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Heterogenous changes in neuropeptide Y, norepinephrine and epinephrine concentrations in the hearts of diabetic rats

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Abstract

The changes in concentrations of neuropeptide Y (NPY), norepinephrine and epinephrine were investigated in the rat hearts 1, 2, 4, 6, 9 and 12 months after administration of streptozotocin (STZ; 65 mg/kg i.v.). About 30% of diabetic animals displayed symptoms of partial spontaneous recovery, i.e. decreasing blood glucose levels and increasing insulin concentrations in the plasma and pancreas. NPY concentrations in the atria of diabetic rats did not differ from those in age-matched control rats 1, 2, 4, 6 months in the right atria and even 9 months after STZ in the left atria. However, uncompensated diabetes led to a significant decrease in NPY levels 9 and 12 months after STZ administration in the right and left atria, respectively. In the ventricles, NPY concentrations were significantly decreased 6 months after the onset of diabetes. Interestingly, partial spontaneous recovery of diabetes was associated with increased NPY levels in the atria. Myocardial norepinephrine concentrations increased 1 month after STZ and then declined reaching ~60% of the respective control values 12 months after the onset of the disease. Partial spontaneous recovery of diabetes had no effect on norepinephrine concentrations. Myocardial epinephrine concentrations did not differ from those found in controls till month 9 of the disease and they became significantly lower at month 12. Partial recovery of diabetes resulted in epinephrine concentrations not differing from the control values at month 12 of diabetes. Regarding to preferential localization of norepinephrine in the sympathetic postganglionic fibers and that of NPY also in intrinsic ganglion neurons, intrinsic neuronal circuits seem to be less susceptible to STZ-induced damage than extrinsic nerves and they might be able to recover after amelioration of diabetes.

Keywords: Neuropeptide Y; Norepinephrine; Epinephrine; STZ-induced diabetes; Rat; Heart

1. Introduction

Neuropeptide Y (NPY) is a 36-amino-acid peptide that was first isolated by Tatemoto (1982) from the porcine brain extract. NPY is co-localized with norepinephrine and ATP in the sympathetic postganglionic nerve fibers supplying the heart, and is particularly abundant in the region of the coronary vasculature (Gu et al., 1984). Moreover, NPY has been also identified in the intrinsic cardiac neurons that exert relatively autonomous local control over the myocardial function and employ a number of neurotransmitters, including acetylcholine (Horackova et al., 2000), various neuropeptides (Forsgren, 1989), epinephrine (Slavíková et al., 2003), nitric oxide (Richardson et al., 2003), and ATP (Burnstock, 1989).

In the mammalian heart, NPY acts as a direct vasoconstrictor of coronary arteries (McDermott et al., 1993) and it exerts species-dependent postsynaptic inotropic and chronotropic effects (Lundberg et al., 1984; Zukowska-Grojec et al., 1987). The peptide also regulates norepinephrine release through the activation of presynaptic NPY receptors via a negative feedback mechanism (Donoso et al., 1988). In addition, NPY seems to be an important trophic factor in the mammalian cardiovascular system as evidenced by NPY-mediated increase in the ventricular L-type calcium current density (Protas et al., 2003), cardiac hypertrophy

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(Bell et al., 2002), and angiogenesis (Zukowska-Grojec et al., 1998).

Diabetes mellitus is frequently associated with cardiovascular autonomic neuropathy (CAN) that represents a serious complication of long-lasting diabetes and contributes significantly to the increased morbidity and mortality of diabetic patients (Ewing et al., 1991; Gerritsen et al., 2001). Furthermore, CAN seems to be an important factor related to the development of diabetic cardiomyopathy, associated with multiple metabolic and electrophysiological abnormalities of the diabetic myocardium that result in diastolic dysfunction preceding the systolic damage, myocardial hypertrophy and heart failure (Price et al., 2003).

Multiple data based on functional testing suggest that both sympathetic and parasympathetic branches of the autonomic nervous system are impaired in the type 1 diabetic humans (Faulkner et al., 2001; Chessa et al., 2002; Diem et al., 2003) as well as in the animal model of the type 1 diabetes induced by a single intravenous administration of streptozotocin (STZ; Hicks et al., 1998; Lo Giudice et al., 2002). Although numerous studies dealing with cardiac norepinephrine concentrations in humans and various animal models of type 1 diabetes have been reported (Neubauer and Christensen, 1976; Ganguly et al., 1987; Akiyama et al., 1989; Wisniewska and Wisniewski, 1996; Patel et al., 1997; Schmid et al., 1999), evidence about putative changes in NPY and epinephrine levels in relation to the shifts in norepinephrine concentrations in the diabetic heart is still lacking.

Multiple origin of NPY in the mammalian heart as well as its potential role in the cardiovascular complications frequently associated with chronic diabetes (Anderson et al., 1992; Feng et al., 1999) thus give rise to hypothesis that the impact of diabetes on the peptide levels in the heart could differ from diabetes-induced changes in cardiovascular concentrations of norepinephrine. The purpose of this study was therefore to determine how diabetes affects concentrations of NPY and epinephrine in relation to norepinephrine levels in the heart compartments of female rats that were injected with STZ at the age of 2 months and then left without any treatment up to the age of 14 months.

2. Materials and methods

2.1. Materials

STZ and other chemicals were from Sigma (St. Louis, MO, USA) or Lachema (Brno, Czech Republic) if not stated otherwise. All chemicals were of analytical grade.

2.2. Animals

Adult female Wistar rats ($n=320$) purchased from VELAZ (Czech Republic) at the age of 50 days were used. The animals were housed five per cage, fed standard laboratory chow ad libitum with free access to drinking water. All animals were left intact to adapt for 10 days before the initiation of the study. All experiments were conducted in accordance with European Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (86/609/EU), the relevant Guidelines of the Czech Ministry of Agriculture for scientific experimentation on animals and were approved by the University Committee for Experiments on Laboratory Animals. At the beginning of experiment, rats were divided into two groups: 200 animals were rendered diabetic by a single intravenous injection of STZ (65 mg/kg body weight) dissolved in citrate buffer (pH 4.5) and 120 animals were injected with vehicle and served as controls. The blood glucose levels were measured after overnight fasting before the administration of STZ, 48 h after STZ injection and then monthly by glucose oxidase method (Bio-La-Test, Lachema, Czech Republic). About 25% of diabetic animals died in the course of the experiment. Surviving diabetic animals ($n=152$) were divided into two major groups: STZa-rats with blood glucose levels above 18 mmol/l till month 12 after STZ injection and STZb-rats having glycaemia above 18 mmol/l for at least 6 months after the onset of diabetes and reaching blood glucose concentrations below 10 mmol/l 9 and 12 months after STZ administration. Remaining animals were excluded from the experiments ($n=16$). Table 1 shows experimental groups.

2.3. Glucose tolerance tests

Glucose tolerance tests were performed in STZ9a, STZ9b, STZ12a and STZ12b rats ($n=4-6$) and their age-matched controls (C9 and C12; $n=6$). The rats were fasted from evening before and anaesthetized with urethane 1.5 mg/kg b.w. Glucose (2 g/kg) was administered intraperitoneally and blood samples were collected from the orbital sinus at 15, 30, 60, 90, 120, 150, and 180 min after the glucose challenge.

2.4. Extraction of NPY, norepinephrine, epinephrine, and insulin

Rats were anaesthetized with ether and killed by decapitation. Blood was collected on EDTA and centrifuged (1000 $\times g$, 4 °C, 15 min). The plasma was removed and stored at

Table 1
Experimental groups

Controls	Age (months)	n	STZ	Age (months)	After STZ (months)	Glucose (mmol/l)	n
C0	2	14					
C1	3	14	STZ1a	3	1	>18	15
C2	4	14	STZ2a	4	2	>18	15
C4	6	14	STZ4a	6	4	>18	15
C6	8	14	STZ6a	8	6	>18	15
C9	11	20	STZ9a	11	9	>18	18
			STZ9b	11	9	<10	20
C12	14	20	STZ12a	14	12	>18	18
			STZ12b	14	12	<10	20