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Short communication

Effects of the Rho-kinase inhibitors, Y-27632 and fasudil, on the corpus cavernosum from diabetic mice

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Abstract

Relaxant responses to two Rho-kinase inhibitors, (+)-(*R*)-*trans*-4-(1-aminoethyl)-*N*-(4-pyridyl) cyclohexanecarboxamide dihydrochloride monohydrate (Y-27632) and fasudil, were compared in the corpus cavernosum obtained from diabetic and non-diabetic mice. Streptozotocin (100 mg kg^{-1} day^{-1}, for 2 days) induced diabetes with a blood glucose level of 318 ± 55.4 mg dl^{-1}; whereas it was 85.4 ± 4.1 mg dl^{-1} in control mice ($P<0.05$). Electrical field stimulation (40 V, 0.5 ms, 1, 2, 4, 8, 16 Hz for 15 s) and acetylcholine-induced relaxations were markedly attenuated in the corpus cavernosum from streptozotocin-diabetic mice whereas responses to Y-27632 (10^{-9}–3×10^{-5} M) and fasudil (10^{-9}–3×10^{-5} M) were not altered. EC_{50} values for Y-27632 were 2.98 ± 0.89 and 4.19 ± 2.71 μM in the corpus cavernosum from control and diabetic mice, respectively ($P>0.05$). The values for fasudil were 7.42 ± 4.91 and 3.53 ± 1.41 μM in the corpus cavernosum from control and diabetic mice, respectively ($P>0.05$). These results may suggest that, in diabetes, the relaxant effects of the Rho-kinase inhibitors may not be changed and thus, they may have a beneficial therapeutic effect in diabetic erectile dysfunction.

Keywords: Corpus cavernosum; Diabetes; Erectile dysfunction; Fasudil; Rho-kinase; Y-27632

1. Introduction

In response to electrical field stimulation, nitrergic nerves supplying cavernosal smooth muscle release nitric oxide (NO), which is the major neurotransmitter responsible for vasodilatation and penile erection. NO is also released from the endothelium of cavernosal tissue (Büyükafş ar et al., 1999; Göçmen et al., 1997; Roselli et al., 1998). However, inhibition of the action of vasoconstrictor agents, namely, endothelin-1, angiotensin-II and noradrenaline, may cause penile erection (Ari et al., 1996; Cellek and Moncada, 1997). These vasoconstrictors stimulate receptors that are coupled with both Ca^{2+}-dependent pathways and a small GTPase Rho and its downstream effector Rho-kinase (Park et al., 2002; Rees et al., 2001; Wang et al., 2002). The latter signalling pathway may play a role in Ca^{2+} sensitization (Somlyo and Somlyo, 1994). It has been reported that Rho-kinase is expressed in human and rabbit cavernosal smooth muscle (Rees et al., 2002). Moreover, antagonism of Rho-kinase stimulates rat penile erection (Chitaley et al., 2001; Mills et al., 2002; Rees et al., 2001).

Diabetes mellitus causes endothelial dysfunction, including an impairment of NO-mediated vasodilatation (Jack et al., 2002; Saenz de Tejada et al., 1989). In addition, diabetes attenuates nitrergic neurotransmission in human, rat and mouse corpus cavernosum (Göçmen et al., 2000; Saenz de Tejada et al., 1989; Way and Reid, 1999). Streptozotocin-induced diabetes in the rat also results in a marked inhibition of PGI_2 synthesis, which has a vasodilator effect in cavernosal smooth muscle (Khan et al., 1999).

Although a Rho-kinase inhibitor, (+)-(*R*)-*trans*-4-(1-aminoethyl)-*N*-(4-pyridyl) cyclohexanecarboxamide dihydrochloride monohydrate (Y-27632), causes penile erection, its effect on the corpus cavernosum in diabetes has not been studied. Therefore, we aimed to investigate whether the relaxant responses to two Rho-kinase inhibitors, Y-27632 and fasudil, are altered in the isolated corpus cavernosum from streptozotocin-diabetic mice.

2. Materials and methods

2.1. Diabetic protocol

This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals of Mersin

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University Centre for Experimental Medicine. Male balb/c mice initially weighing 32–41 g were caged separately under a 12-h light–12-h dark photoperiod and received standard mice chow ad libitum throughout the experiment. The mice were randomly divided into two main groups. In the diabetic group, the mice ($n=7$) received intraperitoneal streptozotocin (100 mg kg^{-1}) at a 24-h interval for 2 days. In the non-diabetic control groups ($n=10$), 0.1 M citrate buffer (pH = 4.5), the vehicle of streptozotocin, was injected (0.3 ml 30 g^{-1} mouse weight) in the same manner as in the diabetic group. None of the diabetic animals died in 30 days. One control and one diabetic mouse were used for daily experiments. Glucose level was measured in blood taken from the tail vein of each animal on the 30th day using glucose strips (Boehringer Mannheim, Germany).

2.2. Tissue preparation

The mice were killed by cervical dislocation. Penises from diabetic and non-diabetic mice were removed and placed in a Petri dish containing Krebs solution (composition (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, Na_2EDTA 0.01). The glans penis and urethra were excised and adherent tissues were carefully removed, keeping the tunica albuginea intact. Cavernosal strips were suspended through two platinum ring electrodes in organ baths filled with Krebs solution, gassed with 95% O_2 and 5% CO_2 under 0.5 g initial tension. Tension was recorded isometrically with a force transducer (COMMAT, Ankara, Turkey) and displayed on a Biopac acquisition system (Biopac Systems, CA, USA). Tissues were allowed to equilibrate for 45 min before experiments were carried out, during which time the resting tension was re-adjusted to 0.5 g as required, and every 15 min, the bath was replaced with fresh Krebs solution.

2.3. Experimental procedure

Following equilibration, cavernosal strips were pre-contracted with 5×10^{-5} M phenylephrine. After a steady state of contraction was obtained, electrical field stimulation (40 V, 0.5 ms) was delivered for 15 s at the frequencies of 1, 2, 4, 8, 16 Hz at 2-min intervals via two platinum ring electrodes connected to the Biopac stimulator. In another series of experiments, acetylcholine (10^{-9}–10^{-5} M), Y-27632 (10^{-9}–3×10^{-5} M) or fasudil (10^{-9}–3×10^{-5} M) was applied cumulatively.

2.4. Drugs and chemicals

Phenylephrine hydrochloride, acetylcholine chloride and streptozotocin were all obtained from Sigma (St Lois, MO, USA). Y-27632 and fasudil (HA-1077) were purchased from Tocris Cookson (Bristol, UK). All chemicals except streptozotocin were dissolved in distilled water and stored at −20 °C. Streptozotocin was dissolved in 0.1 M citrate buffer (pH = 4.5).

2.5. Analysis of results

Relaxations are expressed as percent reductions of phenylephrine-induced tone, and shown as means ± S.E.M. For statistical analysis, one-way analysis of variance (ANOVA), followed by the Bonferroni post hoc test or Student's *t*-test, if appropriate, was used. *P* values less than 0.05 were considered significant.

3. Results

3.1. Plasma glucose level and body weight alterations in control and diabetic mice

Mice given streptozotocin (100 mg kg^{-1} day^{-1}, for 2 days) had pronounced diabetes. Blood glucose level was 318 ± 55.4 mg dl^{-1}; whereas it was 85.4 ± 4.1 mg dl^{-1} in control mice ($P<0.05$). Their body weight decreased from 35.6 ± 1.2 (control) to 29.2 ± 2.2 g (diabetic, $P<0.05$).

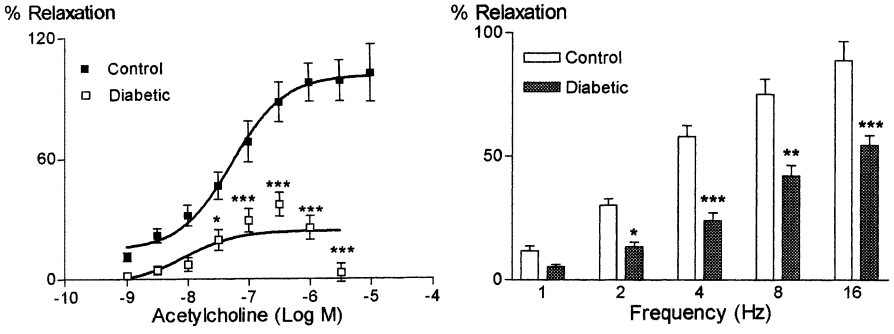

Fig. 1. Effects of acetylcholine (10^{-9}–3×10^{-5} M, left panel) and electrical field stimulation (40 V, 0.5 ms, 1, 2, 4, 8, 16 Hz, for 15 s at 2-min intervals, right panel) on the isolated mouse corpus cavernosum obtained from streptozotocin (100 mg kg^{-1} for two consecutive days)-induced diabetic and non-diabetic mice. Relaxations are expressed as percent reductions of phenylephrine-induced tone. Data represent means ± S.E.M of 10–14 observations. *$P<0.05$, ***$P<0.0001$. Comparison was made by one-way ANOVA followed by Bonferroni post hoc test.

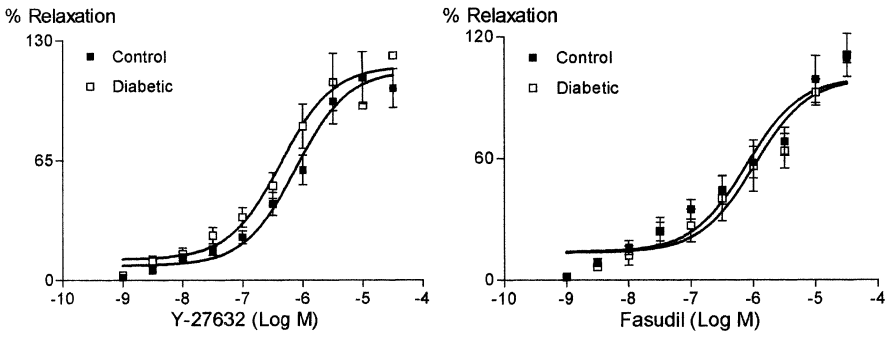

Fig. 2. Effects of Y-27632 (10^{-9}–3×10^{-5} M, left panel) and fasudil (10^{-9}–3×10^{-5} M, right panel) on the isolated mouse corpus cavernosum obtained from streptozotocin (100 mg kg^{-1} for two consecutive days)-induced diabetic and non-diabetic mice. Relaxations are expressed as percent reductions of phenylephrine-induced tone. Data represent means ± S.E.M of 9–10 observations.

3.2. Effects electrical field stimulation, acetylcholine, Y-27632 and fasudil on the corpus cavernosum from diabetic and non-diabetic mice

Electrical field stimulation (40 V, 0.5 ms, 1, 2, 4, 8, 16 Hz at 2-min intervals for 15 s) induced reproducible relaxation of the corpus cavernosum obtained from control as well as diabetic mice. However, in tissues from diabetic mice, the electrical field stimulation-induced relaxation was suppressed (Fig. 1). In addition, the acetylcholine (10^{-8}–10^{-4} M)-evoked relaxation was also inhibited (Fig. 1). However, the relaxation in response to Y-27632 (10^{-9}–3×10^{-5} M) or fasudil (10^{-9}–3×10^{-5} M) was not changed in the corpus cavernosum from diabetic and non-diabetic mice (Fig. 2).

4. Discussion

In the present study, we compared relaxant responses to Y-27632 and fasudil, two Rho-kinase inhibitors, in the isolated corpus cavernosum from diabetic (streptozocin-induced) and non-diabetic mice. There is a great body of evidence that diabetes mellitus adversely affects both endothelial and nitrergic relaxation in the corpus cavernosum isolated from various species (Göç men et al., 2000; Saenz de Tejada et al., 1989; Sullivan et al., 2002; Thompson et al., 2001). In this study, we also detected diminished relaxant responses to acetylcholine and nitrergic nerve stimulation in the corpus cavernosum from diabetic mice, confirming these findings in the literature. The mechanisms by which diabetes mediates erectile dysfunction involve a defect in NO synthesis or quenching of NO through the generation of superoxide radicals and advanced glycosylation end-products (Khan et al., 2001; Seftel et al., 1997; Vernet et al., 1995); upregulation of arginase II, which catalyzes the hydrolysis of L-arginine and thus limits the bioavailability of L-arginine to produce NO (Bivalacqua et al., 2001); and inhibition of prostacyclin synthesis (Jeremy et al., 1985). Treatment with antioxidants and metal chelators restored erectile dysfunction in diabetic animals (Göçmen et al., 2000; Keegan et al. 1999).

Contraction of cavernosal smooth muscle is maintained by noradrenaline released from noradrenergic nerves of the penis as a result of α-adrenergic receptor activation. These receptors are coupled with heterotrimeric G proteins, agonist stimulation of which causes Rho and subsequently Rho-kinase activation. Rho signalling is one of the major Ca^{2+}-sensitizing pathways (Somlyo and Somlyo, 1994). Ca^{2+} sensitization can be defined by the extent that myosin light chain phosphorylation and contractile force are independent of changes in $[Ca^{2+}]_i$ (Somlyo and Somlyo, 2000). It has been reported that Rho-kinase antagonism may be a potential therapeutic target for the treatment of erectile dysfunction (Rees et al., 2002). However, alterations in the levels of Rho and Rho-kinase proteins in the corpus cavernosum from diabetics have yet to be investigated, although it has been reported that these proteins are upregulated in the vascular smooth muscle from diabetic animals (Miao et al., 2002; Sandu et al., 2000). We examined the effects of the Rho-kinase inhibitors, Y-27632 and fasudil, in the corpus cavernosum from diabetic mice and found that the relaxation induced by the inhibitors was not changed in diabetes. Although the use of Rho-kinase inhibitors may cause hypotension as a side effect, their use may have an advantage in the treatment of diabetic erectile dysfunction, in which nitrergic and endothelial vasodilator responses are especially blunted, because other novel treatments such as sildenafil require functional nitric oxide-mediated responses.

In conclusion, diabetic mouse corpus cavernosum exhibited similar relaxation in response to the Rho-kinase inhibitors as tissue from non-diabetic mice. Therefore, the use of these agents may provide a therapeutic benefit in diabetic erectile dysfunction.

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