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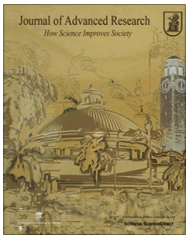

ORIGINAL ARTICLE

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

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KEYWORDS

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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- (SK_{Ca})- and intermediate-conductance (IK_{Ca}) 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; $n \geqslant 8$; $P < 0.001$). NS309 (1 μM) relaxations were also decreased in diabetic arteries (78.5 ± 8.7% control vs 32.1 ± 5.8% diabetics; $n \geqslant 5$; $P < 0.001$). SK_{Ca} and IK_{Ca}-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 SK_{Ca} and IK_{Ca}-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 K_{Ca} channels function. TRP channels possibly contribute to EDHF vasodilatation

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via direct opening of endothelial K_{Ca}. It is possible that EDHF and TRP channels contribute to the regulation of cardiac function and therefore can be considered as therapeutic targets to improve cardiovascular complications of diabetes.

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, (SK_{Ca}) and the intermediate-conductance, calcium-activated potassium channel (IK_{Ca}) are essential for the initiation of the EDHF pathway [5]. The activation of these channels requires an increase in the intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ of endothelial cells [6]. The hyperpolarization-induced by the activation of endothelial K_{Ca} channels increases the driving force for Ca^{2+} influx *via* cation channels belonging to transient receptor potential ion channels (TRP channels) which sustain the Ca^{2+} 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 K_{Ca} channels by a global increase in $[Ca^{2+}]_i$ [13,14].

NS309 is a selective opener of both the SK_{Ca} and IK_{Ca} channels acting by enhancing the sensitively of K_{Ca} channels to intracellular Ca^{2+} [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 $[Ca^{2+}]_i$ and action potential duration (APD) [19]. The expression of different subtypes of SK_{Ca} 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 SK_{Ca} channels lengthens 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 IK_{Ca} and SK_{Ca})-mediated relaxation of mesenteric arteries using activators that work by two different mechanisms namely ACh (by causing a global increase in $[Ca^{2+}]_i$) and NS309 (acting by direct activation the K_{Ca} channels). Both K_{Ca} channels are activated by increase in $[Ca^{2+}]_i$ in order to initiate EDHF pathway. Therefore, we tested whether any change in NS309 or ACh-induced EDHF response is due to change in Ca^{2+} influx mechanism especially TRP channels; one of the main pathways for Ca^{2+} 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 K_{Ca} 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 of the animal 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, $CaCl_2$ 2.0, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.18, glucose 11) bubbled with 95% O_2 and 5% CO_2. 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% O_2–5% CO_2) 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,