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Cystathionine-γ lyase-derived hydrogen sulfide mediates the cardiovascular protective effects of moxonidine in diabetic rats

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ABSTRACT

Blunted cystathionine-γ lyase (CSE) activity (reduced endogenous H_2S-level) is implicated in hypertension and myocardial dysfunction in diabetes. Here, we tested the hypothesis that CSE derived H_2S mediates the cardiovascular protection conferred by the imidazoline I_1 receptor agonist moxonidine in a diabetic rat model. We utilized streptozotocin (STZ; 55 mg/kg i.p) to induce diabetes in male Wistar rats. Four weeks later, STZ-treated rats received vehicle, moxonidine (2 or 6 mg/kg; gavage), CSE inhibitor DL-propargylglycine, (37.5 mg/kg i.p) or DL-propargylglycine with moxonidine (6 mg/kg) for 3 weeks. Moxonidine improved the glycemic state, and reversed myocardial hypertrophy, hypertension and baroreflex dysfunction in STZ-treated rats. Ex vivo studies revealed that STZ caused reductions in CSE expression/activity, H_2S and nitric oxide (NO) levels and serum adiponectin and elevations in myocardial imidazoline I_1 receptor expression, p38 and extracellular signal-regulated kinase, ERK1/2, phosphorylation and lipid peroxidation (expressed as malondialdehyde). Moxonidine reversed these biochemical responses, and suppressed the expression of death associated protein kinase-3. Finally, pharmacologic CSE inhibition (DL-propargylglycine) abrogated the favorable cardiovascular, glycemic and biochemical responses elicited by moxonidine. These findings present the first evidence for a mechanistic role for CSE derived H_2S in the glycemic control and in the favorable cardiovascular effects conferred by imidazoline I_1 receptor activation (moxonidine) in a diabetic rat model.

1. Introduction

Diabetes predisposes to hypertension and baroreflex dysfunction along with cardiovascular oxidative stress in humans and in experimental animals (Erejuwa et al., 2011; Musial et al., 2013; Shida et al., 2014). Notably, oxidative stress predisposes to hypertension (Yamakawa et al., 2000), and interventions that inhibit oxidative stress (Shida et al., 2014) or sympathetic activity (Ganguly et al., 1986) or enhance nitric oxide (NO) production (Cao et al., 2012; Shida et al., 2014) confer cardiovascular protection. Interestingly, centrally acting imidazoline I_1 receptor agonists (moxonidine and rilmenidine), lower blood pressure (BP) and improve cardiac function, at least partly, via enhanced central and peripheral NO generation (Mukaddam-Daher et al., 2009). On the other hand, much less attention has been given to the potential beneficial cardiovascular effects of another endogenous gaseous cellular modulator, hydrogen sulfide (H_2S), in diabetes in general, and specifically following chronic administration of imidazoline I_1 receptor agonists in diabetic rats.

H_2S, generated by cystathionine-γ-lyase (CSE), cystathionine-β-synthase and 3-mercaptopyruvate sulfur transferase, is suppressed in diabetes (Jain et al., 2010). H_2S confers protection against deleterious consequences associated ischemia/reperfusion injury (Elrod et al., 2007), and hypertension induced myocardial hypertrophy (Huang et al., 2012; Streeter et al., 2013). It is also notable that H_2S alleviates myocardial dysfunction associated with insulin resistance (Hu et al., 2014), and improves glycemic control in diabetic rats (Xue et al., 2013b). Importantly, imidazoline I_1 receptor agonists inhibit sympathetic outflow to the heart and vasculature (Honda et al., 2013), and in obese hypertensive subjects, sympathetic inhibition ameliorates insulin resistance (Esler et al.,

Abbreviations: LV+dp/dt$_{max}$, Maximum rate of left ventricle pressure rise; BGL, Blood glucose level; BP, Blood pressure; BRS, Baroreflex sensitivity; CSE, Cystathionine-γ-lyase; DAPK3, Death associated protein kinase-3; ERK, Extracellular signal-regulated kinase; HR, Heart rate; HW/BW, Heart weight/Body weight; MAP, Mean arterial pressure; MAPK, Mitogen activated protein kinase; OGTT, Oral glucose tolerance test; SHR, Spontaneously hypertensive rats; STZ, Streptozotocin

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2006). Notably, despite common signaling cascades triggered by imidazoline I_1 receptor activation (Mukaddam-Daher et al., 2009) and H_2S (Hua et al., 2013), there are no reports on two possible mechanisms by which endogenous H_2S might mediate the anti-inflammatory and the favorable glycemic and cardiovascular effects of imidazoline I_1 receptor agonists in diabetes. First, low H_2S is linked to lower levels of the anti-inflammatory peptide adiponectin (Jain et al., 2012), and reductions in adiponectin contribute to oxidative stress in diabetes (Akiyama et al., 2010; Liu et al., 2015). Second, it is possible that the favorable cardiovascular and antioxidant effects of H_2S involve suppression of the death associated protein kinase-3 (DAPK3), an upstream activator of mitogen activated protein kinases (MAPKs), which are implicated in oxidative stress and hypertrophy in hypertension (Usui et al., 2012).

In this study, we tested the hypothesis that enhanced generation of cellular H_2S underlies the favorable cardiovascular and glycemic effects of moxonidine in a rodent model of diabetes. To test this hypothesis, we conducted integrative cardiovascular and molecular/biochemical studies in STZ (55 mg/kg) diabetic rat model, and employed two doses of moxonidine to determine if the drug effects were dose-dependent. Finally, we elucidated the mechanistic role of the CSE-derived H_2S in the imidazoline I_1 receptor-mediated effects by investigating the ability of the H_2S synthesis inhibitor DL-propargylglycine to abrogate the favorable cardiovascular and glycemic effects caused by moxonidine in STZ diabetic rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (225–250 g, Charles River Laboratories, Raleigh, NC), were used in the present study. Rats were housed individually in standard plastic cages upon starting treatment and allowed free access to water and Purina chow (St. Louis, MO). Rats were maintained on a 12–12-h light-dark cycle with light automatically turned off at 7:00 p.m. and the temperature was maintained at 22 ± 1 °C. All procedures were approved by the institutional animal care and use committee and conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the U.S. National Institute of Health, and the National Research Council Commitee Update of the Guide for the Care and Use of Laboratory Animals, 2011).

2.2. Induction of diabetes

One week after arrival, rats were fasted overnight (~16 h) and received freshly prepared STZ (55 mg/kg i.p) in 0.1 M citrate buffer (pH 4.0) or the buffer (control). Drinking water was replaced with 5% dextrose on the day of STZ injection, and 2 days later blood glucose level (BGL) was measured by a Blood Glucose Monitoring System (ReliOn®, Prime). Onset of diabetes was identified by polydipsia, polyuria and BGL > 250 mg/dl. Rats that exhibited those characteristics were considered diabetic, and buffer-treated rats were used as non-diabetic controls as reported (Liang et al., 2013).

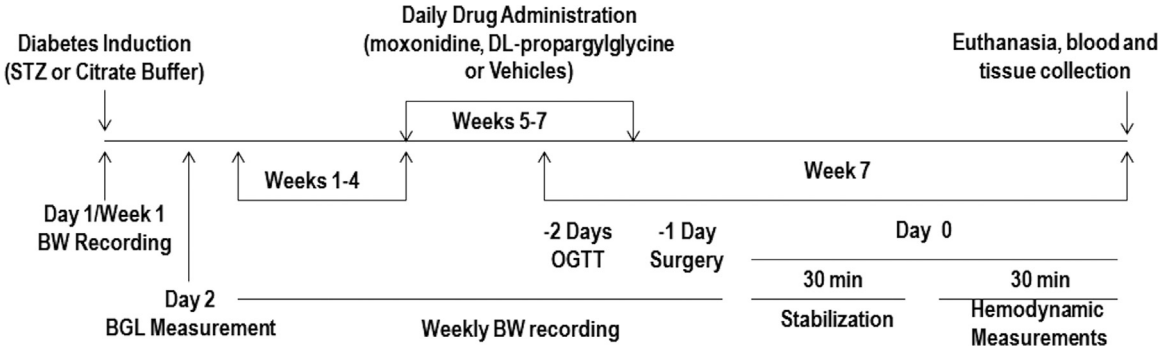

Fig. 1. A schematic presentation of diabetes induction, pharmacologic intervention, and the biochemical and molecular studies conducted to investigate the glycemic and cardiovascular effects of moxonidine (2 or 6 mg/kg/day, p.o) in diabetic male Wistar rats in the absence or presence of H_2S-synthesis inhibitor, DL-propargylglycine (37.5 mg/kg/day, i.p) during the last 3 weeks of the experiment. STZ, streptozotocin; BW, body weight; BGL, blood glucose level; OGTT, oral glucose tolerance test.

Table 1
Effects of 3-week treatment with moxonidine (2 or 6 mg/kg/day; gavage), starting 4 week following diabetes induction, on heart weight (HW), body weight (BW) and HW/BW ratio "index of hypertrophy" in STZ diabetic male Wistar rats. Shown also are the effects of DL-propargylglycine alone in non-diabetic and diabetic rats, and in combination with moxonidine (6 mg/kg/day) in diabetic rats.

	Heart weight (HW) (g)	Body weight (BW) (g)	HW/BW Ratio
Nondiabetic+Vehicle (n=8)	**1.44 ± 0.07**	**462.5 ± 19.2**	**3.113 ± 0.048**
Nondiabetic+DL-Propargylglycine (n=7)	**1.33 ± 0.05**	**432.3 ± 13.6**	**3.086 ± 0.101**
Diabetic+Vehicle (n=8)	**1.27 ± 0.02**[a]	**339.6 ± 12.8**[a]	**3.854 ± 0.126**[a]
Diabetic+Moxonidine (2 mg/kg) (n=8)	**1.11 ± 0.05**[a,b]	**298.5 ± 16.04**[a]	**3.504 ± 0.188**[a]
Diabetic+Moxonidine (6 mg/kg) (n=8)	**1.08 ± 0.05**[a,b]	**315.3 ± 17.9**[a]	**3.333 ± 0.143**[b]
Diabetic+DL-Propargylglycine (n=8)	**1.11 ± 0.04**[a,b,c]	**299.6 ± 14.8**[a,c]	**3.906 ± 0.186**[a,c]
Diabetic+DL-Propargylglycine+ Moxonidine (6 mg/kg) (n=8)	**0.87 ± 0.052**[a,b,c,d]	**265.8 ± 12.0**[a,b,c,d]	**3.345 ± 0.081**[a,b]

Values are means ± S. E. M. (n=7–8 rats/group).

[a] $P < 0.05$ versus vehicle treated non-diabetic rats.
[b] $P < 0.05$ versus vehicle-treated diabetic rats.
[c] $P < 0.05$ versus DL-Propargylglycine treated nondiabetic rats.
[d] $P < 0.05$ versus moxonidine (6 mg/kg) treated diabetic rats.