the Mϕ. Given the recent demonstration of biological conversion of native LDL to a form recognized, taken up, and degraded by Mϕ by way of the Mal-BSA receptor, one potential source of this ligand in vivo has already been identified (*22*). The relevance of the novel function for this receptor remains to be established. The increased secretion of neutral proteases conceivably could contribute to the formation of atheromatous lesions, either directly by causing tissue damage or indirectly by mediating prolonged inflammation. Alternatively, increased fibrinolysis mediated by augmented secretion of plasminogen activator, a potent lytic agent of thrombi, could retard atherogenesis (*23*). We have already observed one biological consequence of the interaction of maleylated-acetylated proteins with Mϕ—the induction of tumor cytolysis. The full range of biological responses induced by engagement of scavenger receptors on a variety of cells remains to be established.

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Coronary Vascular Reactivity After Acute Myocardial Ischemia

Abstract. *Exogenous thrombin produced a biphasic response (a potent dose-related vasodilatation followed by vasoconstriction) in nonischemic canine coronary arteries. The vasodilatation was not blocked by propranolol, atropine, or indomethacin, but was completely blocked by heparin or denudation of the intimal endothelial cells. A similar loss of vasodilating response to thrombin occurred in ischemic coronary arteries with a concomitant enhancement of vasoconstriction. This study indicates that altered responses to thrombin in coronary arteries with damaged endothelium may play an important role in the pathogenesis of coronary vasospasm.*

Much of the recent interest in the pathogenesis of coronary artery vasospasm and its role in the development of acute myocardial infarction (*1, 2*) stems from a report by Prinzmetal (*3*) that a variant form of angina pectoris may be precipitated from coronary vasospasm without detectable severe coronary obstruction. Many investigators have attempted to identify the mechanism as well as the factors that provoke vasospasm. Several naturally occurring spasmogenic factors, such as noradrenaline, serotonin, and thromboxane A_2 and other derivatives of prostaglandins, have been postulated to play an important role in the modulation of coronary arterial tone (*1, 4, 5*). To date, however, none of these has been shown to be the primary factor in the initiation or maintenance of coronary vasospasm. Recently, thrombin, a naturally occurring factor that is readily activated during vascular injury, was shown to be a potent vasoconstrictor and to be responsible for the development of the cerebral vasospasm (*6*). To determine whether thrombin plays a similar modulating role in the coronary circulation, I studied the effects of thrombin on the coronary vascular reactivity of ischemic and nonischemic canine coronary arteries.

Experimental myocardial infarction was induced in male mongrel dogs (23 to 30 kg) by a temporary coronary occlusion and reperfusion model as described (*7*). The development, as well as the extent, of myocardial ischemic injury during the 90-minute coronary occlusion and 1 to 2 hours of reperfusion was estimated by recording the changes in the epicardial electrocardiogram and was subsequently confirmed by staining of the ventricular muscle with nitro-blue-tetrazolium and examining the loss of intracellular dehydrogenases (*7*). This model of myocardial ischemia generally results in a transmural myocardial necrosis that constitutes approximately 20 to 30 percent of the total left ventricular mass. After completion of the temporary myocardial ischemia, the heart was excised, and the epicardial coronary arteries, with an outside diameter of approximately 1.5 to 2.5 mm, were isolated from the ischemic (left anterior descending) and the nonischemic (left circumflex) coronary arteries. The coronary vessels were cleaned of adhering fats and adventitial tissues, and ring preparations of these vessels were prepared as described (*8*). All vessels were passively stretched to generate a resting or basal tension of 2.0 g and were allowed to equilibrate in 30 ml of Krebs-Henseleit solution at 37°C with continuous oxygenation (95 percent O_2 and 5 percent CO_2) for 60 to 90 minutes before the start of the experiment (*9*).

The effects of purified bovine plasma thrombin (Sigma) on the contractile activity of the nonischemic coronary arteries are shown in Fig. 1. In contrast to a potent vasoconstrictory effect of thrombin as reported in isolated cerebral arteries (*6*), the addition of 5.0 units of thrombin to isolated canine coronary arteries resulted in an immediate vasodilatation followed by gradual recovery of the basal tone. The vasodilating response to thrombin was directly proportional to the resting or basal tone, as evidenced by the proportionally greater dilation when the basal tone was increased from 2.0 to 4.0 g (Fig. 1B). Similarly, addition of thrombin (0.5 to 5.0 units) to coronary arteries previously contracted with either 20 m*M* KCl (Fig. 1D) or 3×10^{-7} *M*

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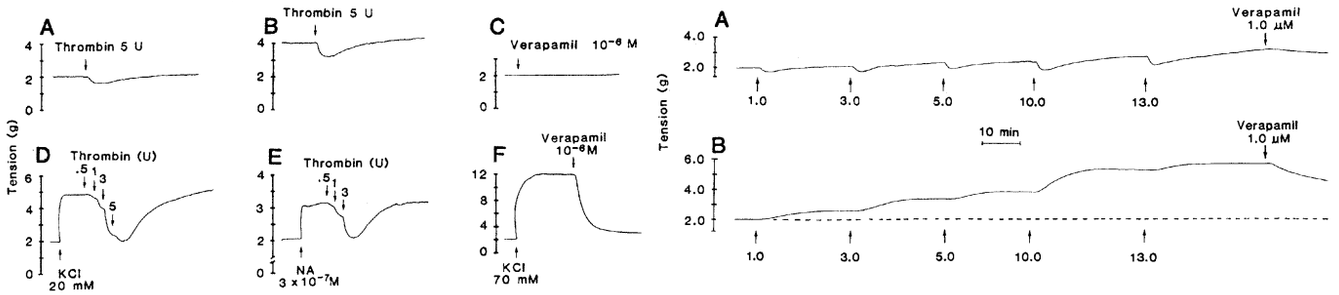

Fig. 1 (left). Comparative arterial vascular responsiveness to bovine plasma thrombin and verapamil in nonischemic canine coronary arteries. Thrombin units represent the total per 30-ml bath. The basal tension of all coronary arterial ring preparations was adjusted at 2.0 g except for (B), which was set at 4.0 g; *NA*, noradrenaline. Similar results were obtained in at least six other experiments. Fig. 2 (right). Loss of vasodilating response of nonischemic canine coronary arteries to thrombin after mechanical denudation of the intimal endothelium. Thrombin was added cumulatively, and the units represent the total per 30-ml bath. Denudation of intimal endothelial cells was performed by rubbing the intima with a specially prepared wooden applicator stick. Similar results were obtained in 11 other experiments.

noradrenaline (Fig. 1E) also resulted in a dose-dependent vasodilatation. These findings are markedly different from those observed with the calcium channel blocking agent, verapamil, which produced a sustained relaxation only in previously contracted coronary vessels (Fig. 1F) and had no significant effect on their basal tone (Fig. 1C).

In all studies with thrombin, the onset of the vasodilating effect was relatively fast, reaching a maximum within 1 to 3 minutes, and was followed by a gradual recovery toward the level before drug application. Repeated addition of various doses of thrombin consistently produced the dose-related transient vasodilatation (Fig. 2A), suggesting that this transient effect was probably not due to desensitization of the vessels to thrombin. Mechanical denudation of the intimal endothelium of these coronary vessels completely abolished the vasodilating responses to thrombin (Fig. 2B), indicating that the initial coronary vasodilatation effect of thrombin was probably mediated by way of its interaction with the endothelial cells. Indeed, thrombin has recently been shown to have numerous biochemical effects, such as release of adenine nucleotides and prostacyclin (*10, 11*) by way of its interaction with the endothelial cells of various vascular tissues.

How the interaction of thrombin with endothelial cells leads to marked coronary vasodilatation is not clear. Prior treatment of coronary vessels with propranolol ($1 \times 10^{-6}M$), which blocks the β-adrenergic receptor, or with indomethacin (50 μg/ml), which inhibits cyclooxygenases, did not abolish the thrombin-induced coronary vasodilatation (data not shown). These data suggest that the observed thrombin-induced vasodilatation was probably not mediated by way of the activation of the β-adrenergic receptor or the prostaglandin system. A similar obligatory role of endothelial cells in the relaxation of isolated arteries caused by acetylcholine has also been reported (*12*). However, in contrast to acetylcholine-mediated vasodilatation, the thrombin-induced coronary vasodilatation was not blocked by high doses of atropine ($1 \times 10^{-5}M$), but was inhibited by heparin (data not shown). Heparin, in contrast, did not inhibit the vasodilating effects of acetylcholine. Thus, these results suggest that the intimal endothelial cells of the coronary arteries contain different receptor sites for acetylcholine and thrombin and that alterations of the thrombin receptor in various pathologic states could play an important modulating role in the coronary arterial tone.

Histologic studies have shown that endothelial cells of coronary vessels are highly susceptible to ischemic injury (*13*). Extensive endothelial cell swelling and derangements were observed after as little as 10 minutes of myocardial ischemia in the capillary vascular beds (*13*). The functional significance of these coronary vascular ischemic injuries on the control of coronary circulation, however, has not been studied previously. The vascular responses of the ischemic coronary arteries to thrombin are shown in Fig. 3. In contrast to the immediate coronary vasodilatation caused by thrombin in normal, nonischemic coronary arteries, the addition of thrombin to the ischemic arteries resulted in a potent vasoconstriction. The addition of thrombin to ischemic coronary vessels previously contracted with KCl also resulted in minimal relaxation, whereas the vasoconstrictory effect was greatly enhanced. A similar loss of vasodilating response with a concomitant enhancement of vasoconstrictory response to thrombin was also observed in small ischemic coronary arteries (outside diameter, approximately 0.9 mm) (data not shown). These results were remarkably similar to those observed in normal coronary arteries with denuded endothelium (Fig. 2B), suggesting that coronary vascular endothelium may be damaged after an acute coronary occlusion. The present data demonstrate that an ischemically injured coronary vascular endothelium may lead to an altered coronary vascular responsiveness to vasoactive agents.

The mechanism of thrombin-induced coronary vasoconstriction is not well understood. As shown in Fig. 2B, the onset of this vasoconstriction was relatively slow, with a lag of 2 to 3 minutes, and required 20 to 25 minutes to reach its

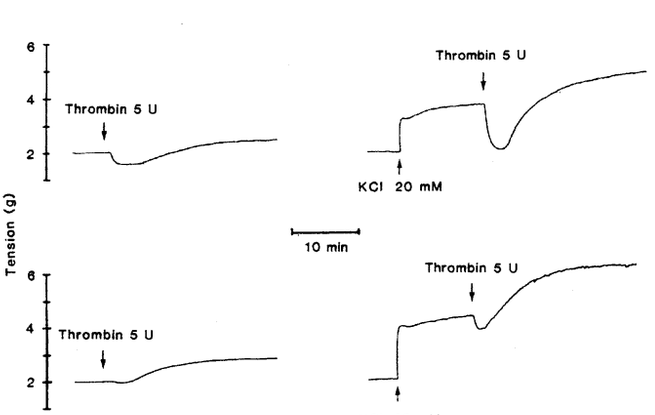

Fig. 3. Effects of temporary myocardial ischemia on coronary vascular responsiveness to thrombin. The ischemic coronary vessels were isolated from the ischemic myocardium after 90 minutes of occlusion and 1 to 2 hours of reperfusion. Similar results were obtained in at least eight other experiments.

maximum effect. Prior treatment with phentolamine ($1 \times 10^{-6} M$) or indomethacin (50 μg/ml) did not inhibit this effect (data not shown), suggesting that it was probably not mediated by the activation of the α-adrenergic receptor or the prostaglandin system. Furthermore, addition of verapamil ($1 \times 10^{-6} M$), which reversed completely the maximum KCl-induced contraction (Fig. 1F), was able to reverse only approximately 40 percent of the thrombin-induced contraction (Fig. 2B). These results suggest that an increase in intracellular calcium necessary for thrombin-induced vasoconstriction may not have been mediated entirely by way of the activation of voltage-dependent calcium channels.

These results indicate that ischemic damage following acute coronary occlusion occurs not only in myocardial cells but also in coronary vasculature. The functional alterations in coronary vascular reactivity to vasoactive agents, such as thrombin, after acute myocardial ischemia have not, to my knowledge, been reported previously. These results also suggest that similar ischemic damage with corresponding altered vascular responses may occur in the endothelium of the small intramyocardial resistance vessels as well as in the epicardial coronary vessels. The enhanced vasoconstrictory response to thrombin in coronary vessels with damaged endothelium may play an important role in the pathogenesis of coronary vasospasm and contribute to the development of coronary "no reflow" phenomenon during reperfusion of the previously ischemic myocardium.

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A Pheromone Influences Larval Development in the Nematode *Caenorhabditis elegans*

Abstract. *A* Caenorhabditis*-specific pheromone and the food supply influence both entry into and exit from a developmentally arrested juvenile stage called the dauer larva. The pheromone increases the frequency of dauer larva formation and inhibits recovery but does not affect adult behavior such as chemotaxis and egg laying. The fatty acid–like pheromone has been partially purified and characterized by a new bioassay. If similar developmental control mechanisms are used by parasitic nematodes, such mechanisms might be exploited to develop highly selective anthelmintic agents.*

Primer pheromones influence the physiology or development of a variety of metazoan organisms. In insects, honeybee queen pheromones influence the physiology of workers (*1*), and termite societies use pheromones as mediators in caste control (*2*). In rodents, primer pheromones have been shown to play an important role in reproductive strategies (*3*). The existence of sex attractants has been reported in several nematode species (*4*). We now report the existence of a nematode pheromone that controls development.

Under conditions of abundant food and low population density, *Caenorhabditis elegans* develops through four larval stages (L1 through L4), reaching the hermaphroditic adult stage 3 days after hatching (*5*). However, in response to starvation or overcrowding, development can be arrested at the second molt. The arrested stage, called the dauer larva (German, "enduring" larva) is a non-feeding form specialized for survival and dispersal (*6*), and such larvae may survive for months until they encounter food, at which time they molt and resume development (*7*). More than 20 genes influencing the developmental sequence leading to the dauer larva have been identified (*8*, *9*). Some mutants which fail to form dauer larvae are defective in chemotaxis and have morphologically abnormal afferent processes of specific sensory neurons (*10*). These genetic defects affect both entry into, and recovery from, the dauer larva stage.

We describe an environmental cue, a "crowding" pheromone, which triggers formation of dauer larvae in wild-type nematode cultures and prevents the dauer larvae from resuming development. Dauer larvae are found in starved cultures but may also be induced by high population density on enriched agar plates and in high-density liquid cultures before exhaustion of food. When placed

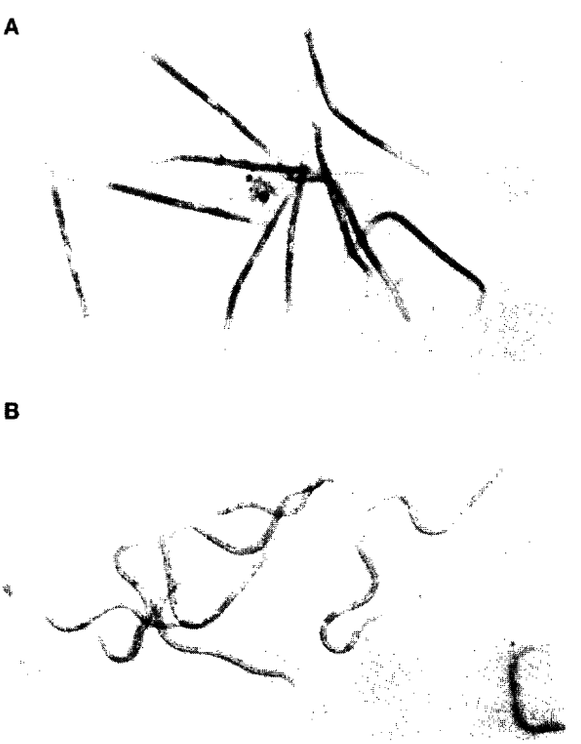

Fig. 1 (A) *Caenorhabditis elegans* nematode larvae incubated in microtiter wells containing a pheromone responsible for maintenance of the developmentally arrested dauer larva stage. (B) In the absence of pheromone, dauer larvae recover to resume development within 4 hours (× 70).

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