Effects of stress and the sympathetic nervous system on coronary artery atherosclerosis in the cynomolgus macaque

Epidemiologic evidence increasingly implicates psychosocial variables in the development of coronary heart disease in human beings, an association that appears to be independent of the effects of other coronary disease risk factors. It has been hypothesized that behavioral influences on coronary heart disease are mediated by activation of the sympathetic nervous system, perhaps through exacerbation of coronary artery atherosclerosis. This article summarizes several studies of the effects of stress and sympathetic arousal on atherosclerosis in a nonhuman primate model of atherogenesis. The application of a behavioral stressor involving periodic reorganization of social group memberships resulted in worsened coronary atherosclerosis among male cynomolgus monkeys *(Macaca fascicularis)* fed a cholesterol-containing diet, relative to control animals housed in groups of fixed (stable) membership, but only among those monkeys that retained dominant social status during the course of the study. This effect could not be attributed to concomitant variability in blood pressure or serum lipid concentrations. When the same experimental procedures were applied to males fed a diet low in saturated fat and cholesterol, the manipulation of group memberships similarly led to development of greater atherosclerosis in the coronary arteries. In related observations, monkeys that exhibited the largest heart rate responses to a standardized behavioral challenge had more extensive coronary atherosclerosis than animals showing a less pronounced cardiac responsivity to stress. In a final investigation, we observed that the exacerbated atherosclerosis of dominant monkeys consuming an atherogenic diet and housed in unstable social groups could be prevented by long-term administration of a β-adrenoreceptor-blocking agent, propranolol hydrochloride. (Am Heart J 1988;116:328.)

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There is epidemiologic evidence that psychosocial factors contribute to the development and clinical expression of coronary artery disease.[1] One such factor is the type A behavior pattern, a constellation of dispositional attributes characterized by extremes of competitiveness, impatience, and hostility. With few exceptions, prospective studies of *initially healthy individuals* have shown type A persons to be at significantly greater risk for later coronary heart disease (CHD) than persons who exhibit a contrasting and more placid type B behavior pattern.[1] This association has been observed in both men and women and over follow-up intervals of 5 to 14 years.[2] Multivariate analyses also demonstrate that the heightened coronary risk of type A persons is independent of concomitant variability on standard CHD risk factors such as hyperlipidemia, hypertension, cigarette smoking, and age. Interestingly, recent findings suggest that this increased risk does not derive equally from the several type A attributes previously cited but stems primarily from the type A person's high propensity to experience hostility or anger.[3,4]

However the behavioral antecedents of CHD may be best characterized, the foregoing epidemiologic associations and their apparent independence of other CHD risk factors invite speculation regarding the physiologic mechanism(s) mediating psychosocial influences on coronary disease. In this respect, it is widely hypothesized that recurrent or pronounced responses of the sympathetic nervous system to behavioral stimuli contribute to CHD, possibly by precipitation of acute clinical events or by an exac-

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erbation of coronary artery atherosclerosis.[1,5-7] Several sources of evidence are consistent with this hypothesis: (1) Type A persons show larger hemodynamic and catecholamine reactions than do type B persons when exposed to frustrating laboratory tasks or other behavioral challenges[8]; (2) persons with coronary disease exhibit larger pressor responses to mental stress than patients without CHD or nonpatient controls[9-15]; and (3) in one prospective investigation, large blood pressure reactions to the "cold pressor test" (immersion of a limb in cold water) predicted the 23-year incidence of CHD.[16] However, these data only indirectly implicate sympathetic activity in the development of CHD and offer no positive evidence that such activity accounts for the heightened coronary risk attributable to behavioral factors such as type A.

Investigations with suitable animal models offer a complementary approach to the study of psychosocial influences on coronary disease and their potential mediation by the sympathetic nervous system. Two major opportunities afforded by animal models are the experimental manipulation of relevant environmental variables and a more precise quantification of lesion characteristics (e.g., atherosclerosis) than is feasible in patient populations. Animal studies also permit assessment of atherosclerosis before its clinical manifestation, an evaluation that is ethically precluded in the study of asymptomatic human beings.

Of the many mammalian species typically used in cardiovascular research, the nonhuman primates, particularly the macaques and baboons, are especially useful for studies of atherogenesis. These animals develop lesions that are similar in their location and morphologic characteristics to those observed in humans and do so readily when maintained on diets that induce moderate hyperlipoproteinemia.[17,18] Described in this article are the results of several studies we have recently conducted examining the behavioral exacerbation of coronary atherosclerosis in one such species, the cynomolgus macaque *(Macaca fascicularis)*.[19-22] Considerations favoring use of the cynomolgus monkey as a model include its high susceptibility when fed a cholesterol-containing diet to development of fatty streaks and the rapid progression of these initial lesions to the formation of fibrous plaques.[17,18] Comparability with man is also evidenced by a similar incidence of myocardial infarction among cholesterol-fed animals[23] and by the greater severity of coronary artery atherosclerosis seen in males than in females of this species.[24]

Another important feature of the cynomolgus monkey and of macaques generally is the complexity of their behavioral repertoires. Relationships among monkeys are defined by elaborate patterns of affiliative and antagonistic interaction. When living in social groups, macaques form stable hierarchies of social dominance in which an animal's relative status is determined by its ability to defeat other group members in competitive interactions: dominant monkeys are those animals most likely to succeed, and subordinates are those most likely to submit to competitors in such encounters.[17] Not surprisingly, status relationships among members of an established social grouping are disrupted with the appearance of unfamiliar animals.[25] Abrupt changes in group membership tend to intensify antagonistic interactions between monkeys (as seen, for instance, in increased rates of aggressive behavior) as animals seek to reestablish generalized hierarchical associations and affiliative coalitions. The disruptive influence of strangers on status relationships of socially grouped animals also provides the basis for a naturalistic social challenge that is amenable to experimental manipulation, namely, the periodic reorganization of social group memberships. We have used this manipulation in the several investigations summarized herein that describe the effects of social dominance and sympathetic nervous system activity on the development of coronary artery atherosclerosis.

SOCIAL STATUS, ENVIRONMENT, AND ATHEROSCLEROSIS

In our first experiment,[19] we assigned 30 adult male cynomolgus monkeys to one of two laboratory conditions for 22 months. In one condition (designated "unstable"), monkeys were housed in five-animal social groups, the memberships of which were reorganized on a regular basis. Redistribution of animals among the affected social groups occurred at 1- to 3-month intervals and in a manner ensuring that each monkey would be placed with either three or four new animals on every reorganization. In contrast, monkeys assigned to the alternate (or control) condition lived in groups of comparable size that retained their original memberships over the course of the investigation; these groups were designated the "stable" social condition.

All monkeys were fed a moderately atherogenic diet that derived 43% of calories from fat and had a cholesterol concentration of 0.34 mg/kcal (equivalent to a daily consumption in humans of approximately 680 mg of cholesterol). Throughout the study, routine measurements were made of the animals' serum lipid concentrations (total serum

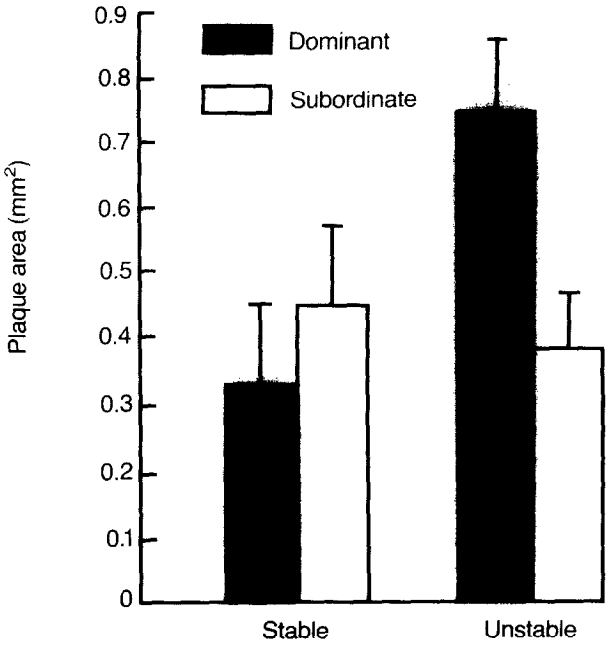

Fig. 1. Mean coronary artery plaque area measurements (± SEM) among dominant and subordinate monkeys in the stable and unstable (i.e., periodically reorganized) social conditions.

cholesterol, high-density lipoprotein cholesterol), blood pressure, fasting glucose concentration, ponderosity, and behavior. The latter evaluations involved frequent assessments of the aggressive, submissive, affiliative, and nonsocial behaviors of each monkey. We also evaluated the social status, or dominance ranking, of individual animals based on the observed outcomes of each monkey's aggressive encounters with all other members of the same social group. For purposes of behavioral classification, monkeys that ranked either first or second in their respective groups over most social evaluations conducted during the study (i.e., animals of consistently high rank) were identified as dominant monkeys; the remaining, lesser ranked animals were labeled subordinates.

At necropsy the animals' coronary arteries were perfused at physiologic pressure. After dissection of the coronary arteries from the heart, five tissue blocks each were cut perpendicularly to the long axis of the left anterior descending, left circumflex, and right coronary arteries. Sections from each block were stained with either hematoxylin-eosin or Verhoeff van Gieson stains. The Verhoeff van Gieson–stained sections were then projected, and the area occupied by intima or intimal lesion (i.e., the area between the internal elastic lamina and the lumen of the artery) was measured with the aid of a Zeiss image analyzer. Our principal index of atherosclerosis is the mean intimal area, expressed in millimeters squared (mm^2), of 15 arterial sections.

The results of this experiment are depicted in Fig. 1. Analysis of variance revealed a statistically significant interaction ($p < 0.04$) between the social condition to which the monkeys had been assigned and the animals' social status. Note that the dominant animals housed in unstable (i.e., periodically reorganized) social groups developed appreciably greater coronary artery atherosclerosis than did either their subordinate counterparts or similarly dominant monkeys assigned to the stable social condition. Hence disruption of group memberships potentiated atherogenesis, but only in those animals that retained positions of social dominance during the course of the study. An important point is that the exacerbated atherosclerosis observed here among unstable dominant monkeys could not be attributed to corresponding alterations in serum lipids, blood pressure, ponderosity, or fasting glucose concentration.

Even though these findings were independent of concomitant variability in the serum lipid concentrations of experimental animals, it is possible that behavioral factors influence atherogenesis only in the presence of a diet-induced hyperlipoproteinemia. Accordingly, in a second experiment[20] we exposed animals to the same psychosocial manipulation (i.e., unstable vs stable social groups) but administered a diet nearly devoid of saturated fat and cholesterol (0.05 mg of cholesterol/kcal). As expected, intimal lesions in this study were minimal in comparison with those seen in our first experiment. Nonetheless, monkeys assigned to the unstable condition developed significantly more coronary atherosclerosis than did animals housed in stable social groups (median intimal areas = 0.021 and 0.004 mm^2, respectively; $p < 0.002$). Intimal lesions made up of small plaques with evidence of smooth muscle cell proliferation were also seen most frequently among the unstable animals ($p < 0.05$). Although dominance relationships in the unstable social condition fluctuated somewhat over successive group reorganizations (as compared with our initial experiment), those unstable animals that retained positions of social dominance throughout the experiment developed more extensive coronary atherosclerosis than all other study animals ($p < 0.05$). Thus the behavioral perturbation that is occasioned by repeated disruption of social group memberships accelerates atherogenesis even in otherwise well-protected (i.e.,

normolipoproteinemic) animals and, again, does so most appreciably among the more aggressive, dominant monkeys.

BEHAVIORALLY INDUCED HEART RATE REACTIVITY AND ATHEROSCLEROSIS

We noted earlier that speculation regarding the mediation by the sympathetic nervous system of psychosocial influences on coronary disease arises from the following: (1) the failure of standard CHD risk factors to account for epidemiologic associations between behavioral factors and subsequent CHD among initially healthy persons and (2) the observation that persons behaviorally predisposed to CHD (e.g., type A persons) and patients with coronary disease exhibit an enhanced cardiovascular responsivity to stress.

As previously described, the first of these observations applies equally to our animal model. We have also examined the relationship of atherosclerosis to animals' cardiac responsivity to behavioral stimulation.[21] For this purpose, the heart rates of monkeys assigned to our first experiment were recorded by radiotelemetry under baseline conditions and during the animals' exposure to a standardized laboratory challenge (a stylized threat of capture). Like human beings, monkeys varied greatly in the magnitude of their heart rate responses to behavioral stress. Moreover, on necropsy animals that had shown the most pronounced cardiac response to our laboratory stressor ("high" heart rate reactors) had more extensive coronary atherosclerosis than did monkeys exhibiting heart rate responses of lesser magnitude under the same stimulus conditions ("low" reactors) ($p < 0.05$). High heart rate–reactive monkeys were also behaviorally more aggressive than their less reactive counterparts, as documented in social observations made during the experiment ($p < 0.05$). Hence severity of atherosclerosis in this animal model is greatest among monkeys that have the largest cardiac reactions to stress, and such reactivity is in turn associated with variability in the animals' aggressive potential.

INHIBITION OF BEHAVIORALLY EXACERBATED ATHEROSCLEROSIS BY β-ADRENORECEPTOR BLOCKADE

It is likely that the heightened cardiac responsivity to stress we have found associated with atherosclerosis is a manifestation of the influences of the sympathetic nervous system on the heart. If so, it is reasonable to hypothesize that administration of a β-adrenoreceptor–blocking agent would inhibit the behavioral exacerbation of atherosclerosis. In our most recent experiment,[22] we tested this hypothesis by administering propranolol hydrochloride on a long-term basis to 15 experimental animals. All these monkeys were fed a cholesterol-containing diet (0.26 mg of cholesterol/kcal) and housed in recurrently reorganized (unstable) social groups for 26 months. Fifteen control monkeys received no propranolol but were otherwise treated identically to the experimental animals. In the propranolol-treated condition, propranolol was added to the diet in the amount of 0.2 mg/kcal/day; this dosage was sufficient to lower heart rate by 20 to 25 beats/min and blood pressure by about 15 mm Hg.

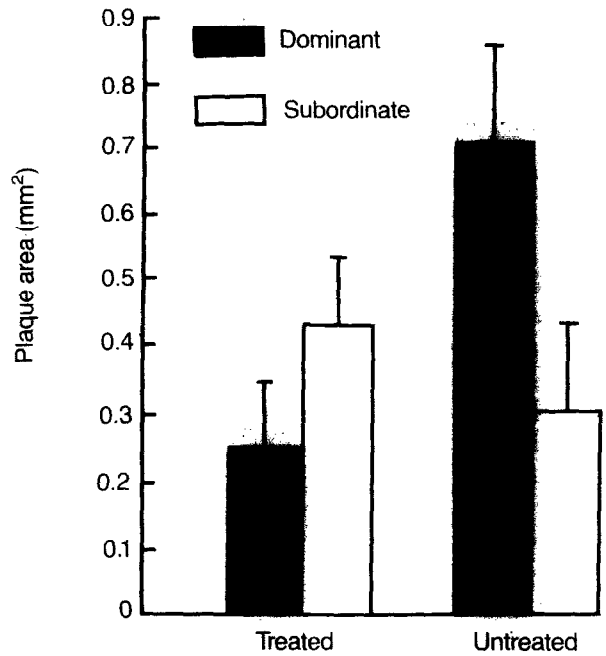

Fig. 2. Mean coronary artery plaque area measurements (± SEM) among dominant and subordinate monkeys in the propranolol-treated and untreated conditions. Note that all animals were housed in unstable social groups.

Behavioral observations were conducted during the investigation and the social status of each animal was determined. Propranolol had no effect on the animals' antagonistic or affiliative behaviors or on the stability of dominance relationships. Regarding atherosclerosis, analysis of variance revealed a significant interaction between treatment condition (i.e., propranolol-treated and untreated) and social status ($p < 0.03$). As illustrated in Fig. 2, dominant monkeys in the untreated condition developed roughly twice the coronary artery atherosclerosis of their subordinate counterparts. This observation replicates the increased atherosclerosis seen among

dominant animals assigned to similarly unstable social groups in our initial experiment (Fig. 1). Yet, social dominance *did not* influence atherosclerosis among the propranolol-treated animals; indeed, the mean intimal area of treated dominant monkeys approximated that of both treated and untreated subordinate animals. Hence we conclude that the exacerbated atherosclerosis characterizing autonomically intact dominant monkeys under unstable social conditions may be prevented by the long-term administration of a β-adrenergic–blocking agent.

At present the selective influence of propranolol on atherogenesis among dominant monkeys cannot be readily explained. This effect is not attributable to concomitant variability in serum lipids, for example, since neither total nor high-density lipoprotein cholesterol concentrations differed significantly between groups. It is conceivable that because of its lipophilicity and associated central nervous system penetrance, propranolol altered those behaviors that are most strongly associated with atherogenesis (e.g., aggression) and in this manner had a protective effect on the coronary arteries of treated dominant monkeys. As previously noted, propranolol did not influence species-typical behavior patterns of these animals, at least as detectable by customary techniques of behavioral observation.

That propranolol lowered heart rate and blood pressure equivalently in the dominant and subordinate monkeys also suggests that the hemodynamic sequelae of β-blockade fail to explain propranolol's delimited amelioration of the atherosclerosis of dominant monkeys. However, we should emphasize that heart rate and blood pressure measurements used to document the cardiovascular effects of propranolol were obtained under controlled conditions of laboratory assessment, often under anesthesia, and at times of convenience to the investigator. In this regard, we have previously noted that the heart rates of freely moving animals fluctuate greatly as a function of varying proximity and physical contact with other monkeys and that during the initial formation of new social groupings the magnitude of such fluctuations is significantly greater in dominant than in subordinate animals.[26] Extrapolating from these observations we speculate that propranolol may have damped cardiac responses to naturally occurring social challenges in the present experiment; this attenuation of behaviorally evoked hemodynamic reactions (and possibly associated metabolic adjustments) may have served in turn to mitigate effects of sympathetic-adrenal activation on atherogenesis, an effect that occurs preferentially among the more highly aggressive and competitive dominant animals.

CONCLUSIONS

The foregoing investigations demonstrate that behavioral factors contribute to atherosclerosis in the male cynomolgus macaque. The application of a psychosocial manipulation involving the recurrent reorganization of social group memberships was found to promote coronary artery atherogenesis among monkeys fed a cholesterol-containing diet, but only in animals that retained positions of social dominance during the experiment. In addition, this effect was independent of variability among animals in both blood pressure and serum lipid concentrations. In a second investigation, we found that the manipulation of group memberships was atherogenic, even among normolipoproteinemic animals (i.e., monkeys fed a prudent diet low in saturated fat and cholesterol). Related observations indicated that animals exhibiting the largest heart rate responses to a standardized laboratory stressor have more extensive coronary atherosclerosis and are more aggressive behaviorally than monkeys that show a less pronounced cardiac responsivity to stress. This finding is consistent with the hypothesis that exaggerated cardiovascular reactions to behavioral stimuli potentiate atherogenesis. The sympathetic nervous system mediation of behavioral influences on atherosclerosis is also supported by results of a third experimental study. Here, long-term administration of a β-adrenergic–blocking agent, propranolol hydrochloride, fully prevented the exacerbated coronary artery atherosclerosis that is characteristic of dominant animals living in unstable social environments and fed a moderately atherogenic diet.

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