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Altered cardiac bradykinin metabolism in experimental diabetes caused by the variations of angiotensin-converting enzyme and other peptidases

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ABSTRACT

The peptidases angiotensin-converting enzyme (ACE) and neutral endopeptidase 24.11 (NEP) mediate most of the kinin catabolism in normal cardiac tissue and are the molecular targets of inhibitory drugs that favorably influence diabetic complications. We studied the variations of those kininases in the myocardium of rats in experimental diabetes. ACE and NEP activities were significantly decreased in heart membranes 4–8 weeks post-streptozotocin (STZ) injection. However, insulin-dependent diabetes did not modify significantly bradykinin (BK) half-life ($t_{1/2}$) while the effect of both ACE (enalaprilat) and ACE and NEP (omapatrilat) inhibitors on BK degradation progressively decreased, which may be explained by the upregulation of other unidentified metallopeptidase(s). *In vivo* insulin treatment restored the activities of both ACE and NEP. ACE and NEP activities were significantly higher in hearts of young Zucker rats than in those of Sprague–Dawley rats. BK $t_{1/2}$ and the effects of peptidase inhibitors on $t_{1/2}$ varied accordingly. It is concluded that kininase activities are subjected to large and opposite variations in rat cardiac tissue in type I and II diabetes models. A number of tissue or molecular factors may determine these variations, such as remodeling of cardiac tissue, ectoenzyme shedding to the extracellular fluid and the pathologic regulation of peptidase gene expression.

1. Introduction

Bradykinin (BK) is a nonapeptide released from high molecular weight kininogen by nonspecific and specific kininogenases. It exerts its vasodilatory effect mainly by stimulation of B_2 receptors (Leeb-Lundberg et al., 2005). However, the pharmacological actions of BK are short-lived due to intense metabolism (Erdös and Skidgel, 1997). At the level of the heart, we have shown previously that two metallopeptidases, angiotensin-converting enzyme (ACE) and neutral endopeptidase 24.11 (NEP) respectively, play important roles in BK degradation (Blais et al., 1997; Dumoulin et al., 1998; Raut et al., 1999). Owing to its kinetic characteristics, ACE is a particularly effective kininase (Brunning et al., 1983; Erdös and Skidgel, 1997). Acute myocardial infarction and chronic left ventricular hypertrophy modify the relative participation of these two zinc peptidases in the inactivation of BK. These observations demonstrate that pathophysiological conditions may influence cardiac metallopeptidase activities, leading to metabolic modifications that are physiologically relevant. In addition to ACE and NEP, aminopeptidase P has been reported by some authors to inactivate BK in the endothelium (Ryan et al., 1994; Prechel et al., 1995), and kininase I, a generic name for several arginine carboxypeptidases, transforms native kinins into their des-Arg^9 fragments which exert their pharmacological actions via B_1 receptors. However, based on our experimental approaches, des-Arg^9-BK formation is only a minor metabolic pathway (Blais et al., 1997; Dumoulin et al., 1998).

ACE and NEP degrade BK, other vasoactive peptides and (in the case of ACE) activates angiotensin. A new class of drugs, the vasopeptidase inhibitors (VPi), suppresses ACE and NEP simultaneously; an agent from this class, omapatrilat, exhibits a similar nanomolar inhibitory constant (K_i) for the two enzymes (Trippodo et al., 1998) and is a useful in dissecting kinin metabolism. ACE inhibitors (ACEi) are in clinical use and have been proven to reduce mortality and morbidity in diabetic patients (Tatti et al., 1998; Gazis et al., 1998; HOPE investigators, 2000). There is accumulating experimental evidence suggesting that the increase in BK concentration resulting from ACE inhibition may play a major role in the

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beneficial effects of these drugs, particularly as it relates to diabetes. For example, Uehara et al. (1994) demonstrated that BK infusion *in vivo* mimicked the impact of ACEi on insulin action and glucose uptake. These effects were inhibited by a B_2 receptor antagonist.

The present study has two objectives: firstly, to define the influence of experimental type I and type II diabetes, and also the effect of insulin treatment, on the activities of ACE and NEP in the rat heart, a representative tissue where ACE blockade may exert kinin-mediated protective effects. Finally, the participation of both metallopeptidases in BK degradation was quantitatively examined in heart membrane extracts from the same animals.

2. Methods

2.1. Drugs, peptides and reagents

BK was purchased from Peninsula Laboratories (Belmont, CA). Enalaprilat, an ACEi, was obtained under the commercial solution form Vasotec IV (Merck & Co.). Omapatrilat, the VPi which inhibits both ACE and NEP, was kindly provided by Bristol-Myers Squibb (Princeton, NJ). Streptozotocin (STZ) in citrate buffer, pH 4.5, for the induction of diabetes was obtained from the pharmacy of the Royal Victoria Hospital (Montréal, QC). Alzet 2ML2 osmotic minipumps were from Alza Corporation (Palo Alto, CA), and insulin from Eli Lilly (Humulin R recombinant source, Indianapolis, IN). Hypnorm (fentanyl citrate 0.315 mg/ml and fluanisone 10 mg/ml) was obtained from Janssen Pharmaceuticals (Belgium), and sodium pentobarbital (Somnotol) from M.T.C. Pharmaceuticals (Mississauga, ON). Glycosuria was measured with Diastix urinary strips (Bayer, Etobicoke, ON), and glycemia by the glucose oxidase method (Lifescan Canada, Burnaby, BC). Heparin and bovine serum albumin, 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate (CHAPS) were purchased from Sigma–Aldrich (Mississauga, ON). Alkaline phosphatase-labeled anti-digoxigenin Fab fragments, *o*-phenanthroline, *p*-chloromercuriphenyl sulfonate (PCMS), and phenylmethylsulfonyl fluoride (PMSF) were from Boehringer Mannheim (Laval, QC). The bicinchoninic acid protein assay was from Pierce (Rockford, IL). Other reagents and chemicals of analytic grade were from Fisher Scientific (Montréal, QC).

2.2. Type I diabetes model

All research protocols conformed to the guidelines of the Canadian Council on Animal Care and were approved by the Committee for Animal Research of the Université de Montréal. Diabetes was induced by a single dose of STZ (55 mg/kg) via caudal vein injection in male anesthetized (Hypnorm, 0 4 ml/kg, i.m.) Sprague–Dawley rats (125–150 g, Charles River Canada, St-Constant, QC, Canada). The animals were housed at constant room temperature (21–23 °C) and humidity for the duration of the experiments. They had free access to normal rat chow and tap water. The evolution of diabetes was monitored each day for the first week and then every week for a period of 8 weeks by measurement of glycosuria and glycemia. Animals presenting glycemia over 17 mM 2 days after STZ administration were considered diabetic (Tschöpe et al., 1996). Age-matched Sprague–Dawley rats receiving no treatment were used as controls. These animals were housed and fed under identical conditions and at the same time as the treated groups.

In some diabetic rats, Alzet miniosmotic pumps containing regular insulin were implanted intraperitoneally in 8 weeks post-STZ injection anesthetized rats (sodium pentobarbital). Insulin was administered at a constant rate (3 U/day) for 7 days. Weight, glycemia and glycosuria were monitored as described above.

2.3. Type II diabetes model

Zucker fa/fa and lean rats (Charles River Canada, St-Constant, QC, Canada) served as type II diabetic animals and their respective controls (Bray, 1977).

2.4. Organ sampling and membrane preparation

Three groups of rats with induced type I diabetes were sacrificed 2, 4 or 8 weeks post-STZ injection along with their age-matched controls. Moreover, another group of 8 weeks STZ-injected rats was sacrificed 7 days post-insulin treatment. Zucker lean and fa/fa rats were sacrificed at 8 weeks of age.

Briefly, all animals were anesthetized with sodium pentobarbital (65 mg/kg, i.p.) before the thorax was opened. Heparin (1000 U/kg) was injected into the jugular vein before they were exsanguinated by transection of the carotid artery. The heart was excised after rapid perfusion in situ with Krebs-Heisenleit buffer through a cannula inserted in the aortic stump. All hearts were frozen at −80 °C until cardiac membrane preparation. Membranes were extracted from cardiac tissues by a method used previously in our laboratory for normal, infarcted and hypertrophied rat and human hearts (Blais et al., 1997; Raut et al., 1999). Briefly, each heart was weighed and then cut in small pieces of 3–4 mm. Heart tissues were homogenized in a 50 mM Tris–HCl buffer, pH 7.4, at 4 °C (10 ml/g of tissue) with a Polyron homogenizer (Brinkman Instruments, Rexdale, ON) at setting 8 for 15 s. The total homogenate was then centrifuged at 40,000×*g* for 20 min at 4 °C and the cytosolic supernatant was discarded. The pellet containing heart membranes was suspended in 50 mM Tris–HCl buffer, pH 7.4, containing 100 mM NaCl, at 4 °C with a Wheaton–Potter–Elvehjem tissue grinder (Fisher Scientific, Pittsburgh, PA) driven by a T-line motorized stirrer (Talboys Engineering, Emerson, NJ) rotating at setting 8 for 60 s. The protein concentration of the cardiac membrane suspensions was determined by the bicinchoninic acid method, before biochemical investigation.

2.5. Measurement of insulin in rat serum

Serum insulin was evaluated by competitive radioimmunoassay (ImmuChem Insulin Assay, ICN Pharmaceuticals Inc., CA) using a rabbit anti-human insulin antibody that presents 90% cross-reactivity with rat insulin.

2.6. Measurement of membrane ACE and NEP activities in myocardium

Heart membrane suspensions (1 ml) were incubated with CHAPS at a final concentration of 8 mM for 2 h and then centrifuged (40,000×*g* for 15 min at 4 °C) for the solubilization of proteins (Costerousse et al., 1994). Protein concentration of the solubilized membranes was assessed by the bicinchoninic acid method. The Buhlmann ACE radioenzymatic test (ALPCO, Vindham, NH) was employed according to the manufacturer's instructions to quantify ACE activity in these protein solutions.

NEP activity was measured using the tritiated substrate (tyrosyl-[3,5-^{3}H]) D-Ala$_2$-Leu-enkephalin (50 Ci/mmol) (Research Product International Inc., Mount Prospect, IL), as described previously by Le Moual et al. (1991). Incubations were conducted in 50 mM MES, pH 6.5, at 37 °C. Incubation time for both the ACE and NEP assays was prolonged to 4 h. Each sample was quantified in duplicate. ACE and NEP activities were expressed respectively as pmol and fmol of hydrolyzed substrate/min/mg of proteins.