The effect of streptozotocin-induced diabetes on the EDHF-type relaxation and cardiac function in rats

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Abstract The endothelium-derived hyperpolarizing factor (EDHF) response is a critical for the functioning of small blood vessels. We investigated the effect of streptozotocin-induced diabetes on the EDHF response and its possible role in the regulation of cardiac function. The vasorelaxant response to ACh- or NS309- (direct opener endothelial small- (SKCa)- and intermediate-conductance (IKCa) calcium-activated potassium channels; main components of EDHF response) were measured in pressurized mesenteric arteries (diameter 300–350 μm). The response to 1 μM ACh was reduced in diabetes (84.8 ± 2.8% control vs 22.5 ± 5.8% diabetics; n ≥ 8; P < 0.001). NS309 (1 μM) relaxations were also decreased in diabetic arteries (78.5 ± 8.7% control vs 32.1 ± 5.8% diabetics; n ≥ 5; P < 0.001). SKCa and IKCa-mediated EDHF relaxations in response ACh or NS309 were also significantly reduced by diabetes. Ruthenium red, RuR, a blocker of TRP channels, strongly depress the response to ACh and NS309 in control and diabetic arteries. RuR decreased SKCa and IKCa-mediated EDHF vasodilatation in response to NS309 but not to ACh. An elevation in systolic blood pressure was observed in diabetic animals. ECG recording of control hearts showed shortening of PR interval. RuR reduced PR interval and R wave amplitude in diabetic hearts. In conclusion, the reduced EDHF-type relaxations in STZ-induced diabetes is due impairment of KCa channels function. TRP channels possibly contribute to EDHF vasodilatation.
Introduction

Endothelial cells have an essential role in the control of tone of the underlying smooth muscle cells via the release of various vasodilators [1,2]. These include nitric oxide (NO), prostacyclin and the endothelium-derived hyperpolarizing factor (EDHF) [3]. Although the exact mechanism by which EDHF acts is controversial [4,5], it is well-established that endothelial small-conductance, (SKCa) and the intermediate-conductance, calcium-activated potassium channel (IKCa) are essential for the initiation of the EDHF pathway [5]. The activation of these channels requires an increase in the intracellular Ca2+ concentration [Ca2+]i of endothelial cells [6]. The hyperpolarization-induced by the activation of endothelial KCa channels increases the driving force for Ca2+ influx via cation channels belonging to transient receptor potential ion channels (TRP channels) which sustain the Ca2+ signal [7].

The contribution of EDHF to the relaxation of blood vessels depends on the size of the blood vessel being of major importance in small arteries [8].

Complications of diabetes (such as nephropathy and retinopathy) are due to dysfunction of small blood vessels [9]. Thus, the impairment of the EDHF responses could have an important impact on the microvasculature. Indeed, Wigg et al. [10] reported a selective impairment of the EDHF-mediated relaxation in the mesenteric artery whereas Shi et al. [11] reported an augmented contribution of EDHF and reduced contribution of NO to endothelium-dependent relaxations. Leo et al. [12] showed an impairment of both, NO and EDHF-dependent relaxation of rat mesenteric arteries. These studies showed a reduced responsiveness to the endothelium dependent vasodilator acetylcholine (ACh) which induces the activation of endothelial KCa channels by a global increase in [Ca2+]i [13,14].

NS309 is a selective opener of both the SKCa and IKCa channels acting by enhancing the sensitively of KCa channels to intracellular Ca2+ [15]. This compound hyperpolarizes smooth muscle cells of rat mesenteric arteries [16] and human endothelial cells [17]. Recently, it has been demonstrated that there is a reduction in EDHF-type relaxation upon ACh or NS309 stimulation of mesenteric small arteries from ZDF rat; an animal model of type II diabetes [18].

Changes in the heart rate are accompanied by alterations in both [Ca2+]i, and action potential duration (APD) [19]. The expression of different subtypes of SKCa channels were demonstrated in rat, murine and human hearts [20–22]. It was hypothesized that based on the high calcium-sensitivity of these channels, they may be involved in the modification of APD of cardiac tissues particularly during cardiac repolarisation. Indeed, based on the observation that the inhibition of SKCa channels lengths the APD, it was suggested that these channels can represent an antiarrhythmic mechanism [21].

The aim of the present study was, therefore, to investigate the effect of streptozotocin (STZ)-induced diabetes on the EDHF (and its main components IKCa and SKCa)-mediated relaxation of mesenteric arteries using activators that work by two different mechanisms namely ACh (by causing a global increase in [Ca2+]i) and NS309 (acting by direct activation the KCa channels). Both KCa channels are activated by increase in [Ca2+]i, in order to initiate EDHF pathway. Therefore, we tested whether any change in NS309 or ACh-induced EDHF response is due to change in Ca2+ influx mechanism especially TRP channels; one of the main pathways for Ca2+ influx into the endothelial cells. The possible role of EDHF response in the regulation of cardiac function was also studied.

Our data suggest that the EDHF response is reduced in rats with (STZ)-induced diabetes. This is attributed to the impairment of direct opening of endothelial KCa channels. TRP channels may be involved in the EDHF-mediated relaxations. EDHF response contributes to the regulation of the electrical conduction of normal hearts whereas the role of TRP channel is more prominent in diabetic hearts.

Methodology

Animals

Animal use in the present study was approved by The Animal Use Committee of Aleppo University and is in accordance with the institutional regulations. Male albino Wistar rats (220–300 g; n = 25) were maintained in the laboratory unit of Aleppo University under standard laboratory conditions, i.e. at 25 ± 2 °C with a 12-h dark-light cycle. They were fed with regular chow, and given free access to water. Diabetes was induced by a single intravenous injection of streptozotocin (STZ; 60 mg/kg of body weight, dissolved in citrate buffer, pH 4.5), into the tail vein. For controls, age-matched rats were injected with the same volume of citrate buffer only. All experiments were performed four weeks after the STZ injection; at that time the tail blood glucose level was above 350 mg/dl.

Preparation of mesenteric arteries

Rats were decapitated. Small mesenteric arteries (second order branch; approximate diameter 300–350 μm) were rapidly removed and placed in ice-cold Krebs solution (composition in mM: NaCl 119, KCl 4.7, CaCl2 2.0, KH2PO4 1.2, MgSO4 1.2, NaHCO3 25, KH2PO4 1.18, glucose 11) bubbled with 95% O2 and 5% CO2. The artery was carefully cleaned of fat and connective tissue, and cut into segments 1–2 mm in length, these were cannulated and mounted in the chamber of a pressure myograph (Model 111P; Danish Myo Technology, Aarhus, Denmark) containing 10 ml of oxygenated (95% O2–5% CO2) Krebs solution. The arteries were left for at least 30 min to adapt before application of drugs; the intraluminal pressure was held at 70 mm Hg and the temperature at 37 °C. The external diameter of the artery was recorded with CCD camera using MyoView software (Danish Myo Technology, Aarhus, Denmark). In order to study the EDHF-mediated response,
Krebs solution containing 300 μM N-nitro-L-arginine and 10 μM indomethacin (non-selective nitric oxide synthase and cyclooxygenase inhibitors, respectively) was used throughout the experiments. Arteries were pre-constricted with an approximate EC₅₀ concentration of phenylephrine (1 μM). Kᵥ and TRP channel inhibitors were applied intraluminally at least 20 min before the application of ACh or NS309.

**Body weight and biochemical measurements**

Body weights were determined before and after the induction of diabetes and at the day of the experiment. Glucose levels were measured in samples taken from blood via the tail vein using the glucose oxidase method (BioSed, Italy). Insulin levels were measured using Ultra Sensitive Mouse Insulin ELISA Kit (Crystal Chem., Inc., IL, USA) with a microplate reader (Multiskan EX Microplate Photometer, Thermo Scientific, Schwerte, Germany).

**Tail-cuff blood pressure measurements**

Arterial blood pressure was measured non-invasively (Volume Pressure Recording; using a CODA 8-channel tail-cuff blood pressure system; Kent Scientific, Torrington, CT, USA). Blood pressure (BP; systolic, diastolic & mean) and heart rate (HR) measurements were performed after pre-warming the rats on a platform kept at 37 °C for 10 min. This allows to de-stressing the rat. However, the rat which exhibits any signs of disturbance after 10 min (such as moving the tail) was excluded from the study. The proximal occlusion cuff constricts the tail artery, while the distal cuff detects changes in tail artery volume when blood flow resumes as the occlusion cuff deflates. Measurements of average of three sessions (each consisting of 15 cycles) were used for statistical analysis.

**Electrocardiogram (ECG) recording**

Control and diabetic rats were anaesthetized with sodium pentobarbette (50 mg kg⁻¹ i.p.), were given heparin (250 IU i.v.), and were killed by cervical dislocation. Their hearts were rapidly excised and placed immediately into an ice-cold perfusion buffer. These were cannulated through the aorta in a Langendorff system, perfused with oxygenated (95% O₂, 5% CO₂) Krebs-Henseleit solution (composition in mM: NaCl 118.5, KCl 4.7, CaCl₂ 1.8, MgSO₄ 1.2, KH₂PO₄ 1.2, glucose 11.0, NaHCO₃ 25.0, pH 7.4) at 37 °C and allowed to stabilize for 30 min after being mounted. Initial perfusion pressure kept constant at 80 mmHg. The isolated hearts from control and diabetic rats were treated with: Krebs solution, Krebs solution containing 300 μM N-nitro-L-arginine and 10 μM indomethacin (in order to inhibit NO and prostaglandin synthesis) with and without 1 μM ruthenium red (a non-selective blocker of TRP channels; also known for its specific inhibition of TRPV channels in low micromolar concentrations) [23,24]. The ECG was recorded using an Animal BioAmp amplifier (Lab/8s, ADInstruments, Oxford, UK).

**Drugs**

Acetylcholine (ACh; as chloride salt), indomethacin, N²-nitro-L-arginine, NS309 (3-oxime-6,7-dichloro-1H-indole-2,3-dione) and ruthenium red were from Sigma–Aldrich, UK. Apamin was from Latoxan, USA, and 1-[2-chlorophenyl] diphenylmethyl]-1H-pyrazole (TRAM-34) from Enzo Life Sciences, UK.

**Data analysis**

All values are given as mean ± SEM. The number of animals is given by n. Data were analysed using analysis of variance (ANOVA) (GraphPad Prism software, version 4) followed by a Bonferroni’s post hoc-test, where applicable. P values of less than 0.05 were considered to indicate statistically significant differences.

**Results**

**Glucose level and body weight**

About threefold higher levels of glucose were measured in STZ-treated rats (diabetic rats) in comparison with untreated controls (Table 1). By contrast, insulin levels were significantly lower in diabetic rats. An approximately 40% reduction in body weight was observed in the latter (Table 1). Assessment of a potential cardiac hypertrophy induced by diabetes, as determined by the heart to body weight ratio, was negative (Table 1). The liver and lung to body weight ratios were not significantly changed.

**Blood pressure**

No significant changes were observed in diastolic and mean blood pressure (BP). In contrast, significant increases in heart rate (HR), systolic were observed (Table 2).

**Relaxations**

**Acetylcholine**

ACh induced a concentration-dependent relaxation of mesenteric arteries from control rats (10⁻⁸–10⁻⁵ M). ACh at 1 μM concentration (which produced submaximal relaxation of control arteries) was decreased by 75% in mesenteric arteries from STZ-diabetic rats (Fig. 1a, Table 3). ACh-induced relaxation mediated by IKᵥCa (in the presence of 100 nM apamin to block SKᵥCa channel activity) [25] was significantly reduced in diabetic arteries in comparison with controls (Fig. 1b, Table 3). Similarly, in the presence of 1 μM TRAM-34 (to block IKᵥCa

| Table 1 | Glucose and insulin levels, body weights, liver/body weight, lung/body weight and heart/body ratios, both in control and diabetic animals (n = 25). Data expressed as mean ± SEM. |
|--------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|         | Control                                                                                       | Diabetics                                                                                       |
| Glucose level (mg/dl) | 130.8 ± 11.2                                                                                 | 433 ± 20.1*                                                                                   |
| Insulin level (μU/ml)  | 19.2 ± 2.3                                                                                   | 3.4 ± 0.3*                                                                                    |
| Body weight (g)        | 259 ± 11                                                                                    | 162 ± 6.1*                                                                                    |
| Liver/body weight ratio (g) | 38.4 ± 3.3                                                                                      | 40.2 ± 2.4                                                                                    |
| Lung/body weight ratio (g) | 5.0 ± 0.5                                                                                      | 6.5 ± 0.4                                                                                    |
| Heart/body weight ratio (g) | 3.5 ± 0.3                                                                                      | 3.7 ± 0.5                                                                                    |

* Significantly different from the control rats (Unpaired t test, P < 0.001).
channel activity and reveal SKCa-mediated responses) [26,27], the relaxation to ACh was reduced by 80% (Fig. 1c, Table 3). In the presence of TRAM-34 plus apamin, ACh-induced relaxation was completely abolished in diabetic but in control arteries (10.3 ± 7.1%; n = 4).

**NS309**

NS309 induced a concentration-dependent relaxation of mesenteric arteries from control rats (10⁻⁸–10⁻⁵M). It was used at 1 lM concentration which produced submaximal relaxation of control arteries. This concentration is well below the IC₅₀ (10 lM) reported to inhibit voltage-dependent calcium channels in urinary bladder smooth muscle cells [28].

NS309-mediated relaxations were reduced by approximately 60% in arteries from diabetic rats (Fig. 2a, Table 3). In the presence of 100 nM apamin, the relaxation to 1 lM NS309 was decreased (Fig. 2b, Table 3). A reduction in the response to NS309 in the presence of 1 lM TRAM-34 was also observed in diabetic arteries in comparison with controls (Fig. 2c, Table 3).

We then tested whether the impairment of EDHF-type relaxation involves dysfunction of Ca²⁺ influx mechanism mainly via TRP channels.

(a) The application of 1 µM ruthenium red (non-selective blocker of TRP channel blocker [29]) reduced the relaxant effect of ACh (Fig. 1a), suggesting an involvement of these channels in EDHF-mediated relaxation of mesenteric arteries. ACh-mediated relaxations in the presence of 100 nM apamin (Fig. 1b) or in the presence of 1 µM TRAM-34 (Fig. 1c) were not affected by ruthenium red.

In the presence of both TRAM-34 and apamin the remaining relaxant response to ACh (10.3 ± 7.1%; n = 4) was not affected by ruthenium red (9.1 ± 2.3%; n = 4).

Ruthenium red did not produce any effect when applied alone indicating the absence of non-specific effects on endothelial or smooth muscle cells at the concentration used.

The above results do not exclude the possibility that Ca²⁺ influx through TRP channels is involved in the activation of KCa channels and consequently the EDHF response. Therefore, a selective opener of IKCa and SKCa channels (NS309) was used [30].

### Table 2: Blood pressure parameters in control and diabetic rats (n = 25).

|                | Control                  | Diabetics                |
|----------------|--------------------------|--------------------------|
| HR (beats/min) | 340.8 ± 6.4              | 383.3 ± 10.0*            |
| Diastolic pressure (mmHg) | 84.9 ± 2.5              | 92.4 ± 6.2               |
| Systolic pressure (mmHg)  | 126.8 ± 2.8              | 148.0 ± 7.5*             |
| Mean pressure (mmHg)     | 96.2 ± 1.7               | 110.9 ± 8.5              |

* Significantly different from the control rats (unpaired *t* test, *P* < 0.05).

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Fig. 1 Changes (in%) of the EDHF-mediated relaxation of mesenteric arteries in control and diabetic rats in response to ACh. (A) ACh (1 µM) induced relaxation of mesenteric arteries from control rats was significantly reduced in diabetics. The IKCa response, in the presence of 100 nM apamin (B) and SKCa response, in presence of 1 µM TRAM-34 (C), were also affected by diabetes. RuR (1 µM) produced a decrease in the response to ACh in arteries from both control and diabetic animals (A) but did not affect either IKCa (B) or SKCa (C) -mediated responses. Results shown are means ± s.e.mean (n ≥ 5). One-way ANOVA; *P < 0.05 was considered significant.
Ruthenium red application resulted in a significant decrease in relaxation to NS309 (Fig. 2a). The application of ruthenium red reduced the relaxant response of 1μM NS309 in the presence of 100 nM apamin (Fig. 2b). A similar reduction in relaxation to NS309 was also detected in the presence of 1 μM TRAM-34 (Fig. 2c).

### Table 3

Changes (in %) of the EDHF- [induced by 1 μM ACh or 1 μM NS309, + 1 μM TRAM-34, + 100 nM apamin in the presence the (+) and in the absence (−) of 1 μM ruthenium red] mediated relaxations of mesenteric arteries from control and diabetic rats. Data expressed as mean ± SEM.

|               | Ruthenium red | Controls (n) | Diabetics (n) |
|---------------|---------------|--------------|---------------|
| ACh           |               |              |               |
| ACh + TRAM-34 | −             | 84.8 ± 2.8%  (8) | 22.5 ± 5.8%*  (10) |
| + apamin      | −             | 55.5 ± 3.7%  (5) | 10.8 ± 3.5%*  (6) |
| ACh + TRAM-34 | +             | 50.6 ± 8.7%  (4) | 15.7 ± 3.3%  (5) |
| + apamin      | +             | 31.1 ± 3.3%  (5) | 9.5 ± 2.5%*  (6) |
| NS309         |               |              |               |
| NS309 + TRAM-34 | −          | 78.5 ± 8.7%*  (5) | 32.1 ± 5.8%  (6) |
| + apamin      | −             | 27.0 ± 4.4%*  (5) | 14.8 ± 2.7%  (6) |
| NS309 + TRAM-34 | +           | 52.6 ± 8.9%*  (4) | 25.4 ± 5.7%  (5) |
| + apamin      | +             | 39.9 ± 9.5%*  (5) | 19.8 ± 3.6%*  (5) |

* Significantly different from the ACh or NS309 response in the mesenteric arteries from control rats (Bonferroni’s test, P < 0.001).

§ Significantly different from the ACh or NS309 response in the mesenteric arteries from control and diabetic rats after treatment with ruthenium red (Bonferroni’s test, P < 0.05).

**Fig. 2** Changes (in %) of NS309-induced responses of mesenteric arteries from control and diabetic rats in response to NS309. (A) NS309 (1 μM)-induced relaxations were reduced in diabetics arteries. The IKCa response, in the presence of 100 nM apamin (B) and the SKCa response, in presence of 1 μM TRAM-34 (C) were also affected by diabetes. Relaxations of arteries mediated by NS309, opening of IKCa (B) or SKCa (C) were markedly reduced by RuR. Results shown are means ± s.e.mean (n ≥ 5). One-way ANOVA; *P < 0.05 was considered significant.

(a) Ruthenium red application resulted in a significant decrease in relaxation to NS309 (Fig. 2a). The application of ruthenium red reduced the relaxant response of 1μM NS309 in the presence of 100 nM apamin (Fig. 2b).
Electrocardiograms parameters of isolated hearts

There was no significant alteration in P duration, QT interval and QRS interval between hearts from control and diabetic rats (Table 4). The application of NO and COX inhibitors (to reveal EDHF pathway) before and after treatment with 1 μM ruthenium red was not accompanied by a change in the above ECG parameters (Table 4). Similarly, ST and T wave amplitude was not significantly changed between groups studied. However, a significant decrease in PR interval was obtained in hearts isolated from control rats after the infusion of NO and COX inhibitors. In addition, following the application of ruthenium red a significant decrease in PR interval and R amplitude was observed in diabetic hearts in comparison with diabetic hearts that were infused with NO and COX inhibitors (Table 4).

Discussion

In mesenteric arteries from control rats, ACh produced a relaxation which was largely due to the opening of SKCa channels. In control arteries, the relaxant response to ACh required the opening of both IKCa and SKCa channels as evident by significant reduction in the relaxation induced by ACh in the presence of both TRAM-34 plus apamin. The remaining ACh response could be due to the involvement of additional pathways (independent of endothelial hyperpolarization). An example is the release of epoxyeicosatrienoic acids (EETs) acting on the potassium channels located on the smooth muscle cells [5]. In contrast, in diabetic arteries the ACh-induced relaxation appeared to be due to EDHF, since ACh failed to produce any response in the presence of TRAM-34 and apamin. This indicates that the contribution of EDHF relaxation of small arteries is becoming more important in pathological conditions. These results are in agreement with recent findings by Leo et al. [12] who showed that endothelium-dependent relaxation was abolished in diabetic arteries, but only slightly attenuated in normal arteries.

The induction of diabetes impaired the EDHF pathway in response to ACh. In the presence of either TRAM-34 or apamin, SKCa or IKCa-mediated relaxations in response to ACh were also compromised in mesenteric arteries from diabetic rats. Our results are in agreement with previous findings [10,12]. It is possible that the impairment of the EDHF response and KCa-mediated relaxation of mesenteric arteries contribute to the development of elevated systolic blood pressure observed in this study, since these arteries are considered to play an important role in regulating blood pressure [30].

Previous studies [10,12] showed that the most commonly observed effect in the resistance arteries is a reduced responsiveness to the endothelin dependent vasodilator ACh [10] which induces the activation of endothelial KCa channels by a global increase in [Ca2+]i [13,14]. This led us to test the following hypothesis:

Is the impairment of SKCa or IKCa-mediated relaxation in response to ACh (and consequently the EDHF response) is due to impairment of global increase in [Ca2+]i, which will in turn affect the activity of KCa channels or is it due to the compromised function of the KCa channels per se?

In order to address this possibility, NS309 (a direct opener of KCa channels) was used. Results showed that NS309 induced a relaxation of mesenteric arteries from control rats. The response to NS309 was largely attenuated in diabetic rats. In addition, SKCa or IKCa-mediated relaxation in response to NS309 was also reduced in diabetics. This indicates that impairment of the EDHF response is due to dysfunction of SKCa or IKCa channels.

It was also observed that NS309, which acts by increasing the channel sensitivity for Ca2+, appears to produce its relaxant response largely via IKCa channels. This in agreement with study by Strobaek et al. [15] and consistent with results of a study in rat mesenteric arteries and human umbilical vein endothelial cells in which IKCa channels play the prominent role with respect to the response to NS309 [27].

Is the impairment of the EDHF relaxation in response to ACh or NS309 is related to a change in the Ca2+ influx mechanism required for the activation of KCa channels?

In order to test this possibility, we examined the EDHF-mediated relaxation of mesenteric arteries from control and diabetic rats in the presence of ruthenium red which is a non-selective blocker of TRP channels. These are considered one of the main pathways for Ca2+ entry into the endothelial cells.

Results showed that ACh- and NS309 produced relaxations were inhibited by ruthenium red. This suggests that TRP channels are involved in the EDHF-mediated relaxation in response to ACh, or via NS309. Ruthenium red also reduced EDHF-mediated relaxation induced by ACh and NS309 in mesenteric arteries from diabetic rats, which suggests that TRP-mediated dilatations of those arteries are also impaired.

Table 4 Summary of ECG parameters of the hearts obtained from control and diabetic rats without and following treatment with: NO and cyclooxygenase inhibitors (EDHF) and NO and cyclooxygenase inhibitors +1 μM ruthenium red [EDHF + RuR] (n = 5). Data expressed as mean ± SEM.

|                        | Controls | Diabetics | EDHF-C | EDHF-D | EDHF-C + RuR | EDHF-D + RuR |
|------------------------|----------|-----------|--------|--------|--------------|--------------|
| P duration (ms)        | 15.3 ± 2.8 | 19.10 ± 1.4 | 16.8 ± 0.001 | 20.4 ± 0.004 | 20.4 ± 0.6 | 8.82 ± 0.002 |
| QT interval (ms)       | 56.4 ± 3.8 | 54.4 ± 4.6 | 62.4 ± 5.6 | 59.1 ± 8.6 | 62.4 ± 5.5 | 51.2 ± 12.4 |
| PR interval (ms)       | 44.1 ± 4.2 | 30.7 ± 4.7 | 14.6 ± 3.9 | 32.8 ± 0.008* | 14.6 ± 3.9 | 16.1 ± 2.9* |
| QRS interval (ms)      | 24.7 ± 1.9 | 23.8 ± 3.1 | 29.4 ± 5.9 | 25.1 ± 2.7 | 29.4 ± 5.9 | 32.2 ± 10.1 |
| ST amplitude (mV)      | 0.18 ± 0.01 | 0.12 ± 0.01 | 0.39 ± 0.09 | 0.14 ± 0.07 | 0.13 ± 0.02 | 0.08 ± 0.03 |
| T amplitude            | 0.35 ± 0.16 | 0.28 ± 0.02 | 0.21 ± 0.06 | 0.16 ± 0.02 | 0.22 ± 0.01 | 0.05 ± 0.001 |
| R amplitude            | 1.56 ± 0.55 | 0.78 ± 0.12 | 1.03 ± 0.23 | 0.62 ± 0.12* | 0.42 ± 0.12 | 0.018 ± 0.006* |

* Significantly different from the corresponding control (P < 0.05).
Ruthenium red did not affect IK_{Ca} or SK_{Ca} induced relaxations of mesenteric arteries caused by ACh whereas it markedly reduced those produced by NS309. This strongly indicates that above and indicates that impairment of the EDHF response is due to compromised opening of KCa channels. It is also possible that the activation of TRP channels leads to dilatation of arteries via a mechanism which involves direct opening of endothelial IK_{Ca} and SK_{Ca} channels, most likely associated with a near-membrane rather than a global increase in [Ca^{2+}]. [14,16,31]; see Fig. 3. Thus, it is possible that these channels may provide and maintain some level of [Ca^{2+}] that is necessary for the activation of endothelial IK_{Ca} and SK_{Ca} by NS309 (see Fig. 3). This direct interaction between KCa and TRP channels and the resulting adequate levels of [Ca^{2+}] are possibly impaired in diabetes.

This interpretation is in agreement with the results of Earley et al. [32] in rat cerebral arteries. They showed that Ca^{2+} influx via TRPA1 (which co-localizes with KCa3.1) produces a vasodilatation by a mechanism involving the opening of endothelial IK_{Ca} and SK_{Ca} channels [32]. Another channel of the TRP family, TRPV4, produces EDHF-mediated vasodilation in small-sized Arteria gracilis vessels, in which EDHF plays a significant role [24].

It is unlikely that TRP channels-mediated relaxation of mesenteric arteries from control rats is due the opening of K^- channels that are located on vascular smooth muscle cells since ruthenium red did not change the relaxant response to ACh following the blockade of endothelial KCa channels.

Is there any effect of STZ-induced diabetes on the function of rat hearts?

The most known metabolic disturbance associated with STZ-induced diabetes is hypothyroidism [33–35]. This was linked to cardiovascular disturbances [34], whereas other studies showed no effect of hypothyroidism on diabetes-induced cardiac dysfunction [36,37]. However, in a study by Ramanathan et al. [38], cardiac dysfunction was observed in diabetic rats in the absence of hypothyroidism.

Zhang et al. [39] reported that, in contrary to previous studies, streptozotocin-induced diabetes protected the ex vivo heart against ischemia–reperfusion induced arrhythmias. They observed signs of clinical hypothyroidism (including decreased heart rate, prolonged QT interval and decreased rectal temperature) in STZ-diabetic rats. These signs were absent from our study. Moreover, there was no change in ECG parameters in hearts isolated from STZ-diabetic rats in comparison with those obtained from control rats which exclude the possibility that STZ is associated with metabolic disturbances that affect cardiac function.

Our data showed a significant weight loss in STZ-diabetic rats despite the fact that these animals exhibited normal food intake and grooming. Weight loss also does not seem to affect cardiac function as evident by comparable ECG parameters (such as P duration and PR interval) between control and STZ-rats. The weight loss in STZ-diabetic rats observed in our study is in agreement with previous findings by Wang et al. [40] in which STZ-induced weight loss was attributed to reduction in adipose tissue mass and gene expression of proteins that play important role in the regulation of adipocytes and adipose tissue function including leptin and adiponectin receptors.

Is there any role of the EDHF in the regulation of cardiac function in control and diabetic hearts?

Both endothelial KCa channels have high sensitivity to [Ca^{2+}] which leads to the expectation that the opening of these channels (and consequently the generation of EDHF) may affect Ca^{2+} handling, cardiac repolarisation and hence the electrical conduction of the cardiac myocytes [41]. In the presence of NO and COX inhibitors (in order to reveal

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**Fig. 3** Summary of suggested pathways for the activation of KCa channels and generation of EDHF. ACh leads to a global increase in [Ca^{2+}] as a consequence of Ca^{2+} release from inositol trisphosphate (IP3) sensitive Ca^{2+} stores within endothelial cells. The rise in [Ca^{2+}] triggers the activation of IK_{Ca} and SK_{Ca} and consequently the generation of EDHF response. Depletion of intracellular stores triggers Ca^{2+} entry via TRP channels, which consequently participates in the global increase of [Ca^{2+}]. KCa are possibly localized in close vicinity to TRP channels. The opening of TRP channels maintains some level of localized increase in [Ca^{2+}], which is essential for KCa channels to be activated by NS309 (2).
the EDHF pathway), the P wave duration (which represents the wave of depolarization of the atria that is created by sino-atrial nodal action potentials) [42] remained unchanged which indicates that EDHF does not contribute to the propagation of the action potential through the atria.

After atrial activation the action potentials reach the atrioventricular node and His bundle and the time during which they are activated corresponds the PR interval on the ECG [42]. In our study, the observed decrease in PR interval in hearts isolated from control rats, indicates that under normal conditions EDHF may contribute to the regulation of atrioventricular conduction.

NO and COX inhibitors had no significant effect on the duration of QRS complex (which represents the propagation of the action potential through the ventricles) [42]. The QT interval (representing the time for ventricular depolarization and repolarization to occur) [42] was also not changed after the application of NO and COX inhibitors which suggest that EDHF does not contribute to the rate of propagation of excitation in the perfused rat heart. Since Ca\(^{2+}\) influx is essential for the activation of K\(_{Ca}\) channels and the EDHF response, we tested the effect of TRP channels inhibition (in the presence of both NO and COX inhibitors plus RuR) on the function of hearts from control and diabetic rats.

The inhibition of TRP channels seem to shorten the PR interval and decrease the R wave amplitude in diabetic hearts. These changes indicate that TRP channels may have a role in the regulation of the electrical conduction through the AV node as well as intraventricular conduction particularly in diabetes. Indeed, a recent study on the developing chick heart showed that TRPC channels have a role in the regulation ventricular activity and their inhibition leads to ventricular arrhythmias [43]. However, other ECG parameters were comparable between control and diabetic hearts in the presence and absence of NO and COX inhibitors and ruthenium red. Future studies are needed to elucidate the type of K\(_{Ca}\) and/ or TRP channels involved in there regulation of cardiac electrical activity in isolated myocytes.

There are other important questions that remain to be answered including which TRP channel/s are involved in the EDHF response and whether the function and/or protein expression of these channels are also affected by diabetes.

### Conclusion

The results of the present study demonstrate: (1) the impairment of EDHF-mediated relaxation of rat mesenteric arteries with streptozotocin-induced diabetes; (2) diabetes affects the direct opening of both IK\(_{Ca}\) and SK\(_{Ca}\) channels; (3) a possible involvement of TRP channels in the EDHF-mediated relaxation of rat mesenteric arteries; (4) TRP-induced relaxations are likely mediated via the opening of endothelial K\(_{Ca}\) channels and are affected by diabetes (5) the EDHF response is possibly involved in the regulation of the electrical conduction between the atria and the ventricles in hearts from control rats. In contrast, TRP channels are more important in diabetic state and may play a role in the regulation of ventricular activity of the heart.

Based on the findings of the recent study, it is possible that K\(_{Ca}\)-mediated EDHF response and TRP channels could provide potential therapeutic targets for the treatment of cardiovascular complications associated with diabetes.

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### References

[1] Alvarez DF, King JA, Weber D, Addison E, Liedtke W, Townsley MI. Transient receptor potential vanilloid 4-mediated disruption of the alveolar septal barrier: a novel mechanism of acute lung injury. Circ Res 2006;99:988–95.

[2] Baylie RL, Brayden JE. TRPV channels and vascular function. Acta Physiol 2011;203:99–116.

[3] Brayden JE. Potassium channels in vascular smooth muscle. Clin Exp Pharmacol Physiol 1996;23:1069–76.

[4] Matchkov VV, Rahman A, Bakker LM, Griffith TM, Nilsson H, Aalkjaer C. Analysis of effects of connexin-mimetic peptides in rat mesenteric small arteries. Am J Physiol Heart Circ Physiol 2006;291(1):H357–67.

[5] Edwards G, Félotou M, Weston AH. Endothelium-derived hyperpolarising factors and associated pathways: a synopsis. Pflügers Arch 2010;459:863–79.

[6] Busse R, Edwards G, Félotou M, Fleming I, Vanhoutte PM, Weston AH. EDHF: bringing the concepts together. TRENDS Pharmacol Sci 2002;8:374–80.

[7] Nilius B, Droogmans G. Ion channels and their functional role in vascular endothelium. Physiol Rev 2001;81:1415–59.

[8] Félotou M, Vanhoutte PM. Endothelial dysfunction: a multifaceted disorder (The Wiggers Award Lecture). Am J Physiol Heart Circ Physiol 2006;291:H985–H1002.

[9] The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. N Engl J Med 2000;342:381–9.

[10] Wigg SJ, Tare M, Tonta MA, Obrien RC, Meredith IT, Parkinton HC. Comparison of effects of diabetes mellitus on an EDHF-dependent and an EDHF-independent artery. Am J Physiol Heart Circ Physiol 2001;281:232–40.

[11] Shi Y, Ku DD, Man RY, Vanhoutte PM. Augmented endothelium-derived hyperpolarization factor mediated relaxation attenuates endothelial dysfunction in femoral and mesenteric, but not in carotid arteries from type 1 diabetic rats. J Pharmacol Exp Ther 2006;318:276–81.

[12] Leo CH, Hart JL, Woodman OL. Impairment of both nitric oxide-mediated and EDHF-type relaxation in small mesenteric arteries from rats with streptozotocin-induced diabetes. Br J Pharmacol 2011;162:365–77.

[13] Wu CC, Chen SJ, Yen MH. Loss of acetylcholine-induced relaxation by M\(_2\)-receptor activation in mesenteric arteries of spontaneously hypertensive rats. J Cardiovasc Pharmacol 1997;30:245–52.

[14] Oishi H, Budel S, Schuster A, Stergiopulos N, Meister J, Bény JL. Cytosolic-free calcium in smooth-muscle and endothelial cells in an intact arterial wall from rat mesenteric artery in vitro. Cell Calcium 2001;30:261–7.

[15] Stroubak D, Teuber L, Jorgensen TD, Ahring PK, Kjær K, Hansen RS, et al. Activation of human IK and SK Ca\(^{2+}\)-activated K\(^+\) channels by NS309 (6,7-dichloro-1H-indole-2,3-dione 3-oxime). Biochim Biophys Acta 2004;1665:1–5.

[16] Absi M, Burnham MP, Weston AH, Harno E, Rogers M, Edwards G. Effects of methyl beta-cyclodextrin on EDHF responses in pig
and rat arteries; association between SK_{Ca} channels and caveolin-rich domains. Br J Pharmacol 2007;151:332–40.

Sheng JZ, Ella S, Davis MJ, Hill MA, Braun AP. Openers of SK_{Ca} and IK_{Ca} channels enhance agonist-evoked endothelial nitric oxide synthesis and arteriolar vasodilation. FASEB J 2009;23:1138–45.

Brøndum E, Kold-Petersen H, Simonsen U, Aalkjaer C. NS309 restores EDHF-type relaxation in mesenteric small arteries from type 2 diabetic ZDF rats. Br J Pharmacol 2010;159:154–65.

Bers DM. Calcium fluxes involved in control of cardiac myocyte contraction. Curr Res 2000;87(4):275–81.

Wang W, Watanabe M, Nakamura T, Kudo Y, Ochi R. Properties and expression of Ca^{2+}-activated K^{+} channels in H9c2 cells derived from rat ventricle. Am J Physiol 1999;276(5 Pt 2):H1559–66.

Xu Y, Tuteja D, Zhang Z, Xu D, Zhang Y, Rodriguez J, et al. Molecular identification and functional roles of a Ca^{2+}-activated K^{+} channel in human and mouse hearts. J Biol Chem 2003;278(49):49085–94.

Tuteja D, Xu D, Timofeyev V, Lu L, Sharma D, Zhang Z, et al. Differential expression of small-conductance Ca^{2+}-activated K^{+} channels SK1, SK2, and SK3 in mouse atrial and ventricular myocytes. Am J Physiol Heart Circ Physiol 2005;289(6):H2143–24.

Voets T, Prenen J, Vriens J, Watanabe H, Janssens A, Wissenbach U, et al. Molecular determinants of permeation through the cation channel TRPV4. J Biol Chem 2002;277:33704–10.

Köhler R, Heyken WT, Heinau A, Schubert R, Han S, Kacik M, et al. Evidence for a functional role of endothelial transient receptor potential V4 in shear stress-induced vasodilatation. Arterioscler Thromb Vasc Biol 2006;26:1495–502.

Bychkov R, Burnham MP, Richards GR, Edwards G, Weston AH, Féleto M. Characterization of a charybdotoxin-sensitive intermediate conductance Ca^{2+}-activated K^{+} channel in porcine coronary endothelium: relevance to EDHF. Br J Pharmacol 2002;137:1346–54.

Wulff H, Miller MJ, Hansel W, Grissmer S, Cahalan MD, Chandy KG. Design of a potent and selective inhibitor of the intermediate-conductance Ca^{2+}-activated K^{+} channel, IK_{Ca1}: a potential immunosuppressant. Proc Natl Acad Sci USA 2000;97:8151–6.

Stankevičius D, Dalsgaard T, Kroigaard C, Beck L, Boedtkjer E, Misfeldt MW, et al. Opening of small and intermediate calcium-activated potassium channels induce relaxation mainly mediated by NO release in large arteries and EDHF in small arteries from rat. J Pharmacol Exp Ther 2011;339:842–50.

Morimura K, Yamamura H, Ohya S, Imaizumi Y. Voltage-dependent Ca^{2+} channel block by openers of intermediate and small conductance Ca^{2+}-activated K^{+} channels in urinary bladder smooth muscle cells. J Pharmacol Sci 2006;100(3):237–41.

Moran MM, McAlexander MA, Biró T, Szallasi A. Transient receptor potential channels as therapeutic targets. Nat Rev Drug Discov 2011;10:601–20.

Lüscher TF, Dohi Y, Tschudi M. Endothelium-dependent regulation of resistance arteries: alterations with aging and hypertension. J Cardiovasc Pharmacol 1992;19(5):S34–42.

Saliez J, Bouzin C, Rath G, Ghisdal P, Desjardins F, Rezzani R, et al. Role of caveolar compartmentation in endothelium-derived hyperpolarizing factor-mediated relaxation: Ca^{2+} signals and gap junction function are regulated by caveolin in endothelial cells. Circulation 2008;117:1065–74.

Earley S, Gonzales AL, Crnich R. Endothelium-dependent cerebral artery dilation mediated by TRPA1 and Ca^{2+}-activated K^{+} channels. Circ Res 2009;104:987–94.

Sundaresan PR, Sharma VK, Ginold SI, Bannister J. Decreased b-adrenergic receptors in rat heart in streptozotocin-induced diabetes: role of thyroid hormones. Endocrinology 1984;114:1358–63.

Rodgers RL, Davidoff AJ, Mariani M. Cardiac function of the diabetic renovascular hypertensive rat: effects of insulin and thyroid hormone treatment. Can J Physiol Pharmacol 1991;69:346–54.

Rondeel JMM, Degreff WJ, van Der Heide D. Hypothalamohypophyseal thyroid axis in streptozotocin-induced diabetes. Endocrinology 1992;130:216–20.

Barbee RW, Shepherd RE, Burns AH. T3 treatment does not prevent myocardial dysfunction in chronically diabetic rats. Am J Physiol 1988;254:H265–73.

Sato N, Hashimoto H, Takiguchi Y, Nakashima N. Altered responsiveness to sympathetic nerve stimulation and agonists of isolated left atria of diabetic rats: no evidence for involvement of hypothryoidism. J Pharmacol Exp Ther 1989;248:367–71.

Ramanadham S, McGrath GM, McNeil JH. Chronotropic function in spontaneously diabetic BB rats. Can J Physiol Pharmacol 1989;67:519–21.

Zhang L, Parratt JR, Beastall GH, Pyne NJ, Furman BL. Streptozocin diabetes protects against arrhythmias in rat isolated hearts: role of hypothryoidism. Eur J Pharmacol 2002;435:269–76.

Wang HJ, Jin YX, Shen W, Neng J, Wu T, Li YJ, Fu ZW. Low dose streptozotocin (STZ) combined with high energy intake can effectively induce type 2 diabetes through altering the related gene expression. Asia Pac J Clin Nutr 2007;16:412–7.

Nagy N, Szuts V, Horváth Z, Srépényi G, Farkas AS, Acsai K, et al. Does small-conductance calcium-activated potassium channel contribute to cardiac repolarization? J Mol Cell Cardiol 2009;47(5):656–63.

Katz AM. Physiology of the heart. 3rd ed. Philadelphia, USA: Lippincott Williams & Wilkins; 2001.

Sabourin J, Robin E, Raddatz E. A key role of TRPC channels in the regulation of electromechanical activity of the developing heart. Cardiovasc Res 2011;92(2):226–36.