Reduction of plasma norepinephrine levels in response to brief coronary occlusion in experimental dogs

Although an increased plasma norepinephrine (NE) level is sometimes observed during angina pectoris, it is difficult to say whether sympathetic overflow is its cause. The left anterior descending coronary artery was occluded by intracoronary balloon for 3 minutes in 12 closed-chest anesthetized dogs. During occlusion, heart rate did not change but aortic pressure slightly decreased. Occlusion caused a significant reduction in both NE levels in the aorta (177 ± 17 to 134 ± 16 pg/ml, $p < 0.01$) and in the great cardiac vein (GCV) 296 ± 44 to 249 ± 44 pg/ml, $p < 0.01$). After surgical vagotomy, the occlusion increased NE levels in the aorta (227 ± 44 to 278 ± 43 pg/ml, $p < 0.01$) and in GCV (384 ± 76 to 444 ± 81 pg/ml, $p < 0.01$), showing the release of vagal inhibition. These results may be applicable to patients with transient anterior myocardial ischemia; if plasma NE increases without marked hemodynamic changes, it is suggested that the sympathetic overflow is not a result but a possible cause of the ischemia. (Am Heart J 109:1264, 1985.)

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By measuring plasma norepinephrine (NE) levels, sympathetic nerve excitation has been documented in some patients with angina pectoris[1-3] but not in others.[4-6] It is of current interest that there may be a generalized increase in sympathetic outflow associated with the initiation of spontaneous angina in some patients,[7,8] and caution is needed in the use of beta-adrenergic blockade in those patients.[5,9-12] If coronary vasospasm is induced by excessive sympathetic outflow, this excess will be detected as an elevation of the plasma NE level, and these data may be useful as a therapeutic guide. However, for the clinical application of NE measurement, it should be clarified experimentally whether the plasma NE level increases in response to myocardial ischemia; if the NE level increases with a brief coronary occlusion, we can not conclude that the increased plasma NE levels are the cause of the anginal attack. In addition, it should be confirmed whether or not peripheral plasma NE levels are influenced by NE release from the ischemic area, since anoxia-induced catecholamine release has been observed by acute experimental coronary occlusion.[13,14] If the NE level does not increase with transient ischemia, NE measurement will be helpful in distinguishing the cause of the attack. To elucidate these points, we studied the effect of a brief coronary occlusion on plasma NE levels in the aorta and coronary vein of anesthetized closed-chest dogs.

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METHODS

Preparation and analysis. The study was performed in a fasting state in 12 dogs weighing 15 to 30 kg. Anesthesia was initiated with an intravenous pentobarbital infusion of 30 mg/kg and was maintained with continuous drip infusion (4 mg/kg/hr). After intubation, artificial respiration of room air was instituted with a Harvard respirator. Systemic blood pressure and left ventricular pressure were measured with catheters inserted through the femoral artery. A No. 6 French catheter was inserted through the external jugular vein under fluoroscopic guide into the coronary sinus and was advanced into the great cardiac vein. A No. 7 French modified Judkins catheter was inserted through the femoral artery into the ostium of the left anterior descending artery (LAD). A No. 2 French Forgarty catheter was then passed through the Judkins catheter and advanced about 2 cm into the LAD. The Judkins catheter was then withdrawn into the ascending aorta. Heparin (2000 U) was given intravenously.

Plasma NE assay was carried out using a modification

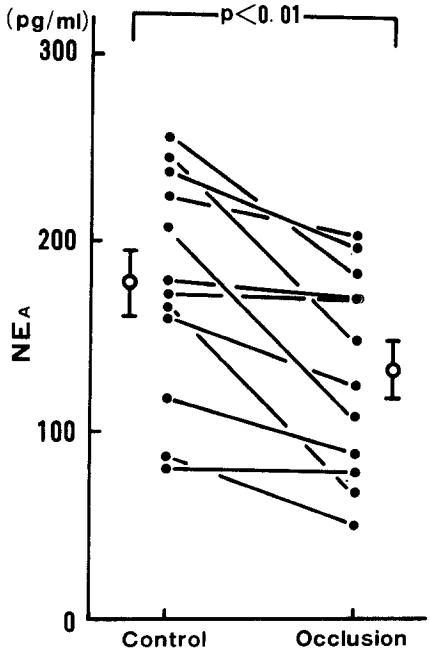

Fig. 1. Changes in norepinephrine levels in the aorta (NE_A) due to 3 minutes of LAD occlusion. LAD occlusion significantly decreased NE_A.

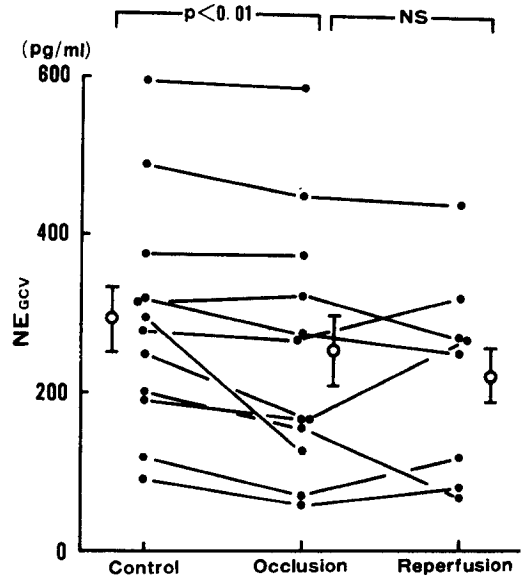

Fig. 2. Changes in norepinephrine levels in great cardiac vein (NE_{GCV}) due to 3 minutes of LAD occlusion and 1 minute of reperfusion. LAD occlusion significantly decreased NE_{GCV}, whereas reperfusion did not change those levels.

of Renzini's original procedure[15] within a fortnight after the blood samples were taken. This method, using a fluorescence spectrophotometer (Model MPF-4, Hitachi) equipped with a high-sensitivity cell assembly (no. 018-0050), permitted accurate measurement of levels as low as 25 to 50 pg of NE/aliquot of plasma.[16] Blood oxygen saturation and concentrations of lactate and potassium were analyzed with a co-oximeter (Model 182, Instrumentation Laboratory Inc., Lexington, Mass.), with a flame photometer (Model 143, Instrumentation Laboratory Inc.), and by the enzymatic method,[17] respectively. The myocardial lactate extraction ratio was calculated from the standard formula. Myocardial potassium balance was expressed as the difference between arterial and coronary venous serum potassium concentrations.

All statistical analyses were performed with the paired Student's *t* test. Mean values are expressed in terms of the mean ± the standard error of the mean.

Protocol. After instrumentation, the control hemodynamics were determined and 10 ml blood specimens were withdrawn simultaneously from the aorta and the great cardiac vein for oxygen, lactate, potassium, and NE determinations. The intracoronary balloon was then inflated for 3 minutes and measurements and samplings were repeated during the 2 to 3 minutes of occlusion. If no ST elevation was detected from the precordial ECG (leads V_3 to V_5), the balloon was deflated and the tip of the catheter was withdrawn to the proximal site of the LAD until ST elevation was obtained.

After 3 minutes of occlusion, the balloon was immediately deflated and blood for the NE assay was taken from the eight dogs after 1 minute of reperfusion. The bilateral cervical vagi were surgically cut in the eight dogs and the same protocol was repeated 30 minutes after vagotomy.

RESULTS

Changes in plasma NE levels in response to LAD occlusion. The hemodynamic and metabolic values of the 12 dogs are summarized in Table I. Although LAD occlusion did not cause a significant change in heart rate, it decreased mean aortic pressure, oxygen saturation in the great cardiac vein, myocardial lactate extraction ratio, and myocardial potassium balance, whereas the occlusion increased left ventricular end-diastolic pressure.

Changes in plasma NE levels due to the short-time occlusion of the LAD are shown in Table I and Figs. 1 and 2. Three minutes of occlusion caused a small but significant decrease in NE levels in the aorta (NE_A) in those dogs. As shown in Fig. 2, NE levels in the great cardiac vein (NE_{GCV}) also decreased in 10 of the 12 dogs. This reduction in NE_{GCV} did not change significantly after 1 minute of reperfusion in eight of the dogs.

In the eight dogs, bilateral cervical vagi were cut and the experiment was repeated. In this condition, LAD occlusion increased heart rate in seven of the eight dogs although no statistical significance could be shown (Table II). In the metabolic data, a significant reduction was found in the lactate extraction ratio and potassium balance during coro-

Table I. Hemodynamic and metabolic changes during LAD occlusion (12 dogs)

	Control	*Occlusion*	*p*
Heart rate (bpm)	144 ± 7*	140 ± 7	NS
Mean aortic pressure (mm Hg)	111 ± 7	97 ± 8	<0.01
Left ventricular end-diastolic pressure (mm Hg)	5 ± 1	8 ± 2	<0.01
Oxygen saturation in GCV (%)	28.9 ± 2.4	24.1 ± 2.0	<0.001
Myocardial lactate extraction ratio (%)	39.1 ± 3.3	19.1 ± 5.3	<0.01
Coronary arteriovenous potassium concentration difference (mEq/L)	0.04 ± 0.07	−0.12 ± 0.06	<0.05
Plasma NE concentration in the aorta (pg/ml)	177 ± 17	134 ± 16	<0.01
Plasma NE concentration in GCV (pg/ml)	296 ± 44	249 ± 44	<0.01

Abbreviations: LAD = left anterior descending coronary artery; GCV = great cardiac vein; NE = norepinephrine; NS = not significant.
*Mean ± standard error of the mean.

Table II. LAD occlusion after vagotomy (8 dogs)

	Control	*Occlusion*	*p*
Heart rate (bpm)	152 ± 13*	159 ± 15	NS
Mean aortic pressure (mm Hg)	130 ± 11	126 ± 9	NS
Left ventricular end-diastolic pressure (mm Hg)	5 ± 2	9 ± 2	<0.05
Oxygen saturation in GCV (%)	21.6 ± 2.3	20.4 ± 2.1	NS
Myocardial lactate extraction ratio (%)	36.8 ± 5.3	20.4 ± 7.8	<0.05
Coronary arteriovenous potassium difference (mEq/L)	−0.03 ± 0.05	−0.25 ± 0.09	<0.05
Plasma NE concentration in the aorta (pg/ml)	227 ± 44	278 ± 43	<0.01
Plasma NE concentration in GCV (pg/ml)	384 ± 76	444 ± 81	<0.01

Abbreviations as in Table I.
*Mean ± standard errors of the mean.

nary occlusion. Fig. 3 shows the effect of bilateral vagotomy on plasma NE changes due to the occlusion. In contrast with Figs. 1 and 2, both NE_A and NE_{GCV} were not decreased, but were increased by the occlusion.

Plasma NE changes in response to LCX occlusion. In an additional eight dogs, we occluded the left circumflex coronary artery (LCX) and carried out the same protocol. With LCX occlusion, hemodynamic data showed changes similar to those obtained with LAD occlusion; blood pressure decreased (125 ± 8 to 112 ± 10 mm Hg, $p < 0.01$) whereas heart rate did not change (126 ± 8 to 129 ± 8 bpm). Both NE_A and NE_{GCV} also decreased with LCX occlusion (261 ± 56 to 218 ± 51 pg/ml in NE_A and 346 ± 90 to 288 ± 88 pg/ml in NE_{GCV}, $p < 0.01$, respectively). However, after vagotomy, these levels increased with LCX occlusion (from 165 ± 43 to 227 ± 53 pg/ml in NE_A and 354 ± 98 to 401 ± 104 pg/ml in NE_{GCV}, $p < 0.05$, respectively).

Effects of repetitive sampling on plasma NE levels. To learn whether repetitive sampling has an effect on sympathetic nerve activity, plasma NE measurements were accomplished repetitively without occlusion in eight dogs which were instrumented in a fashion similar to that described above. In this condition, however, neither NE_A nor NE_{GCV} changed significantly (208 ± 50 to 226 ± 39 pg/ml in NE_A and 199 ± 26 to 175 ± 37 pg/ml in NE_{GCV}, respectively). Similarly, for the eight dogs in which bilateral cervical vagotomy was performed, eight control dogs also underwent repetitive measurement of plasma NE level at similar points in time without vagotomy. In this instance again, neither NE_A nor NE_{GCV} showed any significant change (176 ± 28 to 171 ± 28 pg/ml in NE_A and 210 ± 35 to 204 ± 33 pg/ml in NE_{GCV}, respectively).

DISCUSSION

Changes in plasma NE levels due to brief coronary occlusion. The first aim of this study was to determine whether a short coronary occlusion increases peripheral plasma NE levels. For this purpose, care should be taken to avoid sympathetic nerve suppression due to the experimental procedure. In this study, pentobarbital was chosen as the anesthetic because the inhibitory action of the present doses of barbiturate on cardiovascular reflexes is predominant on vagal rather than on sympathetic outflow from the central nervous system.[18] In addition, these doses of barbiturate do not increase plasma NE levels during the control periods.[19,20] We also chose

LAD as the occlusion site, since sympathetic overactivity may occur more frequently in anterior than in posterior wall ischemia.[21] Moreover, it should be emphasized that the present model keeps the neuroaxis and the pericoronary nerves of the experimental dogs intact. In this condition, a brief coronary occlusion did not increase, but significantly decreased the plasma NE level. This reduction is not simply a result of repetitive sampling, since the control study showed no significant changes in plasma NE level in the absence of coronary occlusion. To determine the role of vagal excitation on plasma NE level, we repeated the LAD occlusion after bilateral cervical vagotomy. As shown in Fig. 3, an increase in plasma NE became evident during coronary occlusion and this result suggests an alternation of the net autonomic nerve balance between both sympathetic and vagal cardiac afferent fibers. In an additional eight dogs, we occluded the LCX. Results were almost the same as those from LAD occlusion, suggesting no regional difference in NE response to acute myocardial ischemia.

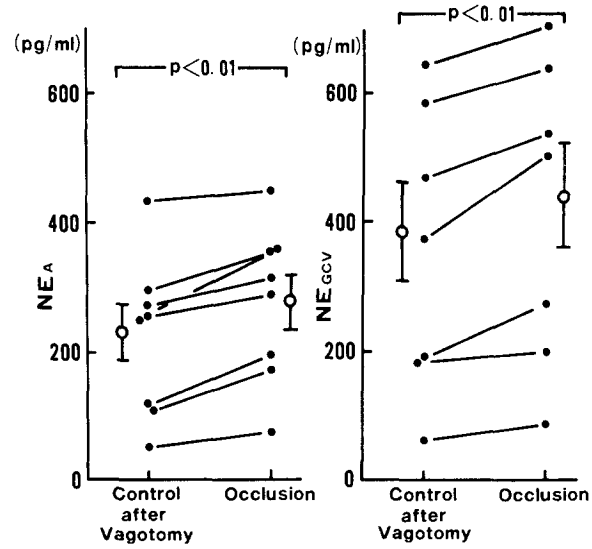

Fig. 3. Changes in NE_A and NE_{GCV} in response to 3 minutes of LAD occlusion after vagotomy. In contrast with Figs. 1 and 2, both NE_A and NE_{GCV} significantly increased with LAD occlusion.

Changes in NE overflow from the myocardium due to brief coronary occlusion. Another aim of this study was to examine whether the change in peripheral plasma NE level is influenced by NE overflow from the ischemic area. Acute experimental coronary occlusion was accompanied by marked catecholamine release into the blood stream,[13,14] and this release can be ascribed to the anoxic effect on the cathecholamine stores in the heart.[14] In the present study, the blood taken from the great cardiac vein showed a significant reduction in oxygen saturation, lactate extraction ratio, and potassium arteriovenous (AV) difference during LAD occlusion. This means that the specimen contained blood from the ischemic myocardium via collateral channels, and thus the NE level could reflect NE overflow from the ischemic areas. Despite the selective sampling, plasma NE in the great cardiac vein did not increase but rather decreased during LAD occlusion. It is possible that we did not capture the NE overflow from the ischemic area because of insufficient washout. However, no increase in NE level was evident after 1 minute of balloon deflation in the seven of eight dogs studied in this way. It should be noted that the previous studies were performed using a Langendorff preparation or an open-chest procedure, which were far from the physiologic condition. Thus it is unlikely that rapid liberation of NE is induced by short-time coronary occlusion under physiologic conditions. After cervical vagotomy, the occlusion increased NE_{GCV} as well as NE_A, suggesting that both coronary venous and peripheral plasma NE levels are largely controlled by the same factor during acute ischemia.

Clinical implications. From the present results, we can speculate that transient myocardial ischemia, such as angina pectoris, does not increase plasma NE levels despite a fall in blood pressure. This is in striking contrast with NE response to acute myocardial infarction in which there is a marked increase of plasma NE level.[2] Probably this difference depends on the time course after the onset of ischemia and hemodynamic changes. Pressure responses to coronary occlusion in the present study showed trends similar to those seen in clinical data obtained during spontaneous angina, in which mean aortic pressure was unchanged or fell, whereas pulmonary capillary pressure rose significantly.[23-26] The observation that heart rate did not change in response to coronary occlusion was also similar to clinical reports of patients with spontaneous angina,[25,26] although a slight increase in heart rate was noted in other reports.[23,24] One might question whether the plasma NE level can promptly decrease with the inhibition of sympathetic nerve excitation. However, there have been several reports which show a reduction in plasma NE in response to vagal excitation.[27,28] One might also assume that the NE level would increase due to anxiety or pain during the attack, which would not have occurred in this experimental model. However, with respect to pain, clinical data do not appear to suggest an increase in plasma NE level.[29] Schwartz et al.[4] observed no increase in plasma NE

levels during pacing-induced anginal pain, and Robertson et al.[5,6] reported that pain did not increase NE level in patients with spontaneous angina. We have also observed a patient in whom the coronary sinus NE level decreased during pacing-induced severe anginal pain.[30] Thus it is reasonable to assume that the pain sensation does not significantly affect the plasma NE levels.

All of these results enable us to assume that plasma NE levels will provide significant information for differentiating the causes of angina pectoris; acute ischemia itself will not increase plasma NE level. Therefore, if plasma NE levels increase in those patients without marked hemodynamic changes, this increase would not be the result but the cause of acute ischemia. Recent advances in the methodology of NE assay are making it possible routinely to measure plasma NE in patients with angina pectoris. We believe that measurement of peripheral plasma NE levels is helpful in evaluating the initial cardiac sympathetic tone in spontaneous angina.

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