tissues, such as luminal glucose transport (*5*) or the ion transport in renal medullary cells mediated by Na- and K-dependent adenosinetriphosphatase (*6*), which have been studied extensively with membrane vesicle preparations that allow direct manipulations of the intra- and extracellular milieu. We now describe a preparation of liposomes derived from synaptosomal plasma membranes that exhibit both high- and low-affinity choline uptake activities and is thus amenable to investigation at the molecular level.

Rat cortical synaptosomes were prepared as described (*7*), and suspended in 0.32*M* sucrose (*p*H 7.4) containing 100 μ*M* each of tris-HCl and EDTA. The synaptosomes were frozen and thawed twice, centrifuged at 22,000*g* for 10 minutes, and then suspended in a small volume of buffered sucrose (20 mg of protein per milliliter). Portions of a tissue suspension (50 μl) were added to tubes containing 5 m*M* $MgCl_2$, 0.5 percent sodium deoxycholate, azolectin-derived phosphatidylcholine (ICN) (2 mg/ml), 150 m*M* KCl, and 5 m*M* Hepes buffer (*p*H 7.2). After being sonicated for 30 seconds at room temperature (Braun

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