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The Genetic Expression of Alpha-1A Receptors in the Prostate of Diabetic Rats

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摘要

對男性糖尿病患者而言，下尿路功能障礙原因除了膀胱病變以外，攝護腺之結構及收縮狀態同樣是一個重要影響因素。而交感神經亞發 1A 受體為激發攝護腺平滑肌收縮之主要受體。故此，本實驗室進行之 NSC 計畫乃利用西方點墨以及 reverse transcription and polymerase chain reaction (RT-PCR) 方法，針對雄性老鼠攝護腺亞發 1A 受體基因表現進行探討。發現亞發 1A 受體基因表現及 mRNA 明顯存在於大白鼠攝護腺。以 streptozotocin 誘導糖尿病後兩者皆明顯上升，α1A-AR receptor mRNA 增加 2.51 ± 0.21 倍；receptor protein 增加 2.23 ± 0.10 倍 (n=8, p<0.05)。故本研究結果能建立「糖尿病性攝護腺病變」之創新觀念，對糖尿病患者下尿路功能障礙之治療有極大意義。

Keywords: 糖尿病，膀胱，交感神經受體，攝護腺。

Abstract

Diabetes associated alteration in prostate α1A-adrenoceptor gene expression was studied using a streptozotocin (STZ)-induced diabetic rat model. Male Wistar rats were divided into four groups; group I: vehicle-treated normal rats; group II: vehicle-treated STZ-diabetic rats; group III: insulin-treated STZ-diabetic rats (0.5 IU/kg t.i.d. for 4 days); group IV: phlorizin-treated STZ-diabetic rats (1 mg/kg t.i.d. for 4 days). Expression of the mRNA that encoded protein of α1A-AR in the rat prostate was studied using reverse transcription combined with polymerase chain reaction (RT-PCR). The α1A-AR protein expression in the prostate was studied by Western blotting analysis with a polyclonal antiserum. A 2.51 ± 0.21 fold increase in the mRNA level of α1A-AR was observed in the prostate of diabetic rats (n=8, p<0.05). Similarly, there was a 2.23 ± 0.10 fold increase in the α1A-AR receptor protein level (n=8, p<0.05). Both insulin and phlorizin treatments restored the normal levels of mRNA and protein expression. In conclusion, the gene expression of α1A-AR is increased in the prostate of diabetic rats. Hyperglycemia plays a major role in this alteration.

Keywords: Diabetes mellitus, urinary bladder, alpha receptors, prostate.

緣由與目的

Clinically up to 80% of patients with diabetes mellitus (DM) develop lower urinary tract symptoms (LUTS), which include urinary frequency, nocturia, urgency, incontinence, voiding difficulty and residual urine sensation [1]. In a study by Kaplan et al. 55% of diabetic patients had detrusor hyperreflexia, 23% impaired contractility and 10% acontractility [2]. Cystometric evaluation of bladder dysfunction in elderly diabetic patients by Starer et al. showed bladder overactivity in 61% of the subjects, underactivity in 17% and acontractility 9% [3]. LUTS associated with diabetes is often attributed to diabetic cystopathy, a bladder dysfunction closely related with DM neuropathy. In the male patients however, the condition of the prostate is also crucial for determining the functional status of the lower urinary tract. How diabetes may
affect the prostate is a question rarely addressed. The purpose of this study was thus to investigate possible diabetic effect on the prostate gland with an animal model.

**Method**

Four groups of animals were used for investigation, group I: vehicle-treated normal rats; group II: vehicle-treated STZ-diabetic rats; group III: insulin-treated STZ-diabetic rats; group IV: phlorizin-treated STZ-diabetic rats. STZ-diabetic rats of group III received i.p. injections of long-acting human insulin at 0.5 IU/kg, every 8 h, three times a day. The group IV STZ-diabetic rats received phlorizin dissolved in 20% solution of propylene-glycerol by an i.p. injection at a dose of 1 mg/kg, every 8 h. The rats were sacrificed and the prostate gland was immediately removed, frozen in liquid nitrogen and stored at –70 ºC for subsequent mRNA extraction. Total RNA was extracted from the prostate tissue using the Ultraspec™-II RNA extraction system. Oligonucleotide primers for amplifying α₁A-AR mRNA were synthesized commercially by BRL (Life Technologies Inc., Grand Island, NY). Primers were designed to amplify ~200-base pair (bp), a third-loop segment of the α₁A-AR subtype. The sequences for each primer were as follows: α₁A-AR sense, 5’-CGAGTCTACGTAGTAGCC-3’; antisense, 5’-GTCTTGGCAGCTTTCTTC-3’ [15]. The PCR was performed with a Perkin-Elmer GeneAmp PCR system 2400. The primers used for β-actin were 5’-ATGGTGGGAATGGGTCAGAAG-3’ for the sense primer and 5’-CACGCAGCTCATTGTGTAGAAGG-3’ for the antisense primer. The PCR products were visualized with florescent illumination and densitometrically measured. For Western blotting analysis, the rat prostate α₁A-AR protein level was measured by Western blotting analysis with a polyclonal antiserum as previously reported [4].

**Result and Discussion**

For RT-PCR for the rat prostate α₁A-AR mRNA, the optical density for the band of the vehicle-treated STZ diabetic group is increased with comparison to the control non-diabetic group. Treatment with either insulin or phlorizin reduced the increase. Quantitative analysis shows a 2.51-fold increment of the prostate α₁A-AR mRNA with induction of diabetes (n=8, number of animals used in each group). Role of hyperglycemia in the alteration of α₁A-AR mRNA level was investigated by administrating either insulin or phlorizin to the diabetic rats. Both treatments restored the expression of α₁A-AR mRNA down to levels not significantly different from that seen in normal rats. Thus, correction of hyperglycaemia in diabetic rats can counteract the elevated gene expression of α₁A-AR. Western blot yielded a single band about 60 kDa for the α₁A-AR protein on SDS-PAGE in the membrane-enriched fractions of the prostatic tissue. Similar to the mRNA expression, the receptor protein in the STZ diabetic group was significantly increased compared to the control group. Quantitative analysis shows a 2.23-fold increase in the diabetic group (n=8). Again, treatment with either insulin or
phlorizin restored the protein level back to near control values. The finding indicates that hyperglycemia is responsible for the increase of $\alpha_{1A}$-AR protein synthesis in STZ diabetic rat prostate.

The important finding of the present study is the up-regulation in genetic expression of the rat prostate $\alpha_{1A}$-AR associated with diabetes. The implication would be that diabetic cystopathy is not the only cause of LUTS in diabetic male patients. Alteration in the prostatic functional status, or a ‘diabetic prostatopathy’, is also a possible etiologic factor leading to lower urinary tract dysfunction (LUTD). In a recent report by Michel et al. [5], it was shown that the severity of lower urinary tract symptoms in patients with BPH and the likelihood of having diabetes are significantly associated. The authors also demonstrated that treatment with tamsulosin, an $\alpha_1$-AR antagonist, is effective in reducing the LUTD. It is difficult to discern whether the presence of bladder outlet obstruction in these patients is inherent to concomitant benign prostatic hyperplasia or secondary to diabetes. Therefore it is worthwhile to conduct future urodynamic studies on younger diabetic male patients to define the incidence of BOO.

成果自評

Our study results have provided evidence for the novel concept of ‘diabetic prostatopathy’. This will lead to the development of new treatment strategy for diabetic patients with lower urinary tract dysfunction.

References