Intravascular Platelet Aggregation in the Heart Induced by Norepinephrine
Microscopic Studies

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SUMMARY
Aggregated platelets and occlusive platelet thrombi were found in small myocardial vessels of dogs on electron-microscope examination after prolonged infusion of norepinephrine. The etiology of the myocardial necrosis and fibrosis induced by catecholamines in experimental animals and seen in patients with pheochromocytoma and patients after norepinephrine treatment for shock may be related to this intravascular platelet-aggregating effect of catecholamines. The link between stress and acute myocardial infarction may be via catecholamine-induced intravascular platelet thrombosis. If the thrombogenic theory of atherosclerosis is valid, platelet aggregation induced by catecholamines may be the mechanism whereby arteriosclerotic heart disease is related to stress.

Additional Indexing Words:
Myocardial necrosis       Catecholamines       Norepinephrine       Stress
Platelet aggregation

The precipitating event in acute myocardial infarction is usually the occlusion of an atherosclerotic coronary artery by a thrombus or by hemorrhage into an atherosclerotic plaque. Autopsy studies conducted on patients who have suffered sudden death, however, have frequently shown atherosclerosis in the coronaries but no evidence of acute obstruction. It has been suggested that a thrombus had been present, but, since arterial thrombi are “white thrombi” consisting of platelets and fibrin, these thrombi may break up prior to autopsy, become destroyed during the fixing process, or may be difficult to demonstrate on section, and no occlusion is seen.

Sympathetic catecholamines have been shown to cause platelet aggregation in vitro and to enhance thrombus formation in plastic shunts in vivo. Acute myocardial infarction and sudden death are frequently preceded by severe or prolonged stress. During stress the endogenous secretion of catecholamines is increased. It is possible that stress-induced myocardial infarction is related to the induction by catecholamines of platelet thrombi that form in and occlude narrowed coronary arteries. In order to study the possibility that sympathetic catecholamines can cause intravascular platelet aggregation in the vessels of the heart, we have subjected dogs to prolonged infusions of norepinephrine. It has been known since the early 1900s that...
catecholamine infusion will result in widespread myocardial necrosis. This necrosis may be due to ischemia resulting from platelet aggregation in small vessels. Previous work in this laboratory has demonstrated that drugs that have an inhibitory effect on platelet aggregation (aspirin and dipyridamole) protect against the necrosis induced by catecholamine infusion. In the present study using the electron microscope we have sought to demonstrate morphologic evidence of platelet aggregation in the vessels of the heart following catecholamine infusion.

Methods

Ten mongrel dogs weighing 15–20 kg anesthetized with pentobarbital 30 mg/kg were used for study. The ECG and intraaortic pressure, via a cannula introduced through the femoral artery, were monitored and recorded using a Statham 23D strain gauge and an oscilloscopic photographic recorder. In six dogs norepinephrine, 4μg/kg/min, in normal saline was infused for 4 hours via a cannula in the femoral vein. The total 4-hour dose of norepinephrine was calculated for each dog, added to 480 cc of normal saline, and infused at a rate of 2 cc/min. Four dogs were infused at a similar rate with normal saline and served as controls.

Records of the intraaortic pressure and ECG lead II were taken prior to infusion, at the peak of the blood pressure response 1, 2, 3, and 4 hours after the onset of infusion. Complete standard lead ECGs were recorded during the control period and at the end of the infusion. At the end of the 4-hour infusion the dogs were sacrificed by opening their chests and removing their beating hearts. Sections of the left ventricle near the apex were taken and prepared for electron microscopy by fixing with glutaraldehyde, postfixing with osmium tetroxide, and by staining with uranyl acetate and lead citrate. Approximately 20 random blocks of tissue were prepared from each dog, and approximately 100 sections were studied with the electron microscope for each animal. The presence of capillaries containing platelet aggregates was noted.

Results

All of the dogs receiving the norepinephrine infusion had a marked early blood pressure response (fig. 1). After 2 hours the blood pressure dropped to near the control level and remained at that level or below through the remainder of the infusion in a manner similar to that reported by Moss et al. The control dogs infused with saline alone had no significant change in their blood pressure. By the end of the infusion five of six dogs receiving norepinephrine had electrocardiographic changes. Three had evidence of myocardial infarction with the appearance of new Q waves and marked S-T and T-wave changes. One of the dogs had T-wave inversions, and one had a marked rightward axis shift. The control animals had no significant changes on ECG.

All of the dogs infused with norepinephrine were found to have capillaries containing platelet aggregates on electron-microscopic examination. Among the 20 blocks of tissue studied for each animal, at least 50% of the blocks were found to have capillaries that contained aggregated platelets. The degree of intravascular aggregation varied from a few platelets adherent to each other and to the vessel wall to complete occlusion of the lumen of the vessel with vessel-wall breakdown. The
presence of myocardial damage (as manifested by myofilament fragmentation, contraction bands, hyalinization, edema, and degenerative changes of the mitochondria) correlated with the presence of capillaries occluded by platelet thrombus. In contrast, none of the 20 blocks studied for each of the control animals was found to have capillaries containing aggregated platelets, or myocardial damage.

Figure 2 is an electron micrograph of a capillary containing two platelets (P) and a red cell (R). Both platelets are ovoid and have well-formed architecture with pseudopods. The round structures within the platelets are granules that contain substances such as ADP and enzymes that are released from the platelet during the second stage of aggregation. The empty structures are part of the canalicular system and communicate with

Figure 2
Electron micrograph of intramyocardial capillary after norepinephrine infusion. Note the two well-formed platelets (P) adherent to each other and to the intact vessel wall. See text for discussion.
the surface of the platelet. Although adherent to the capillary wall and to each other, there is not yet any centralization of the granules or rounding of the platelets that precedes release of the substances in the granules as the platelet undergoes viscous metamorphosis. The capillary endothelial cells (C) appear continuous and intact.

Figure 3 is an early platelet thrombus. The platelets are loosely aggregated and many have pseudopods. The granules still retain their contents. The dark-staining pinpoint-sized particles are glycogen. There are two leukocytes present, at 12 o'clock and medial to 9 o'clock. The vessel wall appears to be intact and continuous.

The capillary in figure 4 is completely occluded by an early platelet thrombus. Most of the platelets remain intact, and borders between individual platelets can be distinguished. The platelets are less ovoid and the granules, in those platelets that still contain them, are larger and more centralized. Some of the platelets have already released their granules although the platelet architecture, for the most part, remains intact. The capillary endothelium (C) appears to be continuous and intact, with no breaks in the wall. There is a small clump of fibrin (F) between the platelets. Only a few of the platelets are labeled (P).

Figure 5 is a longitudinal section of a capillary containing a platelet thrombus, composed of platelets and fibrin. The platelets are in various stages of viscous metamorphosis, some with filled granules, others with pseudopods, others with loss of architecture. The endothelial wall remains intact.

Figure 3

Electron micrograph of early platelet aggregate in small myocardial vessel. The platelets are loosely aggregated, and most retain their fine architecture. See text for discussion.
Figure 4

Electron micrograph of platelet thrombus occluding myocardial capillary in dog after nor-epinephrine infusion. The capillary wall remains intact. See text for discussion.

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Figure 5

Electron micrograph of longitudinal view of mature platelet thrombus occluding a small myocardial vessel. See text for discussion.

A late thrombus is seen in figure 6. The wall of the vessel has degenerated and is indistinct with damaged endothelial cells (C) and a large break in vessel-wall continuity at the tips of the arrows. Platelet material and fibrin are extruding through the broken vessel wall (between the two arrows) into the interstitium. The platelets have undergone viscous metamorphosis and degeneration with loss of granules, and with indistinct borders between the platelets. Red cells (R) and a leukocyte (M) are present in the thrombus.

Examination of the electron-microscopic sections of the myocardium of the control animals showed normal empty capillaries with no evidence of platelets or thrombi.

Discussion

In 1907 Josué demonstrated that infusion of epinephrine in experimental animals will cause diffuse patchy myocardial necrosis. Subsequent workers have found similar results after infusions of norepinephrine, isoproterenol, and epinephrine in a variety of animals including dogs, cats, rats, rabbits, and turtles. In man similar cardiac lesions have been found following prolonged norepinephrine infusion and in patients dying with pheochromocytoma.

Various theories have been suggested to explain these findings. The close resemblance of the lesions to those caused by coronary ligation has suggested that hypoxia plays a
Figure 6

Electron micrograph of late thrombus in a myocardial vessel. Note the poorly formed capillary wall with a break in the wall between the arrows. See text for discussion.
part.19 The inotropic effect of the catecholamines may cause an increase in oxygen consumption that cannot be met by the coronary circulation,20 especially after the blood pressure drops to normal or below after the first hours of infusion.26 Catecholamines may cause inappropriate coronary constriction.16 Possibly the effects of catecholamines on carbohydrate and lipid metabolism of the myocardium play a part.23 The excessive mechanical contraction1, 23 the reflex vagotonia seen at the onset of infusion,15 and a possible direct cardiotoxic effect of the catecholamines14, 15 have also been considered as possible causes of the myocardial necrosis induced by catecholamine infusion.

In addition to their cardiovascular effects, catecholamines have been shown to cause aggregation of platelets.7–9 Epinephrine has been long known to enhance blood coagulation.10, 29 British workers showed that norepinephrine will cause platelet aggregation in vitro,7–9 and found that aggregation occurred in two waves, the first reversible, and the second, due to the release of endogenous ADP, irreversible. Ozge et al.30 showed that epinephrine increased clotting activity in vivo, shortened platelet survival, and increased platelet turnover in rabbits and pigs. O'Brien demonstrated that phenotamine, an antiadrenergic compound, increased the bleeding time in man,8 and Rowsell et al. noted an increase in platelet thrombus formation in extracorporeal shunts in pigs after administration of epinephrine.10

In a study previously reported from this laboratory,24, 25 we demonstrated that drugs known to inhibit platelet aggregation protected dogs from the myocardial necrosis induced by infusion of epinephrine. In the present study we have demonstrated morphologically that infusion of norepinephrine, in doses known to cause myocardial necrosis, will result in the formation of platelet thrombi in the small vessels of the heart. From this work it appears that catecholamine-induced cardiac necrosis is related to intravascular aggregation of platelets in the heart.

Jorgensen et al. reported in 1967 that intravascular platelet aggregation can cause myocardial necrosis.5 These workers infused ADP, a potent platelet-aggregating agent, directly into the coronary arteries of pigs. On study of the hearts by light microscopy they found pathologic changes indistinguishable from those described by investigators who have infused catecholamines intravenously. On electron-microscopic examination their findings were similar to those reported here with aggregated platelets found in small vessels, frequently occluding the lumina.

It is likely that the intravascular platelet aggregation is the primary event with the vessel-wall breakdown seen in some of the capillaries secondary to the release of lysosomal enzymes from the aggregated platelet.31, 32 This hypothesis is supported by the findings that the vessel wall was intact in those capillaries that contained platelets that retained their morphologic integrity and that the capillary wall showed evidence of damage only in those instances where platelets were present which had lost their granules and undergone architectural breakdown. It is possible, however, that norepinephrine also causes direct damage to the endothelial wall of the vessel, and it is the break in the endothelial integrity that initiates platelet thrombosis. Early workers have noted morphologic changes in vessels after prolonged catecholamine infusion.14 and Shimamoto et al. have demonstrated that epinephrine will cause endothelial edema and the release of a thromboplastic substance in rabbit aortas.32 It is possible that the aggregation of platelets seen in this study is due to the combination of the enhanced tendency of platelets to aggregate and minor vessel-wall damage, both induced by the norepinephrine. A redistribution of cardiac blood flow during catecholamine infusion34 may cause regional slowing of flow and possibly further enhance the tendency toward formation of intravascular platelet aggregates. In any case it is evident that intravascular platelet thrombosis is present and strongly suggestive that occlusion of small vessels by these thrombi is important in the pathogenesis of the myocardial necrosis.
seen after prolonged catecholamine infusion. These findings may apply to human disease in a number of ways. It has been demonstrated that the majority of patients who die with pheochromocytoma have evidence of either acute intramyocardial necrosis or of diffuse patchy myocardial fibrosis.\textsuperscript{28} Van Vliet et al. showed that these changes were similar morphologically to the necrosis and fibrosis induced in rats by prolonged catecholamine infusion.\textsuperscript{28} It is conceivable that such myocardial pathology might be prevented in patients with pheochromocytoma by treatment with drugs that inhibit the effect of catecholamines on platelet aggregation.

Morphologic changes in the heart similar to those reported in the experimental animal have also been reported in patients who have died after prolonged norepinephrine infusion during the treatment of shock.\textsuperscript{17, 18} If the pathogenesis of this myocardial damage is related to intravascular platelet aggregation, coincident treatment with a drug such as dipyridamole may protect the hearts of those patients who require prolonged norepinephrine administration.

Less directly, but of greater general importance, is the possible relation of these findings to the occurrence of acute myocardial infarction and sudden death. Acute myocardial infarction occurs not infrequently following severe or prolonged stress.\textsuperscript{11} During stress the endogenous secretion of sympathetic catecholamines is increased.\textsuperscript{12} It is possible that the enhancement of platelet aggregation induced by the higher level of circulating catecholamines in combination with possible endothelial damage induced by the catecholamines or the presence of preexisting atheromatosi may lead to the intravascular formation of platelet thrombi. If one of these thrombi forms at, or is carried to, an area of a major coronary artery that had previously been narrowed by an atherosclerotic plaque, occlusion of the narrowed lumen and infarction of the myocardium distal to the occlusion might occur.\textsuperscript{4} It is of interest that in patients who suffer sudden death pathologic examination usually shows extensive coronary atherosclerotic disease but, although evidence of infarction may be present, frequently acute coronary occlusion is not seen.\textsuperscript{2, 3} A platelet thrombus, although not found at necropsy, may have been the cause of death. At an early stage platelet aggregation is reversible and a platelet thrombus that had been present long enough to cause ischemia might have disagggregated prior to postmortem examination.\textsuperscript{4, 6} Platelet thrombi are relatively fragile and may break up after the damage has been caused or may be lost during handling and fixing of the heart during postmortem examination. Also, platelet thrombi are difficult to visualize histologically and appear as amorphous debris with the usual stains unless a secondary clot containing red blood cells, or organization of the thrombus, has occurred. (This may also be the reason that previous investigators of catecholamine-induced myocardial necrosis did not note intravascular platelet aggregation.) It is of interest that the incidence of occluding thrombi found at autopsy increases with the presence of pump failure and pressor therapy\textsuperscript{25} and as the time from infarction to death increases.\textsuperscript{26} It takes time to form the clots that are found easily at autopsy.

In addition to the link between stress and acute myocardial infarction, chronic stress has also been related to atherosclerosis.\textsuperscript{27, 28} The personality of patients who develop coronary atherosclerosis is described as aggressive, driving, and hardworking, frequently with ambitions that cannot be achieved.\textsuperscript{28} These individuals are high catecholamine secreters.\textsuperscript{12} In 1852 Rokitansky postulated that the initiating event in the development of arteriosclerosis was the development of a mural thrombus.\textsuperscript{39} Duguid revived the thrombogenic theory of atherogenesis in the 1940s after studying arteriosclerotic lesions in human aortas and coronary arteries.\textsuperscript{40, 41} Woolf and Carstairs, in 1958, were able to demonstrate platelet-like material deep in atherosclerotic plaques by the use of fluorescein-tagged antiplatelet antibody.\textsuperscript{42} If the thrombogenic theory is correct it is possible that the link between stress and atherosclerosis is via the platelet-aggregating effect of catecholamines.

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Pharmacologic agents that inhibit platelet aggregation or block the effect of catecholamines on platelets might be useful in slowing the progressive course of vascular disease in patients with evidence of early atherosclerosis.

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