Stress and the Induction of Intravascular Platelet Aggregation in the Heart

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SUMMARY
Intravascular aggregation of platelets similar to that found in dogs after norepinephrine infusion was demonstrated using the electron microscope in the hearts of 20 of 23 rats subjected to two forms of stress (immersion in hot water, 7 of 8 rats; repeated small electric shocks to the feet, 13 of 15 rats). Only one of 14 unstressed rats was found to have similar intravascular platelet aggregates. These findings suggest that catecholamines secreted endogenously during stress are sufficient to cause platelets to aggregate intravascularly and raise the possibility that clinical myocardial infarction occurring during severe or prolonged stress may be caused by catecholamine-induced platelet thrombi which occur at, or travel to, and occlude a coronary artery already narrowed by previous atherosclerosis.

Additional Indexing Words:
Platelet aggregation Stress Myocardial Infarction

SYMPATHETIC catecholamines have been shown to cause aggregation of platelets in vitro.1-3 Infusion of norepinephrine in the anesthetized dog will cause intravascular aggregation of platelets in the small vessels of the heart which can be demonstrated with electron microscopy.4, 5 The cardiac necrosis and fibrosis seen after infusion of epinephrine can be prevented by pretreatment with platelet aggregation inhibitors such as aspirin, dipyridamole6, 7 and clofibrate.8 Thus, such cardiac damage probably is related to the regional ischemia caused by platelet aggregates that occlude small vessels.

The precipitating event in acute myocardial infarction may be the occlusion of an already narrowed coronary artery by a platelet thrombus or embolus.9, 10 With the evidence suggesting that catecholamine-induced platelet aggregation can occur in vivo and with the clinical findings that catecholamine excretion is increased with stress, it is possible that the mechanism of acute myocardial infarction following severe or prolonged stress might be via a catecholamine-induced platelet aggregate that forms in the arterial system and lodges in a coronary artery previously narrowed by atherosclerosis. To determine whether the catecholamines produced endogenously during stress are sufficient to cause intravascular platelet aggregation similar to that seen after exogenous infusion of norepinephrine,4, 5 studies with rats stressed by immersion in ice water were conducted and similar platelet aggregates were noted on electron microscopic examination of the heart.11 It was not clear, however, what effect cold temperature per se had on the tendency for platelet aggregation to occur intravascularly in the heart. To further document that the intravascular platelet aggregates noted in the previous study were indeed due to the stress, 2 other forms of stress—immersion in hot water and repeated electrical shocks to the feet of rats—were studied and reported here. Electron microscopic findings in rats stressed in this way were similar to those found after cold stress, with intravascular platelet aggregates found in the small vessels of the heart.

Material and Methods
Thirty-seven white laboratory rats weighing 300-500 g were used for study.

Eight rats were stressed by immersion in a tub that contained 4 to 5 inches of water kept at 50° by addition of small amounts of boiling water. The four to five inch water level was chosen to allow the rats to just touch bottom and keep their noses above the water. At the first sign of fatigue (inability to maintain head out of water), the rats were promptly removed from the water bath and killed with a blow on the head and removal of
their beating hearts. Sections of the apex of the LV were prepared for electron microscopic study.

Fifteen rats were stressed by placing them in a chamber with an electrified grid floor. The grid was designed so that it was impossible for the rat to be in position where his feet did not come in contact with both the upper grid and the lower copper screen floor of the chamber. One pole of the output of a Grass stimulator was attached to the grid and the other pole to the lower screen floor. By delivering 150 volt (250 ohm) 80 msec electric impulses every 5 sec across the grid and screen floor, the feet of rats were repeatedly stimulated. With each shock the rats would jump and become agitated. The rats were sacrificed after they appeared markedly fatigued as manifested by lying with their feet in the air and not responding to the electrical stimulation.

Fourteen rats were brought to the laboratory and allowed to remain in their cages. They were similarly sacrificed simultaneously with the stressed rats.

The sections of the left ventricular apex were fixed with glutaraldehyde, post-fixed with osmic acid and stained with uranyl acetate and lead citrate. At least 20 blocks were prepared for each rat and approximately 25 sections were examined from each block.

Results

Heat Stress. (figs. 1 and 2) The rats were fatigued after 8 to 10 min in the hot water bath. On electron microscopy 7 of the 8 rats were found to have platelet aggregates in the small cardiac vessels. Aggregation varied in intensity in each rat with some vessels totally occluded by platelet thrombi and other vessels containing platelets adherent to each other and to the vessel wall but not occluding the vessel. No fibrin was noted and no breakdown of the vessel walls was seen. Most of the aggregated platelets appeared intact with retention of their fine architecture. In many of the platelets, however, there was evidence of centralization of granules, a phenomenon seen prior to the release of the substances in the granules and the onset of viscous metamorphosis.12, 13 In those rats with aggregated platelets, approximately 50% of the 20 blocks were found to have intravascular platelet aggregates; and approximately 5 to 7% of small vessels seen contained aggregated platelets.

Shocking Stress. The rats tolerated the stress from 3 to 5 hrs. On electron microscopic study 13 of the 15 rats were found to have platelet aggregates in their cardiac vasculature. The extent of aggregation varied within the individual rat with some vessels completely occluded by aggregated platelets and other vessels containing variable numbers of

Figure 1

Section of a cardiac capillary of a rat stressed by immersion in hot water, containing platelets loosely adherent to the vessel wall and to each other. The platelets are well formed with intact granules without centralization. The vessel wall appears intact. The large dark structures are red cells. (× 10,000)

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platelets adherent to the vessel wall and to each other. Fine platelet architecture was retained with little evidence of platelet breakdown, although centralization of granules was seen. Fibrin was present in the aggregates of 2 of the rats (fig. 3). Arterioles (larger vessels with smooth muscle cells in their walls) were occluded by platelet thrombi in 4 of the rats (figs. 4 and 5). In those animals with aggregation, approximately 50% of the blocks were found to have vessels involved.

Thirteen of the 14 control rats were found to have capillaries that were free of platelet aggregates. One control rat had findings similar to the stressed animals, with aggregated platelets in the small vessels of the heart.

Discussion

The effect of infusion of sympathetic catecholamines has been well documented in the experimen-

Figure 3

Section of a cardiac capillary of a rat stressed by repeated shock to the feet. The capillary is completely occluded by a well formed platelet thrombus. The wavy lines are fibrin. (× 4,800)

Figure 4

Longitudinal section of a myocardial arteriole occluded by a platelet thrombus from a rat after stress with electric shocks. The dark staining cells on the border of the vessel are smooth muscle cells. (× 5,000)

tal animal\textsuperscript{14-18} and in the human,\textsuperscript{19-20} Prolonged infusion results acutely in diffuse necrosis in the myocardium,\textsuperscript{14-20} and repeated exposure leads to diffuse fibrosis. Although various theories, such as the effect of catecholamines on tissue metabolism,\textsuperscript{18} or increased tissue needs not being met by sufficiently increased blood flow,\textsuperscript{16} have been proposed, no one theory has adequately explained the phenomenon. Investigations of platelet function

Figure 5

Longitudinal section of a myocardial arteriole of a rat stressed by repeated electrical shocks. The lumen is entirely occluded by the thrombus. There is fibrin present between some of the platelets. The dark staining cells of the vessel wall are smooth muscle cells. (× 5,000)

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in vitro have shown that the sympathetic catecholamines act as strong platelet aggregating agents.\textsuperscript{1-3} In vivo, it has been demonstrated that catecholamines decrease platelet survival time\textsuperscript{21} and increase the amount of thrombus formation in shunts in the pig.\textsuperscript{22} ADP infused directly in coronary vessels causes intravascular aggregation\textsuperscript{23} and the resultant cardiac necrosis is similar to that seen after prolonged catecholamine infusion. In previous studies performed in this laboratory\textsuperscript{6-8} it has been demonstrated that pre-treatment with the antiplatelet aggregating agents aspirin,\textsuperscript{24, 25} dipyridamole,\textsuperscript{25, 26} and clofibrate\textsuperscript{27} exert a protective effect on the cardiac necrosis induced by infusion of epinephrine. Intravascular aggregation of platelets in the small vessels of the heart has been demonstrated pathologically in dogs using the electron microscope after 4 hr infusion of norepinephrine.\textsuperscript{8} These findings suggest that the cardiac necrosis seen experimentally after catecholamine infusion, clinically in patients dying with pheochromocytoma,\textsuperscript{19} or after prolonged treatment with sympathetic vasopressors,\textsuperscript{20} may be due to the effect of the catecholamines on platelets. The catecholamine-induced platelet aggregates form in or travel to small cardiac vessels, occluding the vessels and causing diffuse ischemic myocardial necrosis.

Men who are aggressive, driving, and frequently stressed tend to be those who are prone to the development of coronary artery disease and acute myocardial infarction.\textsuperscript{28-30} These patients also tend to produce large amounts of catecholamines during stress.\textsuperscript{31} It has been suggested that the initial event in the formation of a clot in the arterial system is the formation of a platelet thrombus.\textsuperscript{9, 10} Pathological studies of coronary thrombi in patients dying from acute myocardial infarction have demonstrated a "white" platelet head to the occlusive thrombus.\textsuperscript{32} It is possible that myocardial infarction seen during stress may be due to the formation of a platelet aggregate at a site of prior narrowing of a coronary artery, or a platelet thrombus that forms elsewhere in the arterial system and travels to and occludes a coronary artery at a point of prior narrowing.

To support the hypothesis that the finding of platelet aggregates in the vasculature of the heart after experimental exogenous infusion of catecholamines is related to the clinical event of myocardial infarction during stress, it was necessary to demonstrate that catecholamines produced endogenously during stressful situations were sufficient to cause similar phenomena. In a study previously reported\textsuperscript{11} it was shown that in 16 rats subjected to immersion in ice cold water until fatigued, platelet aggregates were found in the small vessels of the heart in all the stressed animals. Although it was felt that the platelet aggregates seen in the vessels of the heart in these animals were due to stress alone, it was not entirely clear whether the cold per se could cause this phenomenon. The present study—undertaken to clarify the situation—found that the rats subjected to the stress of repeated electrical shocks without change in temperature had essentially the same results as the rats stressed with ice water immersion.\textsuperscript{11}

During the shock studies, it was evident that the rats were quite uncomfortable and agitated. After a short period of attempting to escape from the chamber they would wander around the chamber with the hair on the back of their necks erected and their eyes dilated as they awaited the shocks. At the impulse they would jump in the air and again start wandering. After 3-5 hrs of this they would appear to give up, would lie on their backs, close their eyes and begin quivering. At this point they were sacrificed. Thirteen of the 15 rats showed marked platelet aggregation in the small vessels of the heart. Why the remaining two rats did not have the intravascular platelet aggregation is unknown, but may be due to variation in the way that the animals responded physiologically to the stress.

It is of interest that fibrin was found diffusely in the platelet aggregates of two of the shocked rats. This was noted in the previous studies after 4 hr norepinephrine infusion\textsuperscript{5} but was not prominent among the rats that were sacrificed after 25-45 min in the ice water bath.\textsuperscript{11} It is possible that stress must be continued for a finite length of time before fibrin will form. This time requirement may be a defense mechanism of the organism against the formation of irreversible thrombi in the small vessels of the heart. Early in the formation of a platelet aggregate, before the second stage of aggregation with release of the substances (e.g., ADP)\textsuperscript{33} in the platelet granules, platelet aggregation is reversible.\textsuperscript{8} At the time of the second stage of aggregation, with the release phenomenon and viscous metamorphosis, the aggregate becomes irreversible\textsuperscript{9} and fibrinogen is converted to fibrin. The absence of vessel wall damage noted in this study and in the rats stressed by cold water immersion may be due to the same phenomenon. Among the substances released from platelets during the second stage of aggregation are proteolytic enzymes that can damage the
vascular wall. With the absence of a substantial release reaction in the stressed rats (the granules were intact in most of the aggregated platelets) damaged vessels would not be expected. It is interesting to note that among rats subjected to ice water stress, but allowed to live and sacrificed one week later, only 2 of 13 showed evidence of myocardial necrosis on light microscopy. This suggests that although intravascular platelet aggregates may form during stress, patients probably survive repeated stress over years without eventually thrombosing all of their small vessels because after most episodes of stress the intravascular aggregates break up and vascular patency is restored. Only if the platelet thrombi form at or travel to sites of critical narrowing of a coronary artery do they cause obvious myocardial infarction.

Among the rats subjected to immersion in hot water (50°C), 7 of the 8 developed platelet aggregates intravascularly in the heart. It is interesting to note that the animals became fatigued after a shorter period of time in the hot than in the cold water. Even though the period of stress before sacrifice was only 8 to 10 minutes, intravascular platelet aggregates were found. The requirement for only a relatively short period of stress may explain why one control rat was found to have intravascular platelet aggregates. The sacrifice procedure was done by a rapid blow to the head with the animal held upside down by the tail. In most instances loss of consciousness occurred with the first blow. In the control rat that developed intravascular platelet aggregation the sacrifice procedure was prolonged. The first blow missed and the rat became very agitated as it was held. It was 4 or 5 minutes until he was adequately immobilized. There may have been sufficient time for intravascular platelet aggregation to have occurred secondary to the stress of the sacrifice procedure.

The role of stress-induced platelet aggregation in clinical disease may be more universal than just the possible occlusion of an area of an already stenosed coronary artery by a platelet thrombus. Early workers have postulated that atherosclerotic plaques have their origin as mural thrombi. Duguid presented evidence to suggest that thrombi may evolve into atherosclerotic plaques in the aorta and the coronary arteries. Wolff and Carstairs found material deep in atherosclerotic lesions that reacted with fluorescein tagged antiplatelet antibodies. If the thrombogenic theory of atherosclerosis is valid, mural platelet aggregates may be the initial event in the formation of atherosclerotic plaques. This hypothesis may help explain why men who are aggressive and driving and frequently stressed are prone to develop coronary artery atherosclerosis.

References

7. HAFT JI, GERSHENGORN K, KRANZ P, OESTREICHER R: Protection against epinephrine-induced myocardial necrosis by drugs that inhibit platelet aggregation. Am J Cardiol 30: 838, 1972
15. MALING HM, HIGMAN B, THOMPSON EC: Some similar effects after large doses of catecholamines and myocardial infarction in dogs. Amer J Cardiol 5: 628, 1960

Circulation, Volume XLVIII, July 1973
28. Russek HI, Zohman BL: Relative significance of heredity, diet, and occupational stress in coronary heart disease of young adults: Based on analysis of 100 patients between the ages of 25 and 40 years and a similar group of 100 normal subjects. Amer J Med Sci 235: 266, 1958
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