Effect of long-term streptozotocin-induced diabetes on coronary vasoconstriction in isolated perfused rat heart

Katsuo KAMATA1, Yuta OZAWA1, Tsuneo KOBAYASHI1 and Takayuki MATSUMOTO1

1Department of Physiology and Morphology, Institute of Medicinal Chemistry, Hoshi University, Japan

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Abstract

The primary goal of this study was to investigate the effect of long-term (9 months) streptozotocin (STZ)-induced diabetes on the coronary vasoconstrictor responses to vasoactive agents such as high K+, acetylcholine (ACh), endothelin-1 (ET-1), and the calcium-channel activator Bay K 8644. For this, we used isolated rat hearts perfused at constant flow rate. Each of the four agents caused dose-dependent increases in perfusion pressure in isolated hearts from age-matched control and STZ-induced diabetic rats. The dose-response curves for high K+, ACh, and ET-1 were shifted to the left, so that at some lower doses of these agents the increased perfusion pressure was greater in coronary arteries obtained from diabetic rats than in those from control rats. On the other hand, the maximum contractile response induced by each of these agents was lower in the diabetic perfused heart. The Bay K 8644-induced contractile response was significantly greater in the coronary arteries of diabetic rats than in those of control rats. A threshold-constrictor concentration of Bay K 8644 (1 nM) potentiated the ACh-induced vasoconstriction in coronary arteries from both groups, and the potentiated responses were greater in diabetic rats than in controls at lower concentrations of ACh (100 nM and 1 µM). These findings suggest that the coronary artery contractile responses to lower concentrations of ACh or ET-1 are exaggerated in long-term STZ-induced diabetic hearts. These changes may be due to alterations in the activity of voltage-gated Ca^{2+} channels.

Key words: coronary artery, diabetes, perfusion pressure, rat, streptozotocin

Introduction

Ischemic heart disease is a leading cause of morbidity and mortality in the diabetic population, despite insulin therapy (Dubrey et al., 1994). Several factors, such as atherosclerosis of the coronary arteries and autonomic neuropathy, may play important roles in the increased occurrence of cardiovascular disease in diabetes (Fein and Sonnenblick, 1985).
Vascular dysfunction (e.g., alterations in the reactivity of blood vessels to neurotransmitters and hormones) is a well-established complication of diabetes mellitus (Oyama et al., 1986; Kamata et al., 1989; Poston and Taylor, 1995; De Vriese et al., 2000; Matsumoto et al., 2003a, b, 2006a, 2008; Kobayashi et al., 2005), and a failure of the adaptive coronary flow response to cardiac hyperactivity may, in part, be responsible for the higher incidence of ischemic heart disease in the diabetic population (Durante et al., 1989).

Muscarinic receptors, which are present in many vascular beds, commonly produce endothelium-dependent vasodilation when activated (Kamata et al., 2006; Koshita et al., 2007). Although the coronary vasculature contains muscarinic receptors, the response evoked by their stimulation varies according to species and can be affected by coronary artery diseases (Kalsner, 1989; Treasure et al., 1992; Egashira et al., 1995). Muscarinic-receptor agonists such as acetylcholine (ACh) cause endothelium-dependent relaxation of canine coronary arteries (Kalsner, 1989; Feigl, 1998), but vasoconstriction occurs in those of the pig (Kawamura et al., 1989) and rat (Sakai, 1980; Hoover and Neely, 1997; Zhang and Hoover, 2000). For the reason given below, it is most likely that such differences in the response are attributable to variations among species in the relative abundance of muscarinic receptors on between the endothelial and smooth muscle cells of the coronary arteries. Indeed, activation of the endothelial receptors induces vasodilation, mainly mediated by endothelium-derived relaxing factors, whereas stimulation of muscarinic receptors on coronary smooth muscle cells evokes vasoconstriction (Hoover and Neely, 1997; Zhang and Hoover, 2000). Relaxation and contraction of isolated human coronary arteries have been variously reported to occur with muscarinic-receptor stimulation, but vasoconstrictor responses become increasingly predominant with the progression of coronary artery disease (Hodgson and Marshall, 1989; Treasure et al., 1992; Egashira et al., 1995). Because there is anatomical and functional evidence that the coronary vasculature is innervated by cholinergic neurons (Van Charldrop et al., 1987; Kalsner, 1989; Feigl, 1998), it is possible that the parasympathetic nervous system plays a role in coronary vasospasm. The responsiveness of coronary arteries to muscarinic-receptor stimulation is therefore an important issue in research on chronic diabetic states, yet few studies have examined this point.

Endothelin-1 (ET-1), a powerful vasoconstrictor peptide secreted by endothelial cells, is thought to play a pathological role in a number of cardiovascular diseases (Rubanyi and Polokoff, 1994; Goto et al., 1996; Miyauchi and Masaki, 1999; Brunner et al., 2006). Indeed, the plasma level of this peptide is increased in patients with myocardial infarction (Stewart et al., 1991), vasospastic angina pectoris (Toyo-oka et al., 1991), or diabetes (Schneider et al., 2002), and also in animal models of diabetes (Makino and Kamata, 1998; Matsumoto et al., 2007). Moreover, antagonists of endothelin receptors may (a) reduce infarct size and improve the recovery of myocardial performance after myocardial ischemia (Pernow and Wang, 1997) and (b) improve vascular function in diabetes (Kanie and Kamata, 2002). Underlying the deleterious effects of ET-1 may be coronary vasoconstriction and coronary blood flow reduction (Muramatsu et al., 1991). Indeed, the vasoconstrictor response to ET-1 is exaggerated in coronary vessels in diseases such as hypertension (Miki et al., 1998) and diabetes (Verma et al., 2002).

Several studies using streptozotocin (STZ)-induced diabetic animals have shown that the
severity and duration of diabetes and its insulin treatment are important factors affecting both the endothelium-dependent and -independent vascular responses to various vasoactive agents (Rodriguez-Manas et al., 1998; Kobayashi and Kamata, 1999; Pieper, 1999). However, the responsiveness of coronary arteries to ACh and ET-1 in long-term diabetic states remains unclear. The purpose of the present study was to use perfused hearts from STZ-induced diabetic rats to investigate the effect of long-term diabetes (9 months) on the coronary vasoconstrictor responses to ACh and ET-1.

Methods

General

The experimental design was approved by the Hoshi University Animal Care and Use Committee, and all studies were conducted in accordance with “Guide for the Care and Use of Laboratory Animals”, published by the US National Institutes of Health, and with “Guide for the Care and Use of Laboratory Animals” adopted by the Committee on the Care and Use of Laboratory Animals of Hoshi University (accredited by the Ministry of Education, Culture, Sports, Science, and Technology, Japan).

Materials

Bay K 8644 and streptozotocin (STZ) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Endothelin-1 was from Peptide Institute, Inc. (Osaka, Japan), while acetylcholine chloride (ACh) was from Daiichi Pharmaceuticals (Tokyo, Japan). All concentrations are expressed as the final molar concentration of the base in the perfusate.

Animal model of diabetes

Male Wistar rats (8 wk old and 180–230 g body weight) received a single injection via the tail vein of STZ 65 mg/kg dissolved in a citrate buffer, as reported previously (Kobayashi et al., 2000; Matsumoto et al., 2003a, 2005, 2007).

Age-matched control rats were injected with the buffer alone. Food and water were given ad libitum. The experiments described here were performed 9 months after the injection. Nine months after the administration of STZ (diabetic group) or buffer (control group), plasma glucose was determined using a commercially available enzyme kit (Wako Chemical Company, Osaka, Japan), as reported previously (Kobayashi et al., 2006; Matsumoto et al., 2006b).

Preparation of the perfused heart

Rats were anesthetized with diethyl ether and euthanized by decapitation 9 months after treatment with STZ or buffer. Each animal’s heart was rapidly removed and placed into a Petri dish containing ice-cold oxygenated, modified Krebs-Henseleit solution (KHS). This solution consisted of (in mM) 118.0 NaCl, 4.7 KCl, 25.0 NaHCO3, 1.8 CaCl2, 1.2 NaH2PO4, 1.2 MgSO4, and 11.0 dextrose. After washing with ice-cold KHS, the heart was prepared for cannulation of the ascending aorta, then immediately transferred to an isolated heart apparatus for perfusion by the Langendorff technique. The perfusion buffer (KHS) in the system reservoir was
continuously gassed with 95% O2- 5% CO2. A peristaltic pump (PST-100; Iwaki, Tokyo, Japan) was used to perfuse hearts at a constant flow rate of 4 ml/min. Since the flow through the coronary vasculature was kept constant, the recorded changes in perfusion pressure directly reflect alterations in coronary vascular resistance (an increase signifying vasoconstriction and a decrease vasodilation). To maintain its temperature at 37°C, the buffer was passed through a water-heated glass coil. Perfusion pressure was measured by means of a pressure transducer (TP-400T; Nihon-Kohden, Tokyo, Japan) attached to the sidearm of a three-way stopcock located at the proximal end of the aortic cannula. The output from the pressure transducer was sent to a recorder for the monitoring of perfusion pressure.

Following a 40-min equilibration period, the perfusion circuit was transformed into a closed system by connecting the perfusate in a second bath and from thence recirculating it through the heart. The total volume of the closed system was 30 ml, and agents were administered via the bath. Concentration-response curves for KCl (10–80 mM), ACh (10^{-7}–10^{-4} M), ET-1 (10^{-10}–10^{-7} M), and Bay K 8644 (10^{-10}–10^{-5} M) were obtained by cumulatively increasing the total concentration of the agonist in the bath. To investigate the influence of 10^{-9} M Bay K 8644 on the ACh-induced responses in the perfused heart, the tissue was incubated in the appropriate solution for 30 min before the addition of ACh.

**Statistical analysis**

Data are expressed as the mean ± S.E.M. Multiple comparisons between treatment groups were made using an analysis of variance (ANOVA) followed by a Bonferroni test.

**Results**

**General parameters**

At the time of the experiment, all STZ rats (non-fasted) exhibited hyperglycemia, their blood glucose concentrations (703.3 ± 15.6 mg/dl, n=32) being significantly higher than those of the age-matched nondiabetic control rats (also non-fasted) (155.7 ± 5.7 mg/dl, n=32; P<0.001). The body weight of the STZ rats (232.9 ± 10.0 g, n=32) was lower than that of the control rats (684.8 ± 12.1 g, n=32; P<0.001). The heart weight of the STZ rats (1.33 ± 0.05 g, n=32) was also lower than that of the control rats (1.95 ± 0.04 g, n=32; P<0.001).

**Effects of various agents on perfusion pressure in coronary arteries**

The basal perfusion pressure (at a constant flow rate) was not different between hearts from age-matched control (23.7 ± 1.7 mmHg, n=32) and STZ-induced diabetic (28.4 ± 2.1 mmHg, n=32) rats. High K+ (Fig. 1), ACh (Fig. 2), and ET-1 (Fig. 3) each caused a dose-dependent rise in perfusion pressure in both diabetic and control coronary arteries. The maximum response to each agent was significantly weaker in the STZ-induced diabetic group than in the age-matched controls (Figs. 1–3). However, certain lower concentrations of these agents [viz. 30 mM high K+ (Fig. 1), 100 nM ACh (Fig. 2), and 3 nM ET-1 (Fig. 3)], the vasoconstriction was significantly greater in the STZ-induced diabetic group than in the age-matched controls. Comparison of EC_{50} values revealed that for each agent, the vasoconstrictor sensitivity was significantly
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increased in STZ-induced diabetic rats (vs. the age-matched controls) (Figs. 1–3). The calcium-channel activator Bay K 8644 caused a concentration-dependent rise in perfusion pressure in both diabetic and control coronary arteries (Fig. 4), vasoconstriction induced by this agent being significantly greater in the coronary arteries of diabetic rats than in those of age-matched control rats (Fig. 4).
Modulating effects of Bay K 8644 on ACh-induced increased in perfusion pressure in coronary arteries

To investigate whether the increased vasoconstriction response to a lower dose of ACh seen in the hearts of STZ-induced diabetic rats (vs. age-matched controls) might be mediated by a change in calcium-channel activity, we measured the ACh-induced perfusion pressure in the presence of a threshold concentration (1 nM) of Bay K 8644 \[i.e.,\] one that caused minimal vasoconstriction in both STZ-induced diabetic \(1.2 \pm 0.6\) mmHg, \(n=6\) \) and age-matched control \(2.3 \pm 1.2\) mmHg, \(n=6\) \) groups\]. The increase in perfusion pressure evoked by a lower dose of
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ACh was significantly enhanced by 1 nM Bay K 8644 treatment in both groups (Fig. 5; 100 nM and/or 1 µM of ACh). This augmented vasoconstriction in the presence of Bay K 8644 was significantly greater in the diabetic group than in the age-matched controls (Fig. 5).

**Discussion**

The main conclusion to be drawn from the present study is that the responses of the perfused coronary arteries of the STZ-induced diabetic rat to high K⁺, ACh, and ET-1 are altered in animals with disease states of long duration. Hearts isolated from long-term STZ-induced diabetic rats (9 months after induction of the disease) exhibited supersensitivity to these vasoconstrictor agents (when compared to age-matched control rat hearts). In addition, we found (a) that the vasoconstriction induced by the voltage-gated Ca²⁺-channel agonist Bay K 8644 was increased in coronary arteries from such STZ-induced diabetic rats, and (b) a threshold concentration of Bay K 8644 potentiated the vasoconstriction induced by a lower concentration of ACh in coronary arteries from both diabetic and control rats.

A number of studies using STZ-induced diabetic rats have observed abnormalities of responsiveness to several vasoactive agents in a variety of vessels (Kamata et al., 1992; Tomlinson et al., 1992; De Vriese et al., 2000; Kobayashi et al., 2005; Matsumoto et al., 2008). In such studies, diabetes is normally induced at a relatively young age in rats by giving STZ, and the effects of chronic STZ-induced diabetes are generally observed for a period of 8–20 weeks, the consequence being that few reports have assessed vascular responsiveness in longer-term
diabetic states (MacLeod and McNeil, 1985; Chang and Stevens, 1992). Since coronary artery disease is frequently encountered, and is a very important issue, in diabetic states (Bax et al., 2007), we designed this study to investigate coronary vasoconstrictor responses in long-term (9-month) STZ-induced diabetes (using perfused hearts). Although such long-term diabetes decreased the maximal vasoconstriction induced by both receptor-dependent (i.e., ACh and ET-1) and receptor-independent (i.e., KCl) stimuli, it is conceivable that the reduction in heart weight seen in our diabetic rats was responsible for this effect (remembering that we measured absolute values of perfusion pressure at a constant flow rate). However, this idea is speculative at present.

An intriguing and potentially important finding made in this study was that the vasoconstrictor supersensitivity to ACh and ET-1 seen in coronary arteries from long-term STZ-induced diabetic rats was associated with evidence of a marked change in Ca\textsuperscript{2+}-channel activity in these arteries. Vasodilator responses to ACh were not detected in our study and have been reported to be absent or minor under basal conditions in other studies of isolated, buffer-perfused rat hearts (Weselcouch et al., 1995). It is known that ACh can produce vasoconstriction directly by stimulating muscarinic receptors on vascular smooth muscle (Kalsner, 1989) and indirectly through muscarinic receptors located on the vascular endothelium (Luscher et al., 1992). Moreover, Zhang and Hoover (2000) demonstrated that voltage-independent receptor-operated Ca\textsuperscript{2+} channels, voltage-operated Ca\textsuperscript{2+} channels, and protein kinase C (PKC) are major signaling components for muscarinic receptor-mediated contraction in rat coronary resistance arteries. In coronary arteries, ET-1 induces vasoconstriction too (Fukuda et al., 1989; Goto et al., 1989), and abnormalities in this response are involved in several heart diseases (Rubanyi and Polokoff, 1994; Goto et al., 1996; Miyauchi, and Masaki, 1999; Brunner et al., 2006). ET-1 has been shown to increase [Ca\textsuperscript{2+}], and to stimulate the Ca\textsuperscript{2+}-mobilization mechanisms underlying vascular smooth muscle contraction, and such responses are reportedly related to the activities of voltage-independent receptor-operated Ca\textsuperscript{2+} channels, voltage-operated Ca\textsuperscript{2+} channels, and PKC (Goto et al., 1989; Pollock et al., 1995; Miwa et al., 2005). In the present study, the ACh- and ET-1-induced vasoconstrictions were significantly greater (and supersensitivity to these agonists was seen) in perfused hearts from long-term STZ-induced diabetic rats. These findings are supported by for instance, evidence that an exaggerated coronary reactivity to ET-1 is seen in diabetes in rats (Verma et al., 2002; Tickerhoof et al., 2003) and that hearts isolated from rats at a chronic stage of diabetes (i.e., 180 and 240 days) are more sensitive to carbachol than control hearts (Vadlamudi and McNeill, 1983). Moreover, in the present study the vasoconstriction induced by high K\textsuperscript{+}, which is known to cause membrane depolarization and to stimulate Ca\textsuperscript{2+} entry through voltage-gated Ca\textsuperscript{2+} channels (Karaki et al., 1997), was significantly greater in diabetic coronary arteries at a low concentration, and the sensitivity to high K\textsuperscript{+} was greater in the diabetic group than in their controls. Bay K 8644 is now recognized as a Ca\textsuperscript{2+}-channel agonist that promotes a transmembrane influx of extracellular Ca\textsuperscript{2+} through voltage-gated Ca\textsuperscript{2+} channels, possibly by opening channels by acting as an effector or by increasing the channel open-time (Hess et al., 1984; Kanamura et al., 1984). We demonstrated that the concentration-dependent responses to Bay K 8644 were greater in preparations from diabetic rats than in those from age-matched controls. Furthermore, a threshold concentration of Bay K 8644 potentiated ACh-induced
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Coronary vasoconstriction in coronary arteries from both STZ-induced diabetic and control rats. To judge from these results and other relevant evidence, the increased coronary vasoconstrictor sensitivity seen in STZ rats is attributable to increased activity in voltage-gated Ca\(^{2+}\) channels. In fact, an increase in such signaling has been observed in several arteries from STZ-induced diabetic rats (White and Carrier, 1990; Inazu et al., 1991). However, the augmented coronary vasoconstrictor response to ACh in the diabetic heart may be to some degree related to signaling via other calcium channels and/or PKC, because ACh-induced vasoconstriction was still significantly greater in the diabetic heart than in the control heart in the presence of Bay K 8644. Further work will be required on this point.

In conclusion, the present study has demonstrated vasoconstrictor hyperreactivity to KCl, ACh, and ET-1 in the perfused coronary arteries of rats with long-term STZ-induced diabetes. These changes may be due at least in part to alterations in the activity of voltage-gated Ca\(^{2+}\) channels.

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