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Vasomotor action of insulin on the rabbit normal cavernous smooth muscle

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Abstract

Investigations on the effects of insulin on the normal vasculature have produced conflicting results. This study was aimed at establishing the vasomotor actions of insulin on normal cavernous smooth muscle. Insulin produced dose-dependent (10^{-10}–10^{-5} M) relaxation of the norepinephrine-precontracted strips of cavernosum, and of Bay K8644 [methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-2(trifluoromethylphenyl) pyridine-5-carboxylate]-precontracted strips. Endothelial denudation or indomethacin (10 μM) pre-treatment significantly reduced these insulin-induced relaxations, whereas N^G-nitro-L-arginine methyl ester (L-NAME, 5 mM) did not. Moreover, the pre-treatment of the cavernosum strips with a prostacyclin synthesis inhibitor [9,11-diazo-15-deoxy-prostaglandin H_2 (U-51605), 10 μM] significantly reduced insulin-induced response, whereas pretreatment with a cyclooxygenase-2 (COX-2) inhibitor (NS-398, 10 μM) did not. In addition, responses to insulin were not inhibited by K^+ channel blockers, i.e., tetraethylammonium (TEA, 10 mM) or 4-aminopyridine (4-AP, 10 μM). Moreover, L-type Ca^{2+} currents were reduced by prostacyclin (2 μM) but not by insulin (10 μM). We conclude that insulin induces the endothelium-dependent relaxation of cavernous smooth muscles and that this relaxation response may emanate from the direct inhibition of L-type Ca^{2+} channels by prostacyclin.

Keywords: Insulin; Corpus cavernosum; Relaxation; Prostacyclin; Ca^{2+} channel

1. Introduction

One of the most common complications of diabetes is erectile dysfunction which occurs at an earlier age than in non-diabetic men. Prevalence of erectile dysfunction has been estimated to be 20–85% (ranging from mild to complete erectile dysfunction; Romeo et al., 2000). Diabetic erectile dysfunction may be caused by many factors, including autonomic nerve dysfunction, altered smooth muscle and vascular function, and derangements of central nervous system control of behavioral drive or autonomic outflow (Tomlinson et al., 1992).

Insulin inhibits vascular smooth muscle cell contraction and migration in the normal vasculature (Kahn et al., 2002), and a failure of insulin action may contribute to the developments of atherosclerosis and restenosis. Vasodilatory dysfunction is often observed in the arteries of diabetic patients, and insulin is known to cause the relaxation of precontracted aortic and mesenteric segments in both endothelium denuded and intact preparations (Verma et al., 1998). Insulin rapidly stimulates myosin bound phosphatase and simultaneously inhibits RhoA/Rho kinase signaling via the nitric oxide (NO)/cGMP signaling pathway (Sandu et al., 2001). Thus, insulin may regulate many vascular functions under physiological conditions. In particular, it was found to stimulate local vasodilation mediated by NO upregulation or by endothelial NO synthase expression (Kuboki et al., 2000; Scherrer et al., 1994; Steinberg et al., 1994; Zeng and Quon, 1996). Moreover, insulin-induced vasodilation in small rat mesenteric arteries has been reported to be mediated through prostacyclin (Miller et al., 2002).

However, studies on the vascular effects of insulin on normal vasculatures have produced conflicting results. Some studies have concluded that insulin enhances vascular constriction in NO-inhibited rat mesenteric arteries and aortic rings, and in endothelium-denuded isolated arterioles (Schroeder et al., 1999; Wu et al., 1994). On the other hand, it was found that the release of endothelin-1 (a potent vasoconstrictor) by normal rat femoral arteries was stimulated by insulin. Moreover, insulin was found to stimulate the expression of the endothelin-1 gene at the transcriptional level via insulin receptors (Nava et al., 1997; Oliver et

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al., 1991). However, the vascular effects of insulin on normal corpus cavernous smooth muscle have not been previously examined. Thus, the aim of this study was to identify the vasomotor action of insulin on normal cavernous smooth muscle.

2. Material and methods

2.1. Preparations of rabbit cavernous strips and tension recording

The study was approved by the Committee for the Protection of Persons and Animals at the Institute of Medical Science, Chung-Ang University, Seoul.

A total of 38 New Zealand white rabbits (2.5–3 kg) were used in the present study. Rabbits were anaesthetized with an overdose of pentobarbital (60 mg/kg, i.p.) immediately exsanguinated, and penises were harvested for in vitro analysis. Cavernous tissue was carefully dissected free from the tunica albuginea, and the excised cavernous tissues were immediately placed in 100% oxygen-saturated Tyrode's solution (composition in mM: NaCl 140, KCl 4.7, $CaCl_2$ 2.5, NaH_2PO_4 1.2, D-glucose 11, HEPES 5) for 30 min. Strips of cavernous smooth muscle were then trimmed to 2 × 2 × 10 mm and mounted in a 20 ml organ bath filled with physiological salt solution (PSS; composition in mM: NaCl, 114; $NaHCO_3$, 26; KCl, 4.7; $CaCl_2$, 2.5; NaH_2PO_4, 1.2; D-glucose 11). The solution in the bath was bubbled with mixed gas (95% O_2, 5% CO_2) and maintained at 37 °C and pH 7.4, and exchanged at 30 min intervals. Strips were connected to a force transducer (52-9545, Havard, UK) and measurements were recorded on a MacLab 4e recording system (ADInstruments, Australia). Passive tension was adjusted to 1 g over a 30 min equilibration period.

2.2. Determination of the relaxation induced by insulin

To determine the cavernous relaxation effect of insulin, cavernous strips were contracted with 10 μM norepinephrine. After equilibration strips were treated with cumulative insulin concentrations of 10^{-10}–10^{-5} M.

To assess the role of endothelium on the effects of insulin, it was removed using an anoxic method. Briefly, 100% N_2 was bubbled for 30 min (instead of 100% O_2) into the tissue chamber in order to induce anoxic damage. Subsequently, 95% O_2: 5% CO_2 was bubbled for 2 h; solutions were changed every 30 min. When strips did not show a relaxation response to 100 μM acetylcholine, the endothelium was presumed to have been removed. If an acetylcholine-induced relaxation response remained, we repeated the procedure until all acetylcholine-induced relaxation had been eliminated. This anoxic method allows observation of contractile responses to acetylcholine and when compared to the 3-[(3-cholamiopropyl)-dimethylammonio]-1-propane-sulfate (CHAPS) method (Azadzoi et al., 1992) this appears to be more efficient.

To investigate the involvement of the NO and cyclooxygenase (COX) pathway on the vascular action of insulin, we measured the insulin (1 μM) induced relaxation responses of norepinephrine (10 μM)-precontracted strips, after pre-incubating these strips with L-NAME (5 mM) or a non-specific COX inhibitor (10 μM indomethacin). These strips were also pre-incubated with the cyclooxygenase-2 (COX-2) selective inhibitor NS-398 (10 μM) or the prostacyclin synthesis inhibitor 9,11-diazo-15-deoxy-prostaglandin H_2 (U-51605; 10 μM).

2.3. Potassium channels and L-type calcium channels

The possibility that some potassium channels can modulate insulin-induced relaxation was examined by determining strip contractile responses to insulin after incubating strips for 30 min with the voltage-dependent and Ca^{2+}-activated K^+ channel blocker TEA (10 mM) or A-type K^+ channel blocker 4-AP (10 μM). To examine the involvement of calcium influx through L-type calcium channels on insulin-induced relaxation, cavernous strips (with intact endothelium) were pre-contracted using calcium channel opener methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-2(trifluoromethylphenyl)pyridine-5-carboxylate (Bay K8644; 10 μM), and subsequent responses to insulin were then observed.

2.4. Electrophysiological studies

Cavernosal tissues were washed and placed in Ca-free Tyrode's solution cut into 1 mm^3 pieces, placed in the same solution containing 0.1% bovine serum albumin, 0.1% collagenase, 0.05% papain and 0.1% trypsin inhibitor, and then incubated for 40 min in a shaker water bath (37 °C) to disperse the cells. The dispersed cells were then filtered and centrifuged, and the isolated cells so obtained were stored in Ca-free PSS at 4 °C. Experiments were conducted within 8 h of isolation and at room temperature. The conventional tight seal (seal resistance 5 to 20 GΩ) whole cell patch-clamp method was used to record currents, using a Axopatch-1D patch-clamp amplifier (Axon Instruments, Foster City, California). All analog signals were low pass filtered using a 4-pole Bessel filter at 1 kHz and digitized at 2 kHz using pClamp 6 software (Axon Instruments, Foster City, California).

2.5. Drugs

U-51605 was purchased from Cayman (Ann Arbor, Michigan), and all other chemicals were purchased from Sigma (St. Louis, MO).

2.6. Data analysis

Experiments were repeated more than 5 times. Results are presented as the means ± standard error of mean (S.E.M.) of *n* observations. Maximal contractions elicited by the various contractile agents were considered 100% contraction. Cavernous strip relaxation measurements are expressed as percentage reductions in maximal contractions. IC_{50} values were calculated and obtained from five regression lines, and each regression line was constructed using four to five points using the logistic sigmoid fitting model (Origin 6.0). In all experiments, *n* refers to the number of strips. For statistical analysis, the Student's *t*