TEMPORAL EXPRESSION OF MUSCARINIC AND PURINERGIC RECEPTORS IN DIABETIC RAT BLADDER

Hypothesis / aims of study
Diabetes mellitus (DM) causes time-dependent bladder dysfunction. We have previously investigated the time-dependent alterations of detrusor muscle contractility (1). However, the mechanisms behind this change are not clear. Bladder contraction is mediated by both neurogenically-mediated cholinergic and nonadrenergic-noncholinergic (NANC) pathways (2). As muscarinic signaling is responsible for the main part of bladder emptying, ATP, as the main NANC excitatory transmitter, induces purinergic contraction. Five different muscarinic subtypes have been cloned. A predominance of the M2-muscarinic receptor subtype, with a smaller population of M3-receptors, has been reported for urinary bladder smooth muscle in several species. The proportion of muscarinic M2- and M3-receptors is approximately 9:1 in the rat bladder and approximately 3:1 in bladders from humans, guinea pigs, rabbits, and pigs. Although M2-muscarinic receptors are the predominant cholinoreceptor present in urinary bladder; the smaller population of M3-receptors appears to be the most functionally important and mediates direct contraction of the detrusor muscle. The M3-receptors are believed to cause a direct smooth muscle contraction through phosphoinositide hydrolysis and are mainly responsible for the normal micturition contraction. Seven subtypes of purinergic receptor have been cloned and characterized so far. The receptor subtype predominating in both species seemed to be the P2X1 subtype. However, changes in P2X2 receptor subtypes in bladders under pathological conditions have been reported. We aimed to examine the temporal alterations of the dominant receptors, muscarinic receptors (M2, M3) and purinergic receptors (P2X1 and P2X2) in the bladder in diabetic and diuretic rats.

Study design, materials and methods
Male Sprague Dawley rats were divided into three groups: streptozotocin-induced diabetics (n=12), 5% sucrose-induced diuretics (n=12), and age-matched controls (n=10). The bladders were removed at 6, or 20 weeks after diabetes and diuresis induction. The amount of muscarinic receptors (M2, M3) and purinergic receptors (P2X1 and P2X2) proteins were evaluated by immunoblotting 6 and 20 weeks after induction.

Results
Diabetes caused significant reduction of body weight compared to diuresis and controls, although the bladders of diabetic and diuretic rats weighed more than the controls. The level of expression of M2 receptor protein in the diabetic and diuretic bladder was not changed when compared with the control bladder. Whereas the expression of M3 and P2X1 receptor proteins in the diabetic and diuretic bladder were increased after 6-week induction, but not after 20-week induction compared to the control. The expression of P2X2 receptor protein in the diabetic was decreased after 6- and 20-week diabetes and diuresis induction.

Interpretation of results
M3 and P2X1 receptors are main receptors which induced bladder contraction. The present results showed that the expression of M3- and P2X1-receptor proteins in the diabetic and diuretic bladder were increased after 6-week induction, but not after 20-week induction compared to the control, which suggested that two main receptors were upregulated to compensate the significant increase of urine loading in the bladder in the early stage of diabetes. However, M3 and P2X1 receptors go back to control level in the later stage of diabetes.

Concluding message
There is a time-dependent up-regulation of M3 and P2X1 receptors biosynthesis in the early phase of diabetic and diuretic bladder, and down regulation of P2X2 receptor protein in diabetic and diuretic bladder. These alterations may contribute to the functional changes of diabetic bladder described earlier. These data also suggest that diabetes-associated polyuria partially contribute to diabetes induced bladder dysfunction.

References

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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 Cleveland Clinic Institutional Animal Care and Use Committee