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MUSCARINIC RECEPTOR SYSTEMS IN YOUNG AND OLD TYPE II GOTO-KAKIZAKI DIABETIC RAT PROSTATE

Hypothesis / aims of study

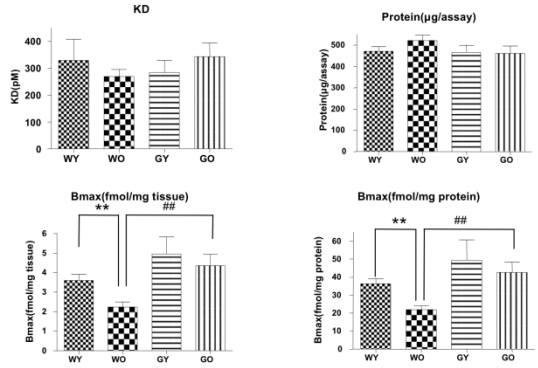
Benign prostatic hyperplasia (BPH) is generally not considered as a preventable disease. However, accumulating evidence suggests that modifiable factors may influence the risk of BPH and lower urinary tract symptoms (LUTS). Parsons reported that obesity, diabetes, physical activity and alcohol intake influence the risk of BPH and LUTS in older men [1]. Recently, increasing evidence is pointing towards the relationship between diabetes and BPH and LUTS. Cross-sectional studies from Sweden demonstrated that the diagnosis of diabetes was significantly associated with concomitant increase of prostate size consisted with BPH [2]. Furthermore, combination therapy of antimuscarinic and alpha-blockers has been highlighted recently as an effective treatment to attenuate LUTS in men with outflow tract obstruction [3]. These data suggest that it may, at least in part, be due to a synergy of action of muscarinic and adrenergic agents at outflow tract smooth muscles action including bladder neck, urethra and prostate. The Goto-Kakizaki (GK) rats represent a spontaneous non-insulin-dependent diabetes model. GK rats are produced from normal Wistar rats by repetition of selective breeding, and are a widely accepted as genetically determined rodent model for human type 2 diabetes. The aim of this study was to investigate the type 2 diabetes-induced changes in the expression of muscarinic receptors and their mRNAs in GK rat prostate, and the relation of these changes in the expression with the advanced rat age.

Study design, materials and methods

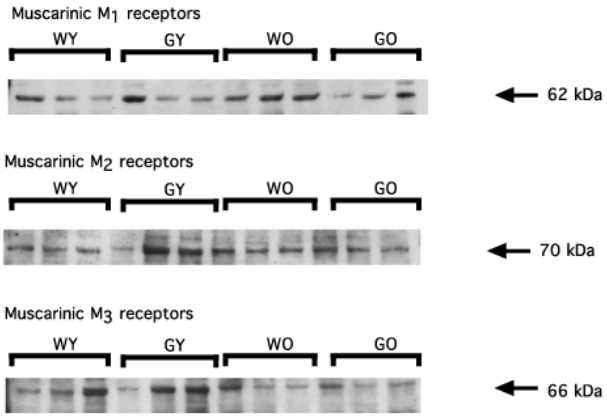
Six-week-old male GK and Wistar rats were purchased from SLC (Shizuoka, Japan). All rats were kept under identical conditions, and had access to food and drinking water *ad libitum*. The rats were divided randomly into four groups (n=6-8). Groups WY and GY consisted of 12-week-old Wistar and GK rats, respectively, while groups WO and GO consisted of 70-week-old Wistar and GK rats, respectively. Upon reaching 12 and 70 weeks of the age, the rats were sacrificed with an overdose of pentobarbital (60 mg/kg i.p.). Blood samples were collected from the vena cava, and the prostate was removed from each animal. The blood serum samples and prostate tissue were frozen at –80 C until used. Serum glucose concentrations in the experimental rats in all groups were measured by the hexokinase method (Glucose CII, Wako Pure Chemical, Osaka, Japan). The insulin concentrations were also measured by ELISA (Rat Insulin ELISA, Mercodia AB, Uppsala, Sweden). Serum testosterone levels were assessed using an enzyme immunoassay kit for testosterone (Oxford Biomedical Research; Enzyme Immunoassay for testosterone Product No. EA 78). The densities of muscarinic receptors (B_{max} values) were determined by saturation studies with [^{3}H]NMS ([N-methyl-^{3}H] scopolamine methyl chloride) in the prostatic membrane particulates. The participation levels of M_1, M_2 and M_3 receptor protein and mRNA levels in the prostate were investigated by immunoblot analysis and real-time polymerase chain reaction (PCR), respectively.

Results

Whereas the serum testosterone levels in the young Wistar rat were significantly higher than those in the young GK or old Wistar rats, there were no significant differences between the other groups.The B_{max} values in 12 week-old Wistar and GK, and in 70 week-old Wistar and GK rat prostates were 36.0±2.8, 49.4±11.4, 22.0±2.2 and 47.0±4.1 fmol/mg protein, respectively. However, there were no significant differences in the affinity constants between any groups. Immunoblot analysis showed the existence of significant amounts of M_1, M_2 and M_3 receptor subtypes in each rat prostate. According real-time PCR studies the rank order of expression levels of muscarinic receptors mRNA subtypes in the prostate were M_3>M_2>M_1 in all groups. In each receptor subtype in each group, diabetes induced up-regulation of mRNAs while the advanced age of the rats was related with down-regulation of mRNAs.



	KD (pM)	Bmax (fmol/mg tissue)	Protein (μg/assay)	Bmax (fmol/mg protein)
Wistar young (WY)	328±79	3.58±0.32	471±22	36.0±2.8
Wistar old (WO)	268±27	2.24±0.26**	522±25	22.0±2.2 **
GK young (GY)	284±15	4.96±0.59	467±32	49.4±11.4
GK old (GO)	342±52	4.35±0.57 ##	473±43	47.0±4.1 ##



WY: twelve-week-old Wistar rats, GY: twelve-week-old GK rats, WO: seventy-week-old Wistar rats, and GO: seventy-week-old GK rats.

Interpretation of results

The up-regulation of muscarinic receptors in diabetes type 2 rat prostate was similar to the findings reported in type 2 diabetic rat bladder and ileum, and therefore they could share the same pathophysiological mechanisms. On the other hand, the above effect of type 2 diabetes on muscarinic receptors in the prostate is quite different from the effect of STZ-induced type 1 diabetes on prostate where a down-regulation of the prostatic muscarinic receptors has been reported. Some possible mechanisms which could explain the up-regulation of muscarinic receptors include the decrease in cholinergic nerve density, or the defective neurotransmitter release mechanism. The diabetes-associated neuropathy may inhibit the release of acetylcholine from cholinergic nerves, inducing subsequently the overexpression of muscarinic receptors in the diabetic prostate.

Concluding message

GK type 2 diabetes induced up-regulation and age-related down-regulation of the expressions of muscarinic receptors and their mRNAs in rat prostate. These alterations may increase the risk of BPH and subsequent development of LUTS.

References

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Specify source of funding or grant	**Non**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**the Tottori University Committee for Animal Experimentation**