Retarding Effect of Lowered Heart Rate on Coronary Atherosclerosis

Abstract. *The role of heart rate in the development of coronary atherosclerosis was assessed in adult male cynomolgus monkeys* (Macaca fascicularis). *Heart rate was lowered in six animals by surgical ablation of the sinoatrial node. A sham procedure, which included all of the surgical steps except for sinoatrial node ablation, was carried out in eight animals. All of the monkeys were fed an atherogenic high cholesterol diet for 6 months, and heart rates were monitored repeatedly by telemetry during 24-hour test periods. Coronary atherosclerosis in animals with postoperative heart rates less than the preoperative mean for all of the animals that underwent surgery was less than half that of animals with heart rates above the mean or of diet-fed control animals not subjected to surgery. Groups did not differ in blood pressure, serum lipids, or body weight. These results suggest that heart rate in itself may contribute to the mechanisms by which behavioral patterns and physical training influence coronary artery disease.*

Atherosclerosis does not affect all human arteries to the same extent. Coronary arteries, the carotid bifurcation, the abdominal aortic segment, and vessels supplying the lower extremities are frequently severely affected, whereas mesenteric, renal, pulmonary, intercostal, and mammary arteries are usually spared (*1*). Locations around branch points and curves are particularly prone to lesion development. These differences in distribution have been attributed, at least in part, to local hemodynamic effects (*2*). Although the mechanisms by which such effects influence plaque formation remain to be elucidated, evidence has accrued from quantitative correlative studies of the human carotid bifurcation that relatively high wall-shear stress is not the principal predisposing hemodynamic factor (*3*). Plaques are most prominent in the proximal internal carotid segment opposite the flow divider, where flow velocity and wall-shear stress are low and where flow separation results in vortex formation, recirculation, and delayed clearance of circulating particles. The hemodynamic features that correlate strongly with plaque formation in this location are accentuated by pulsatile flow (*4*) during which forward and reverse flow patterns are observed, particularly during the down-stroke of systole. Thus, low wall-shear stress and oscillations in shear stress direction between positive and negative values are the hemodynamic features most closely correlated with lesion localization at the carotid bifurcation, a major site of clinically significant disease.

Coronary artery plaques also tend to localize preferentially at the major proximal branch points in zones of presumed relatively low flow velocity and low wall-shear stress opposite the branch flow dividers (*5*). The predisposition of the coronary arteries to severe atherosclerosis, often out of proportion to the involvement of other susceptible vessels in the same arterial tree (*6*), may be attributable to a particularly marked effect of pulsatile flow at this location. Coronary arterial flow decreases initially in systole during the isovolumetric contraction and rapid ejection phases, increases briefly when peak systolic aortic pressure exceeds intracoronary pressure, and decreases again during the remainder of systole as intramyocardial pressure increases the resistance to flow. A net reverse flow has actually been shown to occur during systole with high heart rate (*7*).

In diastole, during isovolumetric relaxation, when both intramyocardial and intraventricular pressures are lowest, coronary blood flow is greatest, but decreases slowly as intraventricular pressure builds during ventricular filling (*8*). Thus, in contrast to other systemic arteries, the coronary arteries are subjected to two systolic episodes and one diastolic episode of flow acceleration and deceleration during each cardiac cycle. If the demonstrated quantitative relationships between flow field properties and plaque thickness about the carotid bifurcation also prevail in the coronary arteries, a relatively high heart rate should amplify atherogenesis selectively in the coronary arteries and a low heart rate should exert a relative sparing effect. This suggestion is strengthened by epidemiologic studies, which have revealed a positive correlation between heart rate and clinical coronary heart disease (*9*), and by experimental studies, which have linked psychosocial stress and marked excursions in heart rate with coronary artery disease (*10*).

To test whether heart rate could modify coronary atherogenesis independent of metabolic changes that may accompany physical conditioning or behavioral stress modification, we lowered heart rates in adult male cynomolgus monkeys by ablation of the sinoatrial node. This was accomplished in six of nine attempts by electrocautery of the crista terminalis at the entry of the superior vena cava into the right atrium and suture of the resulting defect. In eight other monkeys, we performed a sham surgical procedure identical in all respects to the ablation operation, except that the region of the sinoatrial node was not injured. One month after surgery, all of the monkeys that underwent surgery were fed an atherogenic diet that included 25 percent peanut oil and 2 percent cholesterol. The precise composition of the diet is described elsewhere (*11*). Heart rates during 24-hour test periods were recorded by radiotelemetry at four intervals: preoperatively, postoperatively at 4 weeks, and then bimonthly twice. Serum total and free cholesterol, triglycerides, phospholipids, and cholesterol esters as well as body weights were determined monthly. Blood pressure was measured directly at surgery and before animals were killed. Six months after the atherogenic

Table 1. Severity of coronary artery lesions in each experimental group. Differences between the groups receiving the sinoatrial node ablation and the sham operation were not significant for any of the three criteria of lesion severity. Average lesion severity in animals subjected to surgery did not differ significantly from that found in animals fed the atherogenic diet but not operated on (diet-only group). When animals that had been operated on were classified according to whether heart rate was below the preoperative mean (low heart rate) or above the preoperative mean (high heart rate), differences in average lesion severity were significant by all three evaluation criteria. Animals with high heart rates had twice as much disease as those with low heart rates.

Group	Number	Lesion area (mm^2)	Average stenosis*	Maximum stenosis†
Sinoatrial node ablation	6	0.35 ± 0.51	16.4 ± 14.0	31.1 ± 19.0
Sham operation	8	0.31 ± 0.44	20.4 ± 19.9	38.9 ± 28.4
Diet only	8	0.38 ± 0.19	23.8 ± 15.6	49.7 ± 24.4
High heart rate	5	0.48 ± 0.47	28.1 ± 20.9	55.9 ± 23.1
Low heart rate	12	0.21 ± 0.39	13.1 ± 12.2	26.1 ± 18.8
		$P < 0.05$	$P < 0.07$	$P < 0.02$

*Average percent stenosis for all animals within a group. †Average percent stenosis of lesions with the maximum stenosis for each animal within a group.

Table 2. Comparison of blood pressure, body weight, serum cholesterol, and triglyceride values for low heart rate and high heart rate groups. Differences between the groups were not significant.

Group	Number	Blood pressure (mmHg)		Weight (g)	Cholesterol (mg/dl)		Triglycerides (mg/dl)
		Systolic	Diastolic		Total	Free	
High heart rate	5	126 ± 8	74 ± 6	5984 ± 1819	838 ± 324	195 ± 102	9.5 ± 7.4
Low heart rate	12	129 ± 36	79 ± 21	5503 ± 762	656 ± 144	157 ± 45	10.6 ± 4.4
t-statistic		0.289	−0.720	0.751	1.208	0.802	−0.311

diet was started, each animal was anesthetized, and the heart and great vessels were fixed in situ by controlled and monitored pressure perfusion at 100 mmHg with 3 percent gluteraldehyde in Sorensen's phosphate buffer. Six samples of the proximal coronary artery tree, including sections of the right, left circumflex, and left anterior descending branches, were removed as complete transverse rings at standard, anatomically defined, sampling sites (*12*). Coronary atherosclerosis was evaluated from plastic-embedded semithin (1 μm) histologic sections by means of computer-assisted contour-tracing morphometry; the apparatus used has been described (*13*). The total number of coronary intimal lesions was determined for each monkey, and lesion cross-sectional area and percent stenosis were determined for each section. Percent stenosis was defined as the fraction of the area circumscribed by the internal elastic lamina that was occupied by intimal plaque. The vessels of eight control monkeys not operated on, but maintained on the same atherogenic diet for 6 months, were prepared and studied in the same manner as those of animals subjected to surgery. Each of the animals was housed in an individual cage under similar conditions. The investigator who measured the sections had no prior knowledge of treatment group or heart rate of the animals in the study. The Mann-Whitney test was used for statistical comparisons of morphologic measurements.

The preoperative heart rate for all of the animals treated surgically was 136 ± 22 beats per minute (mean ± standard deviation). In the six animals in which sinoatrial node ablation was successful, the heart rate was reduced 31 percent, from 148 ± 11 to 103 ± 20 beats per minute ($P < 0.001$). Reduced heart rate, which was apparent on the initial postoperative determination in these animals, persisted throughout the experimental period. The three animals of the sinoatrial node ablation group that did not have a reduced heart rate postoperatively, as well as the eight animals that had sham operations, maintained heart rates similar to those measured before surgery. The postoperative heart rate for all of the animals subjected to surgery, including successful sinoatrial node ablation, attempted ablation, and sham operation, was 120 ± 26 beats per minute. Five animals had mean heart rates above the preoperative mean for all animals (high heart rate group) and 12 had mean heart rates below the preoperative mean (low heart rate group). All six with successful sinoatrial node ablation were in the latter group (Fig. 1).

Lesion area, percent stenosis, and percent stenosis of the most occlusive lesion, were not significantly different for the animals having sinoatrial node ablation and those having the sham operation (Table 1). However, comparisons between the low and high heart rate groups showed significant differences (Table 1). In animals with heart rates above the preoperative mean for all animals, the number and severity of coronary artery lesions was more than twice that of animals with heart rates below the preoperative mean. The animals in the high heart rate group had an average percent stenosis of 28.1 ± 20.9, whereas those in the low heart rate group had an average percent stenosis of 13.1 ± 12.2 ($P < 0.07$); the average percent stenosis for the control group was 23.8 ± 15.6. Lesions with more than 25 percent stenosis were found in 41 percent of the coronary artery sections from animals with a high heart rate but in only 18 percent of the coronary artery sections from animals with a low heart rate; in the control group, 44 percent of the coronary artery sections had more than 25 percent stenosis. The average of the lesion areas for the animals with a high heart rate was 0.48 ± 0.47 mm^2, compared to 0.21 ± 0.39 mm^2 for those with a low heart rate ($P < 0.05$); in control animals, the average of the lesion areas was 0.38 ± 0.19 mm^2.

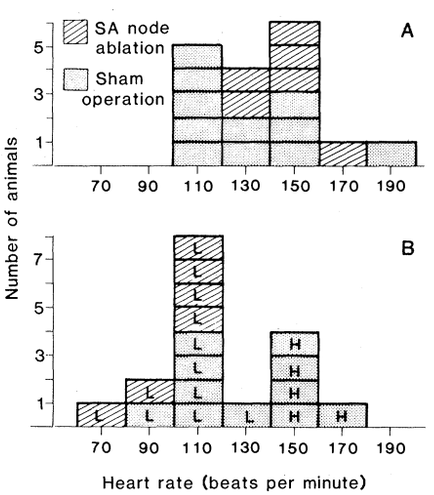

Fig. 1. Distribution of animals according to heart rates (A) before and (B) after surgery. Animals in the sinoatrial (SA) node ablation group are those that underwent successful sinoatrial node ablation as determined by a postoperative reduction in heart rate. Animals that underwent all steps of the operative procedure except sinoatrial node ablation or that experienced no reduction in heart rate after sinoatrial node ablation are in the sham operation group. The average preoperative heart rate for animals that underwent surgery was 136 ± 22 beats per minute. The average postoperative heart rate was 120 ± 26 beats per minute. Twelve of the animals after surgery had an average heart rate on multiple telemetric determinations that was less than the preoperative mean value. These constitute the low heart rate group (L). Five animals with an average heart rate after surgery above the preoperative mean constitute the high heart rate group (H).

The greatest difference between the groups was observed in the comparison of the percent stenosis by the most occlusive lesion (maximum stenosis). The most occlusive lesions averaged 55.9 ± 23.1 percent stenosis in the high heart rate group, 26.1 ± 18.8 percent stenosis ($P < 0.02$) in the low heart rate group, and 49.7 ± 22.2 percent stenosis in the control group. There were no significant differences between the high heart rate and low heart rate groups in blood pressure, body weight, or serum lipid levels (Table 2).

These findings support the hypothesis that a relatively low heart rate for an extended period tends to retard lesion formation in the coronary arteries. Although sinoatrial node ablation was used to produce a sufficient number of animals with a low heart rate, the coronary sparing effect was independent of the

surgical procedure. Such a relationship could help to account for the protective effect of regular physical activity against coronary artery disease in man (*14*) and in experimental animals (*15*), in that such conditioning results in lowered average heart rates (*16*) and therefore in a reduction in total number of heart beats for extended time periods. Lowered heart rate results in more total time spent in diastole when changes in the rate of flow and departures from laminar unidirectional flow are least. Conversely, the association of type A personality traits with coronary heart disease (*17*), and the experimental finding (*18*) that psychosocial stress in monkeys predisposes to coronary plaque formation, could both be the result of frequent stress-related elevations of heart rate that would tend to elevate average heart rate for extended periods. Although the mechanisms are not entirely understood, myocardial oxygen extraction is improved with regular physical activity or exercise training, and physically fit individuals have a lower baseline heart rate, a smaller increase in heart rate when stressed, and a more expeditious return to baseline than do untrained individuals (*19*). The resulting long-term reduced heart rate in physically trained individuals may also retard the progression of atherosclerotic lesions in their coronary arteries. The role of heart rate in the severity and distribution of atherosclerotic lesions merits further investigation.

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Rhodopsin Kinase Activity in the Mammalian Pineal Gland and Other Tissues

Abstract. *Rhodopsin kinase, an enzyme involved in photochemical transduction in the retina, has been found in the mammalian pineal gland in amounts equal to those in the retina; other tissues had 7 percent of this amount, or less. This finding suggests that, in mammals, rhodopsin kinase functions in the pineal gland and other tissues to phosphorylate rhodopsin-like integral membrane receptors and is thereby involved in signal transduction.*

Rhodopsin kinase (RK) is a soluble enzyme that selectively phosphorylates photon-activated rhodopsin (*1–3*). It is thought to play a critical adaptive role in photochemical transduction in the retina by reducing the potency of photon-activated rhodopsin to stimulate guanosine 3,′5′-monophosphate (cyclic GMP) phosphodiesterase (*4*). Rhodopsin kinase is generally considered a retinal enzyme, not present in other tissues. We now report that the mammalian pineal gland has RK activity equivalent to that observed in the retina; its activity in other tissues is less than 7 percent of that in the retina.

Rhodopsin kinase activity was measured by incubating crude homogenates or supernatants (30,000*g*) of rat tissues with [γ-^{32}P] adenosine triphosphate (ATP) and urea-treated rod outer segments (U-ROS) from bovine retina (*5*); phosphorylated opsin was extracted from the U-ROS and isolated by polyacrylamide gel electrophoresis in monomeric and dimeric forms (*6*) (Fig. 1). For quantitation, the monomer and dimer bands of opsin were identified and removed from the dried gel; the amount of radioactive label was then measured by conventional methods, and RK activity was calculated from the sum of the

Table 1. Rhodopsin kinase activity in selected rat tissues. Each assay was performed with about 5 μg of supernatant protein, [^{32}P]ATP (200 μ*M*), and U-ROS containing 0.5 nmol rhodopsin in 50 μl (*4, 5, 7*). The incubation period was 5 minutes.

Tissue	Activity (pmol·mg^{-1}·min^{-1})	Relative activity (percent of retinal activity)
Retina	193 ± 55	100
Pineal	131 ± 49	68
Cerebellum	13.7 ± 2.48	7.1
Cerebral cortex	13.1 ± 2.52	6.8
Hypothalmus	12.0 ± 1.72	6.2
Lung	9.0 ± 1.49	4.7
Olfactory lobe	7.7 ± 0.83	4.0
Brainstem	6.9 ± 0.76	3.6
Anterior pituitary	5.5 ± 1.11	2.8
Posterior pituitary	5.4 ± 1.31	2.8
Spleen	4.4 ± 0.80	2.3
Ovary	2.4 ± 0.29	1.2
Adrenal	2.3 ± 0.44	1.2
Liver	1.4 ± 0.78	0.6
Testes	1.1 ± 0.48	0.6
Harderian gland	0.67 ± 0.054	0.3
Kidney	0.45 ± 0.31	0.2