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Is the Development of Myocardial Tolerance to Repeated Ischemia in Humans Due to Preconditioning or to Collateral Recruitment?

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OBJECTIVES The purpose of this study in patients with quantitatively determined, poorly developed coronary collaterals was to assess the contribution of ischemic as well as adenosine-induced preconditioning and of collateral recruitment to the development of tolerance against repetitive myocardial ischemia.

BACKGROUND The development of myocardial tolerance to repeated ischemia is nowadays interpreted to be due to biochemical adaptation (i.e., ischemic preconditioning).

METHODS In 30 patients undergoing percutaneous transluminal coronary angioplasty, myocardial adaptation to ischemia was measured using intracoronary (i.c.) electrocardiographic (ECG) ST segment elevation changes obtained from a 0.014-in. (0.036 cm) pressure guidewire positioned distal to the stenosis during three subsequent 2-min balloon occlusions. Simultaneously, an i.c. pressure-derived collateral flow index (CFI, no unit) was determined as the ratio between distal occlusive minus central venous pressure divided by the mean aortic minus central venous pressure. The study patients were divided into two groups according to the pretreatment with i.c. adenosine (2.4 mg/min for 10 min starting 20 min before the first occlusion, n = 15) or with normal saline (control group, n = 15).

RESULTS Collateral flow index at the first occlusion was not different between the groups (0.15 ± 0.10 in the adenosine group and 0.13 ± 0.11 in the control group, p = NS), and it increased significantly and similarly to 0.20 ± 0.14 and to 0.19 ± 0.10, respectively ($p < 0.01$) during the third occlusion. The i.c. ECG ST elevation (normalized for the QRS amplitude) was not different between the two groups at the first occlusion (0.25 ± 0.13 in the adenosine group, 0.25 ± 0.19 in the control group). It decreased significantly during subsequent coronary occlusions to 0.20 ± 0.15 and to 0.17 ± 0.13, respectively. There was a correlation between the change in CFI (first to third occlusion; ΔCFI) and the respective ST elevation shift (ΔST): $\Delta ST = -0.02$ to $0.78 \times \Delta CFI$; r = 0.54, p = 0.02.

CONCLUSIONS Even in patients with few coronary collaterals, the myocardial adaptation to repetitive ischemia is closely related to collateral recruitment. Pharmacologic preconditioning using a treatment with i.c. adenosine before angioplasty does not occur. The variable responses of ECG signs of ischemic adaptation to collateral channel opening suggest that ischemic preconditioning is a relevant factor in the development of ischemic tolerance. (J Am Coll Cardiol 1999;33:1027–35) © 1999 by the American College of Cardiology

In 1986 Murry and coworkers introduced the term "ischemic preconditioning," and referred to it as myocardial adaptation to ischemic stress induced by repetitive brief periods of ischemia and reperfusion (1). Warm-up and walking through angina pectoris, traditionally ascribed to coronary vasodilation with opening of collateral channels, are nowadays alternatively interpreted as myoacardial tolerance to ischemia due to preconditioning, a biochemical process possibly triggered and mediated by the release of adenosine from ischemic myocytes, and by the subsequent activation of adenosine A1 receptors (2). The earlier, biomechanical interpretation for the development of ischemic tolerance has been abandoned because the presence of collaterals on angiography could not be shown to predict warm-up angina (3). Moreover, a considerable body of in vitro and experimental evidence has been accumulated

See page 1036

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Abbreviations and Acronyms

CAD = coronary artery disease
CFI = collateral flow index
CR = collateral recruitment
CVP = central venous pressure
ECG = electrocardiography
i.c. = intracoronary
IT = ischemic tolerance (of the myocardium)
P = (ischemic) preconditioning
P_{ao} = (mean) aortic pressure
P_{occl} = distal coronary artery pressure during balloon occlusion (coronary wedge pressure)

showing that ischemic preconditioning leads to ischemic tolerance via a cascade of events involving triggers (such as adenosine, bradykinin, intracellular Ca^{2+}), signaling pathways (via the A1 adenosine receptor, adenosine triphosphate–sensitive K^+ channels, protein kinase C) and end-effector(s) (2,4). Aside from ischemic preconditioning, also pharmacologic induction of myocardial tolerance against ischemia (IT) has been investigated, and, using adenosine before percutaneous transluminal coronary angioplasty, pharmacologic preconditioning has been recently suggested to occur in humans (5). Despite accumulating evidence supporting the occurrence of preconditioning (P) in humans, be it ischemic or pharmacologic, its clinical relevance is still controversial (4,6–8). This may be related to the fact that, so far, the biomechanical term of the equation (i.e., collateral recruitment, CR), IT = P + CR, has not been appropriately taken into account. Although it has been recognized that opening of collateral channels during coronary occlusion does occur in humans (9–11), attempts to assess the contribution of CR in the mentioned equation have generally been omitted (5,12) or have been performed using insensitive means to measure coronary collaterals (13). Measurements of coronary wedge pressure, that is, intracoronary pressure distal to the occluded, collateral receiving (i.e., ipsilateral) vessel obtained simultaneously with aortic pressure, have been demonstrated to accurately determine relative collateral flow in terms of normal flow through the patent vessel (14–16).

The purpose of this study using repeated coronary artery occlusions was to assess the contribution of ischemic as well as adenosine-induced pharmacologic preconditioning and of collateral recruitment to the development of tolerance against myocardial ischemia in patients with angiographically poor collaterals.

METHODS

Patients. Thirty patients (age 57 ± 10 years, 27 men, 3 women) with one- to two-vessel coronary artery disease (CAD) were included in the study. All underwent percutaneous transluminal coronary angioplasty of one stenotic lesion because of symptoms related to CAD. Patients were prospectively selected on the basis of the following criteria: 1) few if any angiographic coronary collaterals before the first occlusion (degree <2 on a 1 to 3 scale according to Rentrop et al. [17]); 2) no previous infarction in the myocardial area undergoing angioplasty; 3) no conduction defects on electrocardiography (ECG); 4) no baseline ECG ST segment abnormalities, and 5) no ECG signs of left ventricular hypertrophy.

The present investigation was approved by the institutional ethics committee, and the patients gave informed consent to participate in the study.

The study population was divided into two groups according to the allocation to a 10-min intracoronary (i.c.) infusion starting 20 min before angioplasty and alternating consecutively between adenosine (2.4 mg/min, adenosine group, n = 15) and normal saline (control group, n = 15).

Cardiac catheterization and coronary angiography. Patients underwent left heart catheterization for diagnostic purposes. Aortic pressure was measured using the angioplasty guiding catheter. Biplane left ventricular angiography was performed followed by coronary angiography. Coronary artery stenoses were estimated quantitatively as percent diameter reduction using the guiding catheter for calibration. Angiographic collateral degrees (0 to 3) were determined according to the extent of epicardial coronary artery filling via collaterals with contrast medium from the contralateral side before angioplasty: 0 = no filling of the distal vessel via collaterals, 1 = small side branches filled, 2 = major side branches of the main epicardial vessel filled, 3 = main epicardial vessel filled by collaterals (17).

Coronary collateral assessment. A 0.014-in. (0.036 cm) fiberoptic pressure monitoring guidewire (Pressureguide, Radi Medical, Uppsala, Sweden) was set at zero, calibrated, advanced through the guiding catheter and positioned distal to the stenosis to be dilated (18–20). The i.c. pressure-derived collateral flow index (CFI, no unit) was determined by simultaneous measurement of mean aortic pressure (P_{ao}, mm Hg, via the angioplasty guiding catheter) and the distal coronary artery pressure during balloon occlusion (P_{occl}, mm Hg, Figs. 1 and 2). Central venous pressure (CVP) was estimated to be equal to 5 mm Hg. Collateral flow index was calculated as (P_{occl} − CVP) divided by (P_{ao} − CVP) (15). Collateral flow index expresses collateral flow relative to normal flow through the patent vessel, an index that has been validated recently (16). Collateral flow index was determined 1 min after the start of each of the occlusions.

Study protocol. After diagnostic coronary angiography including angiographic collateral assessment, an interval of at least 10 min was allowed for dissipation of the effect of the nonionic contrast medium (iopamidol 755 mg/ml) on coronary flow velocity and vasomotion. No i.c. or sublingual nitroglycerin was given before or until after the completion of the study protocol. The pressure guidewire was posi-

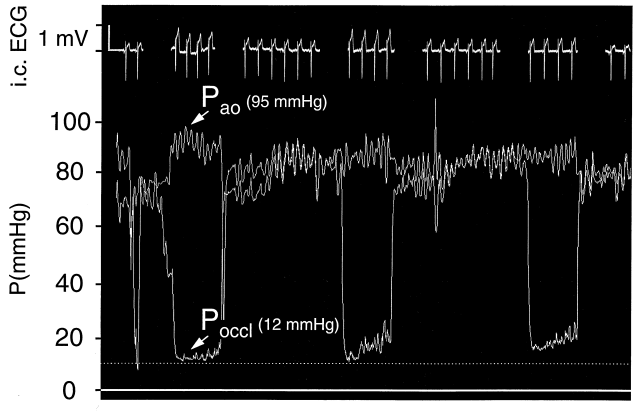

Figure 1. Tracings of simultaneous mean aortic pressure (P_{ao}), distal coronary artery pressure (P_{occl}) and an intracoronary (i.c.) electrocardiographic (ECG) lead obtained in a patient with marked collateral recruitment during the three 2-min coronary artery occlusions (time: **horizontal axis**). The distal coronary artery pressure during the balloon occlusions (i.e., i.c. wedge pressure, P_{occl} = 12 mm Hg) is close to the **dotted line** (= 10 mm Hg) and increases during as well as between each of the occlusions. Meanwhile, P_{ao} remains relatively constant. Collateral flow index during each of the occlusions is calculated as (P_{occl} − CVP)/(P_{ao} − CVP); CVP: central venous pressure = 5 mm Hg. The i.c. ECG lead obtained via the pressure guidewire for the measurement of P_{occl} shows marked ST elevation during the occlusions that decreases between the first and third occlusion.

tioned distal to the stenosis to be dilated. During the entire protocol, an i.c. ECG obtained from the pressure guidewire (21) and a three-lead surface ECG were recorded. In case of atrioventricular node conduction disturbances during adenosine infusion, the i.c. pressure guidewire would have been used for ventricular pacing (21). Adenosine or saline was infused into the proximal portion of the vessel undergoing angioplasty via the angioplasty guiding catheter. Adenosine (Krenosine, 6 mg per 2 ml) was dissolved in sterile saline 0.9% (24 mg in 25 ml) and infused at a rate of 2.4 mg/min over 10 min. The control group received an equivalent volume of normal saline 0.9%. After the infusion and after a 10-min drug-free period, the angioplasty catheter was placed at the site of the stenotic lesion, whereby the 0.014-in. (0.036 cm) pressure guidewire was used to transport the angioplasty balloon. Angioplasty was performed using balloon dilation catheters ranging in diameter from 2.5 to 4.0 mm chosen according to estimates of the size of the vascular regions adjacent to the stenosis. Measurements of coronary wedge (P_{occl}, mm Hg) and simultaneous mean aortic pressure (P_{ao}, mm Hg) as well as i.c. and surface lead ECG recordings were performed during a first 2-min coronary artery balloon occlusion (Figs. 1 and 2). Occlusions were followed by a 5-min reperfusion period. During the reperfusion intervals, the angioplasty balloons were withdrawn from the stenotic lesion, and the pressure wire was left in place. Patients underwent a total of three balloon occlusions.

Myocardial ischemia assessment. Assessment of myocardial ischemia during the three subsequent coronary occlusions was performed off-line using the ST segment change (Figs. 1 and 2; 1 mm = 0.1 mV) of the i.c. ECG (relative to the QRS amplitude). ST segment change was determined at 1 min after the start of each of the occlusions. The ST segment shift was determined 80 ms after the J point.

Angina pectoris assessment. At the beginning of the procedure, patients were informed that they may develop chest pain during balloon inflations. At the end of each occlusion, the intensity of angina pectoris was determined using a visual analog scale (22). Patients were asked to set a mark on a scale of 10 ranging from no pain (0) to the most severe pain (10).

Statistical analysis. Between-group comparisons of continuous demographic, angiographic, hemodynamic, ECG ST shift and i.c. pressure-derived collateral flow index data were performed by a Student *t* test. Coronary collateral data during different time points among patients of the same group were analyzed using analysis of variance for repeated measurements. A chi-square test was used for comparison of categorial variables among the two study groups. Linear regression analysis was used for assessing the relation between collateral flow index changes and i.c. ECG ST segment shift changes during subsequent coronary occlusions. Mean values ± SD are given. Statistical significance was defined at a p value of <0.05.

RESULTS

Patient characteristics and clinical data. There were no statistically significant differences between the adenosine and the control group regarding age of the patients, gender,

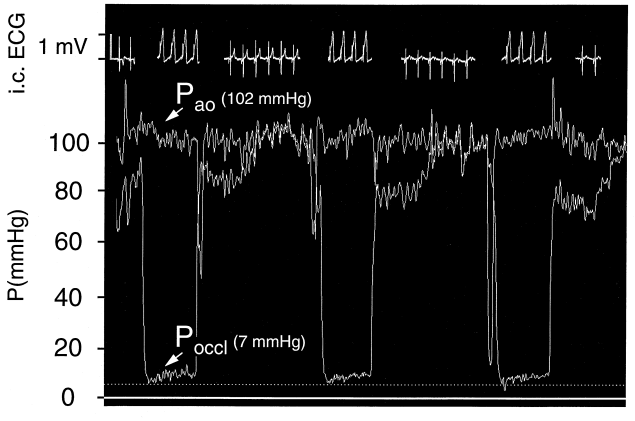

Figure 2. Tracings of simultaneous P_{ao}, distal coronary artery pressure and an i.c. ECG lead obtained in a patient with little collateral recruitment during the three 2-min coronary artery occlusions (time: **horizontal axis**). The **dotted line** indicates a pressure of 5 mm Hg. The i.c. ECG ST segment elevations during the three occlusions remain constant. See Figure 1 for the calculation of the collateral flow index. Abbreviations as in Figure 1.

Table 1. Patient Characteristics and Clinical Data

	Adenosine (n = 15)	NaCl 0.9% (n = 15)	p
Age (yr)	59 ± 10	55 ± 11	NS
Men (%)	14 (93)	13 (87)	NS
NYHA class	1.8 ± 0.7	2.0 ± 0.6	NS
Smoking (%)	2 (13)	4 (27)	NS
Systemic hypertension (%)	4 (27)	8 (53)	NS
Diabetes mellitus (%)	0 (0)	3 (20)	NS
Medication			
Calcium antagonists (%)	4 (27)	2 (13)	NS
Beta-blockers (%)	10 (67)	12 (80)	NS
Nitrates (%)	3 (20)	2 (13)	NS
Lipid-lowering agents (%)	5 (33)	3 (20)	NS

NYHA = New York Heart Association.

New York Heart Association class, the frequency of cardiovascular risk factors and the use of vasoactive and lipid-lowering substances (Table 1). None of the patients used sulfonylurea drugs or nicorandil. No sedatives or analgesics were given during the balloon occlusions.

Angiographic and coronary collateral data. The occurrence of a previous myocardial infarction in nonangioplasty myocardial territory and of a previous angioplasty left ventricular systolic function, the number of vessels affected by coronary artery disease and the severity of the stenosis undergoing angioplasty did not differ between the adenosine and the control group (Table 2). There were no statistical differences among the study groups regarding the frequency of the vessels or the site of the stenosis treated by angioplasty.

Table 2. Angiographic and Coronary Collateral Data

	Adenosine (n = 15)	NaCl 0.9% (n = 15)	p
Previous infarction in non-PTCA territory (%)	4 (27)	4 (27)	NS
Previous PTCA (%)	1 (7)	1 (7)	NS
LV ejection fraction	67 ± 9%	69 ± 5%	NS
Coronary arteries involved	1.9 ± 0.8	1.5 ± 0.6	NS
Diameter stenosis before PTCA	84 ± 9%	82 ± 11%	NS
Coronary artery undergoing PTCA			
LAD (%)	10 (67)	8 (53)	NS
LCX (%)	3 (20)	2 (13)	NS
RCA (%)	2 (13)	5 (33)	NS
Site of PTCA			
Proximal third (%)	8 (53)	5 (33)	NS
Middle third (%)	7 (47)	9 (60)	NS
Distal third (%)	0 (0)	1 (7)	NS
Chest pain score	6.5 ± 1.5	6.3 ± 2.0	NS
Intracoronary ECG ST shift (normalized)	0.25 ± 0.13	0.25 ± 0.19	NS
Coronary collateral data before or during 1st PTCA			
Collateral flow index	0.15 ± 0.10	0.13 ± 0.11	NS
Angiographic collateral degree (0–3)*	1.2 ± 0.6	1.0 ± 0.7	NS

*Assessed before PTCA and before treatment with adenosine.

LAD = left anterior descending coronary artery; LCX = left circumflex coronary artery; LV = left ventricular; PTCA = percutaneous transluminal angioplasty; RCA = right coronary artery.

Table 3. Data on Myocardial Ischemic Adaptation

	Adenosine (n = 15)	NaCl 0.9% (n = 15)	p
Collateral flow index (no unit)			
1st occlusion*	0.15 ± 0.10	0.13 ± 0.11	NS
2nd occlusion	0.17 ± 0.11	0.15 ± 0.10	NS
3rd occlusion	0.20 ± 0.14†	0.19 ± 0.10†	NS
Normalized i.c. ECG ST shift (ST/QRS amplitude)			
1st occlusion	0.25 ± 0.13	0.25 ± 0.19	NS
2nd occlusion	0.25 ± 0.14	0.24 ± 0.18	NS
3rd occlusion	0.20 ± 0.15†	0.17 ± 0.13†	NS
Chest pain score (cm)			
1st occlusion	6.5 ± 1.5	6.3 ± 2.0	NS
2nd occlusion	6.8 ± 1.8	5.8 ± 2.3	NS
3rd occlusion	5.3 ± 2.5†	4.8 ± 1.5†	NS
RPP ($\times 10^3$)			
1st occlusion	9.5 ± 1.7	8.6 ± 2.7	NS
2nd occlusion	9.5 ± 1.7	8.8 ± 2.5	NS
3rd occlusion	9.3 ± 1.6	8.9 ± 2.8	NS

*Values during occlusions obtained 1 min after occlusion. †$p < 0.05$ versus 1st occlusion (for exact p values see Fig. 4, A and B); ECG = electrocardiographic; i.c. = intracoronary; RPP = heart rate × systolic blood pressure.

During the adenosine infusion, blood pressure and heart rate changed from 122 ± 12 over 83 ± 7 mm Hg to 111 ± 15 over 75 ± 10 mm Hg ($p < 0.05$ for systolic pressure), and from 78 ± 12 to 89 ± 14 beats per minute ($p < 0.05$), respectively, whereas it remained unaltered in the control group receiving saline. None of the patients showed atrioventricular node conduction disturbances that required pacing.

The qualitative and quantitative variables for the assessment of the collateral circulation obtained during the first coronary occlusion indicated the presence of low degree collaterals and did not differ between the study groups (Table 2). All patients of both groups did develop chest pain and ST segment shift on i.c. ECG during the first coronary occlusion. Pressure-derived collateral flow relative to normal antegrade flow during vessel patency (CFI) was equal to approximately 15% in both groups, a value that corresponded to an angiographic collateral degree of about 1.2.

Coronary occlusion–induced myocardial ischemic adaptation. Table 3 illustrates that in the adenosine-treated and control patients, there was a similar increase and decrease during subsequent coronary occlusions in the collateral flow index and i.c. ECG ST shift, respectively. These changes became statistically significant when compared between the first and third occlusion. Chest pain during subsequent balloon occlusions of the stenosis diminished similarly among the study groups ($p < 0.05$ between the first and

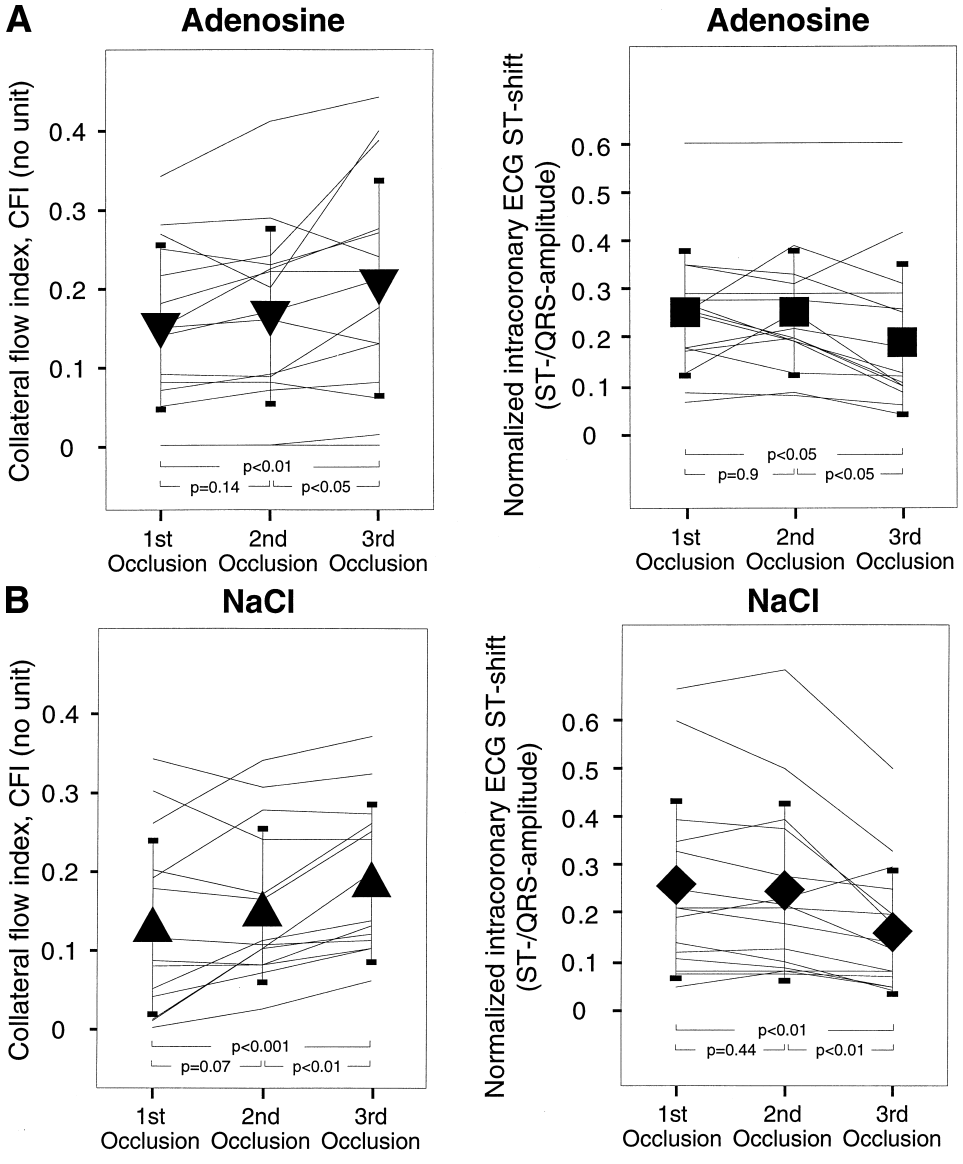

Figure 3. Individual data of collateral flow index (CFI; **vertical axis, left-hand side**) and the ST segment shift (normalized intracoronary electrocardiographic [ECG] ST shift; **vertical axis, right-hand side**) during the three subsequent 2-min coronary artery occlusions **(horizontal axis)** for the patients receiving i.c. adenosine (2.4 mg/min, **panel A**) and for those receiving normal saline (NaCl, **panel B**). The **filled symbols** with **vertical lines** indicate mean values ± SD.

third occlusion), whereas an index for myocardial oxygen consumption during the occlusions, the heart rate–pressure product, remained constant. Figure 3 provides the individual changes of both study groups of the collateral flow index and the normalized i.c. ECG ST segment during the three occlusions. When comparing the adenosine with the control group during each of the occlusions, there was no statistical difference between collateral flow index or i.c. ECG ST segment shift. In both the adenosine and the control group, there was an inverse overall behavior of changes in collateral flow and ST segment shift, that is, an increase in collateral flow index during subsequent coronary occlusions (collateral recruitment) was accompanied by a decrease in i.c. ECG ST segment shift (Fig. 4). Figure 5 shows the significant, inverse correlation between the changes in collateral flow from occlusion #1 to #3 and the corresponding ST segment shifts for all patients. The respective regression equations for the association among collateral changes (ΔCFI) and i.c. ECG ST segments shifts (ΔST) in the two groups were, respectively, adenosine group: $\Delta ST = -0.019 - 0.61 \times \Delta CFI$; $r^2 = 0.34$, $p = 0.03$; control group: $\Delta ST = -0.025 - 0.85 \times \Delta CFI$; $r^2 = 0.26$, $p = 0.05$.

DISCUSSION

The results of this study show that in a population with poorly grown coronary collaterals, the myocardial tolerance to repetitive ischemia is closely related to collateral recruit-

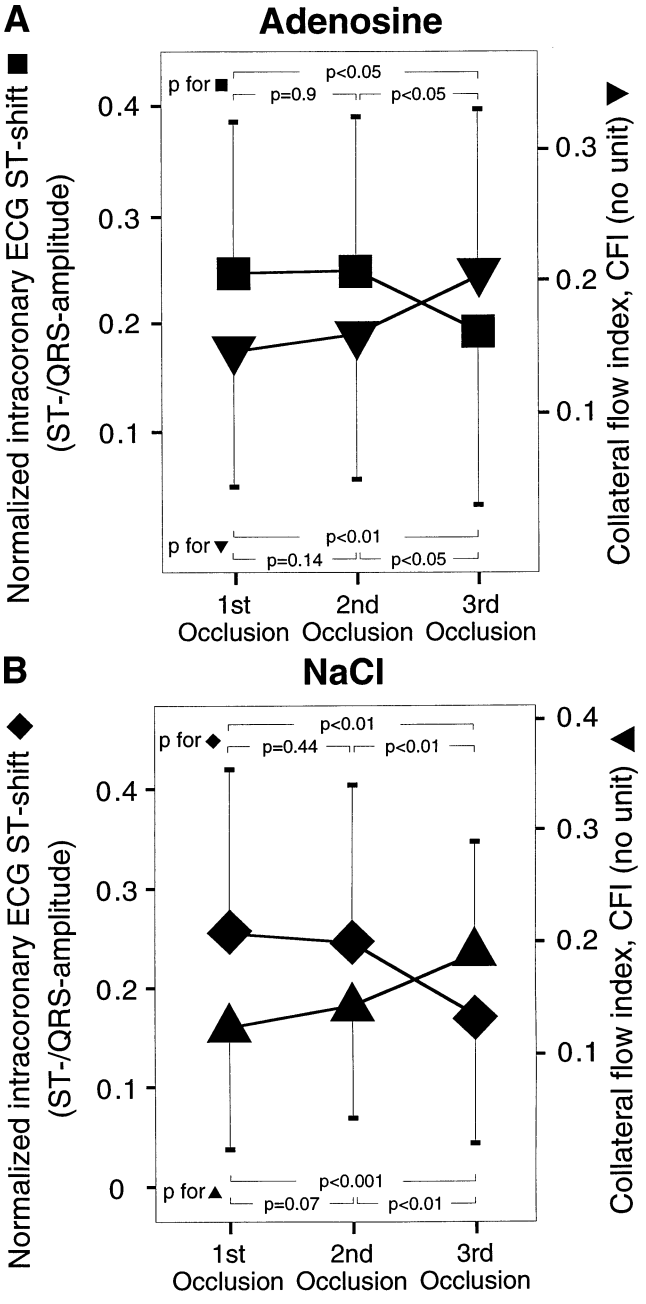

Figure 4. Combined mean values (± SD, **vertical lines**) of the normalized intracoronary electrocardiographic (ECG) ST elevation **(squares, vertical axis, left-hand side)** and the collateral flow index (CFI) **(triangles, vertical axis, right-hand side)** during the three subsequent 2-min balloon occlusions **(horizontal axis)** for the patients pretreated with adenosine **(A)** and for those pretreated with normal saline **(B)**.

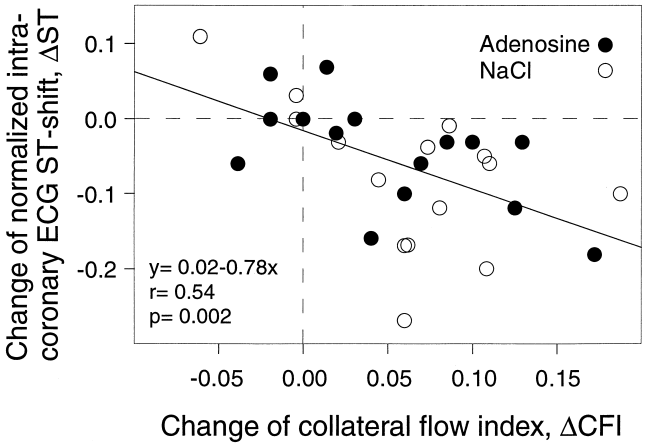

Figure 5. Inverse correlation between the change in collateral flow index (ΔCFI, no unit; **horizontal axis**) and the normalized intracoronary electrocardiographic (ECG) ST-elevation changes (ΔST, no unit; **vertical axis**) determined from the first to the third occlusion. **Filled symbols**: patients pretreated with adenosine; **open symbols**: patients pretreated with normal saline.

ment. Pharmacologic preconditioning using a treatment with i.c. adenosine before angioplasty does not occur in the sense that, compared to untreated patients, signs of myocardial ischemia are attenuated at the first occlusion. The variable responses of ECG signs of myocardial ischemic adaptation to collateral channel opening (Fig. 5) suggest that ischemic preconditioning is a relevant factor in the equation: ischemic tolerance = preconditioning + collateral recruitment.

Mechanisms of myocardial ischemic adaptation during angioplasty: data from the literature. In principle, two components—preconditioning and/or coronary collateral channel opening—may contribute to the myocardial ischemic tolerance developing during subsequent coronary balloon occlusions. Recently, the focus of investigations in humans on myocardial ischemic adaptation has been mainly on the preconditioning term of the previously mentioned equation (7,12,23–29), and rarely on the collateral recruitment term (9–11), as if one or the other component would be entirely responsible for the phenomenon. This situation may relate to the fact that end points for each of the terms of the equation are not clearly defined or that they are difficult to measure. It may even seem justifiable not to account for one of the terms, for example, collateral recruitment, if it seems negligible in size, for example, in the presence of very few collaterals. Then the amount of ischemic myocardial tolerance as measured by, for example, ECG ST segment shift during angioplasty (30,31), would be equal to the preconditioning term. However, collateral recruitment can only be neglected if a sensitive method to measure collaterals can demonstrate that it is insignificant. So far and with the exception of two very recent investigations (13,32), it has been assumed that collateral recruitment contributes insignificantly to ischemic tolerance in patients with angiographically few coronary collaterals. Sakata et al. (13) have used myocardial contrast echocardiography to measure the contribution of collateral opening to ischemic adaptation after repeated coronary occlusions. This method has not been properly validated so far, and it is likely too insensitive to measure or even detect subtle

increases in collateral flow in patients with intrinsically few collaterals. Tomai et al. (32) have demonstrated that ischemic preconditioning does occur in the setting of repeated occlusions by accounting for an index of collateral flow, that is, the absolute flow velocity in the collateral-supplying vessel during the occlusion of the collateral-receiving vessel (33,34). However, changes in absolute flow velocities obtained in the contralateral during occlusion of the ipsilateral vessel do not necessarily reflect collateral flow changes, because the former can be related to alterations in microvascular resistance of the collateral-supplying vessel or to a change in position of the Doppler sensor to measure the velocity (35,36). Conversely, there may be collateral-supplying vessels other than the one interrogated by the Doppler wire. Thus, it is not unexpected that Tomai and coworkers have not found a correlation between an increase in i.c. velocity of the contralateral vessel in some of their study patients during subsequent coronary occlusions and respective ST segment shifts (32). At variance to those results, the presented data very consistently show that collateral recruitment after repetitive myocardial ischemia contributes substantially to its attenuation even in the setting of few collaterals. Except for two, all patients who developed an increase in collateral flow between the first and third occlusion (n = 23, Fig. 5) revealed a decrease in i.c. ECG ST segment elevation during the occlusions. There was only one of 30 patients exhibiting both a decrease in collateral flow and lower ST segment elevation during subsequent occlusions. Those findings were irrespective of the use of adenosine before the first occlusion intended to pharmacologically precondition the myocardium. Collateral recruitment during subsequent coronary occlusions could be predicted very accurately by a decrease in ST segment elevation with 96% sensitivity and 83% specificity.

Pharmacologic preconditioning using adenosine. It has been repeatedly demonstrated that adenosine mimics ischemic preconditioning in experimental animals and in isolated human myocardium or myocytes subjected to substrate-free hypoxia (23,25,37,38). Leesar and coworkers (5) have recently suggested for the first time that adenosine preconditions human myocardium against ischemia in vivo. The principal finding of the mentioned investigation, that is, the induction of resistance to ischemia at the first coronary occlusion expressed by a reduced ST segment elevation, could not be reproduced in our study despite the employment of an equivalent dosage of adenosine infused i.c. over an identical time frame. The variable results of the two studies may, in part, be explained by the slight difference in collateral presentations, which were angiographically absent and mildly developed, respectively. However, angiographic estimation of collaterals cannot be regarded as an adequate method for their measurement (39). Assuming, nevertheless, that the patients in the study by Leesar et al. (5) actually had fewer collaterals than those in the present one, the complete absence of a protective effect of adenosine could only be explained through the effect of few collaterals completely counterbalancing the beneficial effect of adenosine. Theoretically, this scenario could occur during but hardly after the adenosine infusion by a mechanism of collateral steal usually taking place in the presence of copious rather than poor collateralization (40).

Sources of variable myocardial tolerance development in response to collateral recruitment. Figure 5 indicates that in a population with poorly developed collaterals, collateral recruitment accounts for only 30% (regression coefficient $r^2 = 0.29$) of the observed variation in i.c. ECG ST segment shifts, that is, other factors are responsible for 70% of the variability of myocardial ischemic adaptation. Candidates for the mentioned variability are measurement errors in the assessment of collaterals and of i.c. ECG ST segment shifts, the choice of a model with few instead of abundant collaterals for the study of ischemic adaptation and the presence of ischemic preconditioning aside from collateral recruitment. Compared to i.c. Doppler-derived measurements of the collateral flow index, the standard error of estimate is 0.08 (16), a value that is close to the absolute collateral flow index increase during subsequent balloon occlusions determined in this study. By assuming instead of directly measuring central venous pressure for the calculation of the collateral flow index, another source of variability is introduced that weighs more in the lower than the upper range of collateral flow indices. Considering those limitations and the fact that poorly developed collaterals probably tend to exhibit disproportionally fewer vascular recruitment than "good" collaterals, the variability in the association between collateral flow index and ST segment changes is not unexpected. However, from the data of the present study, it can be reasonably assumed that unprecise assessment of the end point of ischemic tolerance development and of the collateral circulation does not account for 70% variability, and the contribution to it by ischemic preconditioning is likely to be substantial. The data recently presented by Dupoy et al. (8) do not support such a conclusion, since they could not find any signs of reduced myocardial ischemia during three subsequent 2-min coronary occlusions in patients with no angiographic collaterals.

Study limitations. Aside from the study limitations implicated above, which are related to the technique used to assess the collateral circulation, the single-blind, uncontrolled study design of the pharmacologic preconditioning part of the investigation is one that has to be considered. Despite the use of objective means to measure myocardial adaptation to ischemia and the collateral circulation, the introduction of a certain bias cannot be entirely excluded. However, a double-blind, controlled study design is hardly feasible since the adenosine group would be easily recognized by the drug's effect on heart rate and blood pressure.

The assessment of myocardial adaptation to repetitive ischemia was based on i.c. ECG ST changes and on the anginal pain severity. The i.c. ECG (21) represents a well

accepted, highly sensitive, simple method for the evaluation of myocardial ischemia during angioplasty that has been demonstrated to very accurately preindicate regional wall motion abnormalities during balloon occlusion of a coronary stenosis (30). The fact that numerous studies have registered reduced signs of ischemia already during the second vessel occlusion whereas we did only during the third occlusion is explained statistically rather than biologically, since they were reduced significantly during the second occlusion when the entire study group was analyzed together.

The presence of vasoactive substances may have influenced the results of this study. However, the distribution and frequency of those drugs among the two study groups were similar.

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