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Mechanisms involved in the relaxant action of testosterone in the renal artery from male normoglycemic and diabetic rabbits

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ABSTRACT

Kidney disease is a frequent complication in diabetes, and significant differences have been reported between male and female patients. Our working hypothesis was that diabetes might modify the vascular actions of testosterone in isolated rabbit renal arteries and the mechanisms involved in these actions. Testosterone (10^{-8} to 10^{-4} M) induced relaxation of precontracted arteries, without significant differences between control and diabetic rabbits. Both in control and diabetic rabbits endothelium removal inhibited testosterone relaxant action. In arteries with endothelium, incubation with indomethacin (10^{-5} M), N^G-nitro-L-arginine (10^{-5} M) or tetraethylammonium (10^{-5} M) did not modify relaxations to testosterone neither in control nor in diabetic rabbits. In endothelium-denuded arteries indomethacin enhanced the relaxant action of testosterone, both in control and diabetic rabbits. In arteries from diabetic rabbits, eNOS, iNOS and COX-1 expression and testosterone-induced release of thromboxane A_2 and prostacyclin were not significantly different from those observed in control rabbits. However, COX-2 expression was significantly lower in diabetic rabbits that in control rabbits. In nominally Ca^{2+}-free medium, cumulative addition of $CaCl_2$ (10^{-5} to 3×10^{-2} M) contracted previously depolarized arteries. Testosterone (10^{-4} M) inhibited $CaCl_2$ contractions of the renal artery both in control and diabetic rabbits. These results show that testosterone relaxes the renal artery both in control and diabetic rabbits. This relaxation is modulated by muscular thromboxane A_2, it is partially mediated by endothelial prostacyclin, and it involves the blocking of extracellular Ca^{2+} entry. Diabetes does not modify the mechanisms involved in the relaxant action of testosterone in the rabbit renal artery.

1. Introduction

Kidney disease is a frequent complication in diabetic patients and gender-associated differences in renal diabetic complications have been reported [1]. Women tend to develop diabetic nephropathy earlier after the onset of diabetes compared with men [2], but the risk of end-stage renal disease is twice as high in men as in women [3]. Recently, it has been reported that it should be considered welcoming low testosterone as a new cardiovascular risk factor in men [4] and that a lower androgen milieu increased the risk of developing diabetes and vice versa [5]. Testosterone concentrations inversely correlate with all-cause and cardiovascular disease-related mortality, as well as with markers of inflammation, and may contribute to the worse prognosis for renal disease in men [6,7]. In addition, it has been reported that while in non-diabetic renal disease absence of testosterone is renoprotective, in the setting of diabetes, low levels of plasma testosterone correlate with the progression of diabetic renal disease [8,9] and androgen replacement therapy could be a potential treatment, particularly in diabetic men [10]. This suggests an important role of testosterone in the pathophysiology of diabetic renal disease although it is accepted that sex hormones act genomically and non-genomically to influence endothelial function, the exact mechanism still remains unclear [11].

Testosterone is well known to elicit direct vasodilation, but its influence upon vascular reactivity may depend upon pathogenesis process or gender and little information is available regarding the effects of acute exposure of the renal arteries to testosterone. There is increasing evidence that endogenous sex hormones regulate vascular reactivity, and several studies have suggested that sex hormones could be related to the renal control mechanisms altered in hypertension, particularly the renin-angiotensin system [12,13]. Testosterone has also been reported to have a favorable direct vasodilator influence on coronary, aortic, renal

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and brachial vasculature by both endothelial-dependent and -independent mechanisms [14,15]. The influence of endothelium, NO and prostanoids in the vasodilatory action of testosterone is still controversial and several reports show that this relaxation does not rely or only partially relies upon endothelial NO and prostanoids depending on the species and vascular bed [16].

Diabetes alters the responsiveness of different vascular beds to several vasoconstrictors and vasodilators, and it has been hypothesized that endothelial dysfunction could partially explain many of these altered responses [17,18]. Previous studies from our laboratory suggest that diabetes-induced changes in renovascular reactivity may be related, at least in part, to differences in the release of NO and vasoconstrictor/vasodilator prostanoids [19,20,21]. Recently we have reported that diabetes induces hyperreactivity of male rabbit carotid artery to testosterone by a mechanism that at least includes an increased modulatory activity of endothelial NO and an augmented release of prostacyclin [22].

Information on the direct actions of testosterone on the renal vascular bed are scanty or conflicting, even more so in the diabetic state. This deficiency forms a rationale for the present study, which aim was to examine the mechanisms involved in the relaxant action of testosterone in the renal artery from normoglycemic and diabetic male rabbits: (1) the vasoactive effects of testosterone in segments of renal artery; (2) the role of endothelium, NO and prostanoids modulating the vascular effects of testosterone; and (3) the involvement of K^+ efflux and Ca^{2+} influx in the vascular action of testosterone.

2. Experimental

Twenty three male New Zealand white rabbits were used in the present study. Animals were randomly divided into two experimental groups: 11 in the control group and 12 destined for induction of experimental diabetes. Housing conditions and experimental procedures were in accordance with the European Union regulations on the use of animals for scientific purposes (86/609/EEC, Article 5, Appendix II) and as promulgated by Spanish legislation (RD 1201/2005).

2.1. Induction of diabetes and control animals

Rabbits weighing 2.77 ± 0.10 kg were sedated with intramuscular injection of ketamine (40 mg; Ketolar) after fasting 24 h. Diabetes was induced by injecting alloxan (100 mg kg^{-1}) into the lateral ear vein. To prevent hypoglycemia, 10 ml of glucose 5% was injected intravenously after alloxan and drinking water was supplemented with 10% glucose for the first 24 h after the alloxan injection. Thereafter, the animals were maintained on tap water, regular food (Global Diet 2030, Harlan Interfauna Iberica) *ad libitum*, and no insulin treatment for 6 weeks. A second group of rabbits (2.79 ± 0.14 kg) was maintained under the same conditions for the same time period to serve as age-matched normoglycemic controls (henceforth "control rabbits"). Control and diabetic animals were well matched with no significant differences at baseline for body weight and glycemia values.

2.2. Plasma glucose and testosterone determinations

Plasma glucose concentrations were weekly measured by the glucose oxidase method with a glucose analyzer (Glucometer Elite, Bayer). For plasma testosterone determinations, just before euthanization, blood samples (3 ml) were collected from the central ear artery in Vacuum Blood Tubes with EDTA. All samples were taken at the same time in the morning to avoid the influence of circadian variations in the plasmatic levels of testosterone. The blood was centrifuged at 1900 rpm for 15 min and the plasma stored at −80 °C. Concentrations of free and total testosterone in plasma were determined in duplicate by radioimmunoanalysis and chemoluminescence, respectively (Gestión Sanitaria Integral, S.L., Valencia, Spain).

2.3. Isometric tension recording

Six weeks after diabetes induction, diabetic and control rabbits were anaesthetized with 2% i.v. sodium pentothal (Tiobarbital Braun) and euthanized by injection of potassium chloride (10 mEq, 0.5 ml/kg, i.v.). The renal arteries were dissected free and cut into cylindrical segments measuring 3–4 mm in length. Each segment was prepared for isometric tension recording in an organ bath. Two stainless steel L-shaped pins (diameter, 207 μm) were introduced through the arterial lumen. One pin was fixed to the organ bath wall and the other pin was connected to a strain gauge for isometric tension recording (isometric tension transducer models Panlab UF-1 and Letica TRI 201). Isometric tension was conveniently amplified (Panlab 40154 and Letica ISO 506), digitized (PowerLab/8SP, ADInstruments), recorded and stored in an IBM PC compatible computer by means of the appropriate software (Chart 5, ADInstruments) for later analysis. The organ bath contained 5 ml of Ringer-Locke solution that was continuously bubbled with 95% O_2 and 5% CO_2 to provide a pH of 7.3–7.4. Temperature was kept at 37 °C. A resting tension of 2 g was applied to the arterial segments, and they were allowed to equilibrate for a period of 60–90 min before the experiments were started. Tension was readjusted when necessary and the bath fluid was changed every 15 min. After this period of equilibration, the reactivity of the arterial segments was checked by depolarization with 50 mM KCl. There were not significant differences in the response to KCl between arteries from control and diabetic rabbits. Then, the functional integrity of endothelium was checked by examining the relaxant action of acetylcholine (10^{-5} M) in arteries precontracted with phenilephrine (10^{-6} M).

2.4. Concentration–response curves

The experiments were carried out with renal arteries from both control and diabetic rabbits. Concentration–response curves for testosterone (10^{-8} to 10^{-4} M) were obtained cumulatively in arteries at basal tone and in arteries previously contracted with norepinephrine (10^{-7} to 3×10^{-6} M). The active tone induced by norepinephrine in arteries from control rabbits (4206 ± 395 mg) was not significantly different from that obtained in arteries from diabetic rabbits (4022 ± 427 mg). One concentration–response curve to testosterone was obtained for each arterial segment. To assess the influence of the endothelium on the effect of testosterone, concentration–response curves were obtained in precontracted arteries from which the endothelium had been removed by rubbing the intimal surface with a scored stainless steel rod (rubbed arteries). The participation of NO synthesis activation in the effects of testosterone was studied by incubating arterial segments (20 min) with the inhibitor of NO synthase (NOS) N^G-nitro-L-arginine (L-NOArg, 10^{-5} M). To examine the possibility that some arachidonic acid derivative could modulate the arterial response to testosterone, concentration–response curves for testosterone were obtained after incubation (20 min) of the arterial segments with indomethacin (10^{-5} M), an inhibitor of cyclooxygenase. In addition, to examine the involvement of large conductance calcium-activated K^+ channels (BK_{Ca}) and voltage-sensitive K^+ (K_V) channels in vasorelaxation to testosterone, concentration–response curves to the hormone were obtained in the presence of the commonly used blocker of these channels tetraethylammonium (TEA, 10^{-5} M). Finally, to investigate the possible Ca^{2+} channel antagonistic effect of testosterone, concentration–response curves to $CaCl_2$ (10^{-5} to 3×10^{-2} M) were