Intravascular Platelet Aggregation in the Heart
Induced by Stress

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SUMMARY
Sixteen rats, stressed by immersion in ice-cold water for 25–45 min, were found to have platelet aggregates in myocardial small vessels on electron microscopic study. None of six similar non-stressed control rats had platelets in myocardial vessels. It is concluded that stress, probably via catecholamine secretion that enhances platelet stickiness, can induce intravascular platelet aggregation. It is possible that this mechanism plays a part in the relationship between stress and acute clinical myocardial infarction.

Additional Indexing Words:
Stress  Myocardial infarction

Numerous epidemiologic studies have demonstrated that coronary atherosclerosis and acute myocardial infarction occur more frequently in men who are subject to acute and recurrent stress.1–5 Such individuals have been found to produce high levels of sympathetic catecholamines.4 Infusion of sympathetic catecholamines in experimental animals is known to produce widespread cardiac necrosis.5–8 In man, patients with pheochromocytoma are frequently found at postmortem examination to have similar necrosis in the myocardium.9 Patients who receive prolonged infusions of norepinephrine for the treatment of hypotension are also frequently seen to have similar lesions in their myocardium at autopsy.10 In vitro a number of investigators have found that sympathetic catecholamines will increase the “stickiness” of platelets and will cause platelet aggregation.11–13 A number of investigators have postulated that the precipitating event in acute myocardial infarction may be the formation of a platelet thrombus intravascularly, that forms at or travels to, and occludes a segment of a coronary artery already narrowed by atherosclerosis.14–15

Considering the above evidence, we have investigated the possibility that sympathetic catecholamines may cause cardiac damage by inducing intravascular aggregation of platelets in the heart. We have previously reported that pretreatment with the antiplatelet aggregating drugs aspirin and dipyridamole16–18 will protect against the cardiac necrosis seen after epinephrine infusion,19–20 and using the electron microscope we have demonstrated the presence of platelet aggregates in the small vessels of the heart in dogs infused with norepinephrine.20–22 It was the purpose of the present study to determine if stress alone, without the infusion of exogenous catecholamine, would also lead to intravascular platelet aggregation in the heart.

Materials and Methods
Twenty-two 300–500-g rats were used for study. Sixteen rats were stressed by immersing them in 4–5 in. of ice-cold water in a glass bell jar. The level of the water was chosen such that the rats were able to keep their heads out of the water by balancing themselves to an upright position. The animals were kept in the water until they showed signs of marked fatigue, usually within 25–45 min. They were then sacrificed by a blow on the head followed by immediate removal of their beating hearts and sections of the left ventricle were prepared for electron microscopy. Six similar rats were brought to the laboratory but kept at rest in their cages for the same period of time, then sacrificed and similar sections taken. The sections were prepared for electron microscopic study by fixing them in glutaraldehyde, postfixing with osmic acid, and staining with uranyl acetate and lead citrate. At least 20 blocks of tissue were prepared for each rat, and approximately 25 sections were taken from each block.

Results
All 16 stressed rats were found to have myocardial small vessels that contained aggregated
platelets. The degree of aggregation varied from two to three platelets adherent to the vessel wall and to each other to thrombi consisting of more than 20 platelets and complete occlusion of the vessel lumen (figs. 1–3). In all of the vessels containing aggregated platelets, the endothelial surface appeared to be intact. Most of the aggregated platelets retained their fine architecture, although there was evidence of centralization of granules in many of the platelets (a phenomenon seen prior to release of the substances in the granules and viscous metamorphosis).23, 24

In contrast to the findings after prolonged infusion of norepinephrine, very few of the platelets showed architectural breakdown and there was very little fibrin found in the thrombi.20 Approximately 5% of the small vessels examined contained platelet aggregates. On study of the sections taken from the six control rats none of the small vessels examined contained aggregated platelets.

Discussion

The findings reported here demonstrate that stress can cause intravascular aggregation of platelets in the heart. All of the 16 stressed rats were found to have platelet aggregates within the small vessels of the heart, whereas none of the controls had similar findings. The possibility exists that the physical effect of cold per se may have played a part in the formation of the platelet
aggregates. We feel that this is unlikely for two reasons: (1) On sacrifice, after a rapid blow to the head and opening of the chest, the hearts were found not to have been cooled and to be beating rapidly as they were removed. (2) Although there are little data in the literature concerning intravascular platelet aggregation in vivo, and none to our knowledge of the effect of cold per se on aggregation in vivo, studies of platelet preservation in vitro have shown that mild refrigeration (10–25°C) causes less aggregation of platelets during centrifugation and short-term storage than does maintenance at body temperature.\textsuperscript{25}

During stress catecholamine secretion is increased. Since the electron microscopic findings reported here are similar to those found after catecholamine infusion,\textsuperscript{20} it appears that the mechanism of stress-induced intravascular platelet aggregation is via the release of endogenous catecholamines that, either through a direct effect of the catecholamine on the platelets to make them more adhesive,\textsuperscript{11} or by a damaging submicroscopic effect on the vessel wall,\textsuperscript{26} causes intravascular aggregation of platelets.

The question arises, however, as to why it is possible for rats (and man) to survive repeated stress.

**Figure 2**

*Electron micrograph of a myocardial capillary from a stressed rat. The lumen of the capillary is completely occluded by platelets. The platelets remain intact, and most of the granules continue to contain dark-staining material. The granules have become grouped together, a phenomenon seen prior to release of the substances within the granules and the onset of viscous metamorphosis. The endothelial wall appears to be intact (× 10,000).*

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stress without rapidly dying of ischemic cardiac damage if with each severe stress 5% of the capillaries of the heart may be occluded. First, severe stress occasionally does cause sudden death in animals and in man. More significant, however, is the fact that platelet aggregation induced by catecholamines in vitro has been shown to be a two-phase process. During the first stage platelets release the substances in their granules, such as serotonin, ADP, catecholamines, and lysosomal

Figure 3

Electron micrograph of two myocardial capillaries from a stressed rat. The top capillary contains a loose aggregation of platelets, many of which have prominent pseudopods (commonly seen in platelets in the early stage of aggregation). The granules are prominent and are dispersed throughout the platelets. In this plane of section only the pseudopods of some platelets are seen. A red blood cell is seen at the lower border of the partially occluded capillary. A normal patent capillary containing one red blood cell is seen at the lower left. The capillary walls of both vessels appear intact. The patent capillary is similar to those seen in the control animals. Capillaries containing platelets were seen only among the stressed rats (× 5000).
STRESS-INDUCED PLATELET AGGREGATION

enzymes.  With the release of the material contained in the granules the aggregation becomes irreversible. Morphologically the platelet aggregates noted in the stressed rats appear to be in an early, probably reversible, stage of platelet aggregation, in that most of the platelets contained intact granules. Moreover, in a group of 14 rats subjected to similar stress that were not sacrificed, one was found dead 18 hours later, 13 appeared to recover completely, and on sacrifice 8 days later only two of the rats were found to have small foci of necrosis and early fibrosis of the heart on light microscopic examination. (Haft JI, Oestreicher R: Unpublished data.) Hence, it is possible that during the normal life of a rat (or man) intravascular platelet aggregates may form and break up repeatedly without doing significant ischemic damage. It may be that damage occurs either when stress is markedly prolonged or when the platelet thrombi fortuitously occur at or travel to an area of a large coronary artery that is already sufficiently narrowed that a small thrombus will cause complete occlusion and thereby a significant infarction.

The observation that many patients dying soon after acute myocardial infarction are not found to have thrombi in their coronary arteries at autopsy, and the suggestion that infarction of myocardium may precede coronary thrombosis, rather than thrombosis being the initiating event, does not necessarily obviate extrapolation of our experimental findings in rats to clinical myocardial infarction in man. All patients dying soon after infarction are found to have severe coronary atherosclerosis with areas of significant stenosis of the coronary lumen. In those patients in whom thrombi are found, the thrombus is composed distally, at the point of initial occlusion, of platelets and fibrin, and proximally of red cells, fibrin, and leukocytes. Platelet thrombi without red cells are difficult to demonstrate pathologically, are fragile, and may either disaggregate prior to fixing or if not firmly adherent to the intima may fall away during processing or be destroyed during the fixing process. Even if they persist in situ it is usually difficult to differentiate a platelet thrombus from amorphous debris by light microscopy. In patients dying soon after infarction in whom thrombi are not found it is possible that narrowed areas of the coronary tree had been occluded during life by platelet thrombi, and that these platelet thrombi were lost during the postmortem period. Patients who survive longer after infarction may have enough time for an easily identified red thrombus to be produced proximal to the platelet occlusion. The findings of Spain and Bradess that the longer the period from clinical infarction to death the higher the incidence of coronary thrombosis identified at autopsy would favor this hypothesis.

In the rat, we found platelet thrombi in small vessels of the heart. Rats have normal coronary arteries, and it would be expected that any thrombi forming in the bloodstream would pass through the normal large coronaries and lodge only at a point where the diameter of the thrombus exceeds the cross section of the vessel. In man, occlusive disease occurs in the large extramural vessels. It is possible that thrombi similar to those demonstrated in the small vessels of the rat heart might, in man, lodge in areas of the extramural coronary arteries that, though patent, are markedly narrowed by prior disease. Such platelet thrombi would act as the final insult, completely occluding an already narrowed coronary artery and precipitating a myocardial infarction. In cases of sudden death such an occlusion may occur, ischemia sufficient to trigger ventricular fibrillation may eventuate, and the early platelet thrombus may disaggregate prior to autopsy study.

It is also possible that stress-induced platelet aggregates may form on the vessel wall and if they do not disaggregate may, as they release lysosomal enzymes, cause damage to the underlying endothelium and change the permeability of the vessel wall, thereby allowing lipids to cross the endothelium and possibly progress to atherosclerotic plaques. Moreover, if the thrombogenic theory of atherosclerosis is valid such platelet aggregates may form the nidus for the development of atherosclerotic plaques. Stress-induced intravascular platelet aggregation may be the mechanism that explains the findings that aggressive, driving, frequently stressed individuals are prone to coronary artery atherosclerosis, acute myocardial infarction, and sudden death.

Addendum

Since submission of this manuscript similar studies were performed on 23 additional rats using forms of stress other than immersion in ice water. Eight rats were immersed in hot water (50°C); 15 rats were placed in a chamber with an electrified grid floor, and their feet shocked with 80-msec impulses of 150 V (250 ohms) delivered every 5 sec for 3–5 hours. Seven of the eight heat-stressed animals and 13 of the 15 shocked rats were found on electron microscopic examination to have intravascular platelet aggregates in their hearts similar to those seen after cold stress, as described in this paper.

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