Cardiac kinin level in experimental diabetes mellitus: role of kininases

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Cardiac kinin level in experimental diabetes mellitus: role of kininases. Am J Physiol Heart Circ Physiol 285: H418–H423, 2003. First published March 13, 2003; 10.1152/ajpheart.00677.2002.—Diabetes mellitus impairs the cardiac kallikrein-kinin system by reducing cardiac kallikrein (KLK) and kininogen levels, a mechanism that may contribute to the deleterious outcome of cardiac ischemia in this disease. We studied left ventricular (LV) function and bradykinin (BK) coronary outflow in buffer-perfused, isolated working hearts (n = 7) of controls and streptozotocin (STZ)-induced diabetic rats before and after global ischemia. With the use of selective kininase inhibitors, the activities of angiotensin I-converting enzyme, aminopeptidase P, and neutral endopeptidase were determined by analyzing the degradation kinetics of exogenously administered BK during sequential coronary passages. Basal LV function and coronary flow were impaired in STZ-induced diabetic rats. Neither basal nor postischemic coronary BK outflow differed between control and diabetic hearts. Reperfusion after 15 min of ischemia induced a peak in coronary BK outflow that was of the same extent and duration in both groups. In diabetic hearts, total cardiac kininase activity was reduced by 41.4% with an unchanged relative kininase contribution compared with controls. In conclusion, despite reduced cardiac KLK synthesis, STZ-induced diabetic hearts are able to maintain kinin liberation under basal and ischemic conditions because of a primary impairment or a secondary downregulation of kinin-degrading enzymes.

bradykinin; kininase; myocardial ischemia

AN INCREASE IN CARDIAC KININS might be a mechanism for protecting the heart during hypertension, cardiac failure, or acute myocardial infarction (MI). Several authors have reported increased endogenous levels of kallikrein (KLK), kininogen, and bradykinin (BK) and an upregulation of B1 and B2 receptors in response to cardiac ischemia (1, 12, 15, 18, 25, 34, 35). Direct application of BK in perfusion medium improves cardiac left ventricular (LV) function, coronary flow, and myocardial metabolism and reduces the infarct size of isolated working rat hearts during and after induction of myocardial ischemia via activation of the prostaglandin and nitric oxide (NO) axis (36). Likewise, the inhibition of kinin breakdown by kininase inhibitors has been shown to exert cardioprotective effects in in vivo models of MI (11, 42).

Several changes of the cardiac kallikrein-kinin system (KKS) have been found under diabetic conditions that may contribute to the profoundly altered myocardial and vascular integrity during the development of diabetic cardiopathy (30, 31, 38). Reduced effectiveness of exogenously applied BK on vascular dilation has been reported in diabetic subjects with endothelial dysfunction (14, 39). Moreover, we and others (30, 31, 38) have described reduced endogenous cardiac kininogen and KLK levels and/or alterations in the activation of cardiac tissue KKL in diabetic animals. Thus a reduction in the BK precursor content and in the activity of the BK-forming enzyme KLK may indicate a decreased capacity for generating cardiac BK and may be among the mechanisms involved in the development of coronary endothelial and myocardial dysfunction under diabetic conditions. On the other hand, we (33) found an upregulation of myocardial BK B1 and B2 receptor mRNA levels, which may belong to an attempt of the organism to compensate reduced cardiac kinin levels. However, concentrations of BK are determined not only by its enzymatic formation but also by the activities of degradation. Therefore, the aim of the present study was to examine the ability of the cardiac KKS to generate BK under basal and ischemic conditions after the induction of diabetes mellitus (DM). We also investigated the influence of streptozotocin (STZ)-induced DM on the activity of the three most important cardiac kinin-degrading enzymes, angiotensin-converting enzyme (ACE), aminopeptidase P (APP), and neutral endopeptidase 24.11 (NEP), in isolated hearts and correlated coronary BK outflow with the parameters of LV function and coronary flow.

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MATERIALS AND METHODS

Animals

Experiments were performed in male Sprague-Dawley rats weighing 280–350 g (Charles River; Sulzfeld, Germany). All animals had free access to distilled water and were maintained on a 12:12-h light-dark cycle. This investigation conformed with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996).

DM was induced as previously described (37). Briefly, a single intraperitoneal injection of STZ (70 mg/kg ip) diluted in 0.4 ml of sodium citrate buffer (0.1 M, pH 4.5, Sigma; München, Germany) was used to induce severe hyperglycemia, which was confirmed 48 h later by a reflectancemeter (Acuretrend, Boehringer Mannheim; Mannheim, Germany).

Bradykinin Assay

Coronary effluent was sampled in 5-ml fractions and was immediately supplemented with 1% trifluoroacetic acid. Kinins were adsorbed to phenyl-silica (Isolute SPE, International Sorbent Technology; Mid Glamorgan, UK) and eluted in 50% acetonitrile and 0.1% trifluoroacetic acid. After lyophilization and reconstitution of the samples in radioimmunoassay buffer, BK was quantitated by a specific radioimmunoassay, as previously described (2). The antiserum displayed a 36% cross-reactivity to T-kinin and had no affinity to smaller kinin fragments such as [1–8]-, [1–7]-, or [1–5]-BK. The detection limit of the assay, based on the amount of BK that produced at least 10% tracer displacement, was 1 pg per tube. Data are given without consideration to extraction recovery, which typically amounted to 85%. Intra- and inter-assay variability was 9% and 11%, respectively.

Statistical Analysis

Data on the hearts from nondiabetic control and diabetic rats are given as means ± SE. Rates of BK degradation were calculated by monoeXponential fits of the complete kinetics (41). Reduction of the BK degradation rate brought about by the addition of a kininase inhibitor was regarded as the activity of the respective enzyme. Student’s t-test for unpaired samples was performed to compare data from control and diabetic groups. The experimental time course and the pretreatment with STZ were regarded as independent determinants of BK release that were evaluated by two-dimenSional ANOVA.

RESULTS

Basal Characteristics of Diabetic Rats

Throughout the 4-wk study period, STZ-treated rats showed severe hyperglycemia (>27.5 mmol/l). This was accompanied by an increase in the heart-weight-to-body weight ratio (Table 1).

Experimental Protocol

After a 15-min stabilization period in the working heart mode, LV hemodynamic parameters were measured via the Millar tip catheter, and coronary effluent was collected for the BK assay. A 15-min global zero-flow ischemia was then started, followed by a reperfusion period in Langendorff mode changing after 10 min into the working heart mode. Hemodynamic parameters were measured again in the working heart mode. In the reperfusion period, the coronary effluent was collected at 20 s, 40 s, 60 s, 2 min, and 6 min of reperfusion.

Control perfusion period. As shown in Table 2, LV dP/dtmax, LV dP/dtmin, HR, and coronary flow were
impaired in diabetic rats 4 wk after STZ injection. Aortic flow and maximal LVP did not change significantly. Similar findings have been reported by several authors (26, 31).

Ischemia-reperfusion period. Global ischemia was induced by stopping perfusion for 15 min. During the reperfusion period, hearts were subjected to 10 min of retrograde perfusion via the aorta. The working heart mode was then reinitiated with perfusion through the left atrium. As shown in Table 2, all LV parameters except LV dP/dt min reached baseline levels after 10 min of reperfusion, so that the preischemic depression in cardiac functions of STZ hearts was maintained. A higher susceptibility of STZ hearts to ischemic damage was seen in diastolic functions, as LV dP/dt max further decreased compared with preischemic values.

Influence of Diabetes on Bradykinin Outflow

The basal coronary BK concentration 2 min before the induction of global ischemia was 0.48 ± 0.1 pg/ml in STZ hearts, which was not significantly different compared with controls. In the first 20 s of reperfusion, BK outflow in control and diabetic hearts increased markedly to the same extent compared with basal values (4.53 ± 1.24 vs. 3.70 ± 0.68 pg/ml, P = not significant). After 1 min of reperfusion, BK levels in both groups returned to preischemic values. Although postischemic kinin levels intended to be higher in diabetic hearts compared with controls, it was not significant between both groups with the exception of the second minute after reperfusion (Fig. 1). Evaluation of the time course of BK release by two-dimensional ANOVA also confirmed a significant, ischemia-related variability among different sampling times but refuted any influence related to the conditions of diabetes.

Table 1. Baseline values of diabetic and control rats

<table>
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<th>Controls</th>
<th>Diabetic</th>
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<tr>
<td>Heart weight/body weight, mg/g</td>
<td>4.36 ± 0.08</td>
<td>4.69 ± 0.11*</td>
</tr>
<tr>
<td>Plasma glucose, mmol/l</td>
<td>4.5 ± 0.6</td>
<td>30.5 ± 0.6†</td>
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Values are means ± SE; n = 7 rats/group. *P < 0.05 and †P < 0.01 compared with control rats.

Influence of Diabetes on Kinin-Degrading Enzyme Activity

The participation of ACE, NEP, and APP in BK degradation during sequential coronary passages was determined in independent experiments by cumulative administration of specific inhibitors. The decline in effluent BK concentrations after coronary passages of control hearts is presented in Fig. 2. Monoexponential regression revealed a degradation rate of 54.7 ± 3.2% per coronary passage in control hearts, which corresponds to a total kininase activity of 27.3 ± 1.6 nmol/min. In STZ hearts, BK was degraded by 32.1 ± 3.2% per passage, and the total kininase activity of 16.0 ± 1.6 nmol/min reflected a significant impairment of kinin breakdown in diabetic rats (Fig. 3). Degradation rates under different inhibitor conditions allowed the determination of the contributions of ACE, APP, and NEP to kinin degradation in control hearts, which amounted to 62 ± 3.5%, 28 ± 2.4%, and 2.7 ± 0.9%, respectively. The distribution of ACE, APP, and NEP was virtually identical (67.5 ± 2.4%, 23.6 ± 1.8%, and 2.8 ± 0.6%) in STZ hearts (Fig. 3). Residual kinin degradation was not inhibited by the combination of ramiprilat, mercaptoethanol, and phosphoram-
In view of the vascular dysfunction and impaired KLK activity in this condition, the present finding of a maintained coronary BK outflow in STZ-induced diabetic hearts was unexpected and demanded further clarification. In one previous investigation (3), even increased kinin levels in cardiac homogenates were found under diabetic conditions. The apparent discrepancy to our findings may be related to the fact that insulin-treated STZ-induced diabetic rats were investigated in that study, which do not develop KLK down-regulation (13). Furthermore, kinin determinations in tissue homogenates can be influenced by plasma-borne BK. In contrast, we could exclude influences from the circulating KKS by using buffer-perfused isolated working hearts. As such, no comparable data on coronary BK levels have been obtained so far under severely diabetic conditions.

To characterize the ability of the diabetic heart to regulate BK formation, we investigated the coronary outflow of BK after the induction of cardiac ischemia. We analyzed BK generation in terms of effluent concentrations rather than release rates; however, calculated basal release rates did not significantly differ between both groups (data not shown).

An increase in coronary BK outflow of isolated normoglycemic rat hearts after the induction of ischemia has already been reported in studies by Baumgarten et al. (1) and Lamontagne et al. (18). The ability of STZ-induced diabetic hearts to increase coronary BK outflow after global ischemia could be verified. Diabetic and nondiabetic hearts did not differ in their peak levels of coronary BK outflow or in the postischemic decline of BK release to basal values. Thus, despite a significant reduction in cardiac KLK activity, ischemic stress can still engender an increase in BK formation under diabetic conditions.

In view of the findings discussed above, the question arises as to the mechanism by which maintained cardiac BK levels originate under STZ-induced diabetic hearts. An independent cardiac KKS has previously been described (22), which is able to induce BK formation after myocardial ischemia (1, 18). This may be particularly important under diabetic conditions. Its potential for NO-mediated reduction of oxygen radicals, for endothelium-dependent vasodilation, and for improvement of glucose transport and utilization makes BK an important mediator for reducing the consequences of diabetes-related oxidative stress on both the myocardium and vessels (21). We and others have found that cardiac KLK mRNA expression and immunoreactivity as well as cardiac kininogen protein levels are reduced up to 40% in STZ-induced diabetic rats, and this therefore suggests that a reduced capacity for local generation of kinins might contribute to the pathogenesis of diabetic complications in this model. The assumption of an insufficient coronary generation of kinins in diabetes is consistent with the detection of endothelial dysfunction that has been attributed to an impaired production of PGI2 and NO (17, 28), which both belong to typical second messenger systems of the KKS.

**DISCUSSION**

A new finding of this study is that despite reduced cardiac KLK activity, the BK concentration in the coronary outflow from severe STZ-induced diabetic hyperglycemic rats does not differ compared with normoglycemic controls. Likewise, identical rates of BK outflow have been observed during ischemia, indicating that not even the capacity to stimulate BK generation is impaired in diabetic hearts.

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conditions. Previous determinations of cardiac KLK and kininogen in this condition permit an estimation of an ~30–40% impairment of kinin generation (31). When a 40% reduction of kinin degradation is assumed to fully compensate for this effect, kininase activities must essentially determine the amount of BK released. While kininases in the rat heart hardly affect kinins within the coronary system, they effectively degrade BK eluted from the interstitial space (6). The most important BK-degrading enzymes in rat myocardium, ACE and APP, are vascular enzymes that degrade BK during passage through the vessel wall by as much as 92% (6). Because only the remainder is released, both enzymes have the capacity to increase kinin overflow substantially and to effectively compensate for a reduction in kinin generation.

Under STZ-induced diabetic conditions, myocardial ACE levels have been found to be unchanged and/or increased, depending on the experimental design (8, 9). These inconsistent results may be the consequences of uncontrolled intracellular protein activation of the used homogenates. To avoid this problem, we analyzed in our study the in situ kininase activity because it considers also the interstitial and intravascular BK origin.

The significance of ACE for kinin degradation is well established. This enzyme accounts for 50–85% of BK-degrading activity in rat plasma or myocardium. The present study also demonstrates a significant contribution of APP to myocardial kinin degradation. This confirms previous determinations that have attributed ~30% of BK degradation to the vascular APP activity in both the pulmonary and coronary circulation of the rat (6, 7, 26). Because of the apparent minor significance of NEP or carboxypeptidases for myocardial BK breakdown, these enzymes cannot be responsible for the reduction in total kininase activity in diabetic hearts. Our study also showed that no major kininase is specifically affected in the diabetic state, thus connecting the mechanism of regulation to their conjoint localization at the vascular endothelium.

Endothelial function seems to be of great importance for the formation (23) as well as for the degradation of BK. Therefore, endothelial dysfunction, which is already apparent at the beginning of diabetic microangiopathy and vascular rarefaction (20, 40), appears to be accompanied by a parallel reduction in the activities of KLK and kininases. This correlation may be interpreted as a general impairment of endothelial regulatory functions, but downregulation of kininases may reflect a pathophysiological mechanism of compensation as well. A comparable pharmacological reduction of ACE activity by an ACE inhibitor substantially attenuated endothelial dysfunction and was able to increase coronary flow in STZ-induced diabetes (29).

In any case, endothelial dysfunction seems to be paradoxically involved in maintaining normal BK outflow. Nevertheless, reduced responses of exogenously applied BK in STZ-induced diabetic rats (31, 38) may suggest that maintained endogenous BK levels does not necessarily indicate a sufficient BK function despite a cardiac upregulation of both BK B1 and B2 receptors found under this condition (33).

In conclusion, despite reduced cardiac KLK synthesis, STZ-induced diabetic hearts are able to maintain kinin liberation under basal and ischemic conditions because of a primary impairment or a secondary downregulation of kinin-degrading enzymes. The known impairment of kinin-mediated vasodilation in diabetes must rather be related to alterations in kinin signal transduction or to a general functional or structural vasomotor dysfunction.

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REFERENCES


