

IMMUNOHISTOCHEMICAL ANALYSIS OF GAP JUNCTIONAL PROTEIN, CONNEXIN-43 IN RAT DETRUSOR MUSCLE WITH STREPTOZOTOCIN-INDUCED DIABETES

Aims of Study

Metabolites, ions and second messengers are directly mediated between the cells via gap junctions [1]. Gap junctions composed of Connexin (Cx) proteins have an essential role in intercellular communication. They are believed to play an important role in the maintenance of cell homeostasis. It is reported that cardiac myocytes are interconnected in the synchronous contraction by gap junctional intercellular communication [2]. It is considered that it will also be an important role concerning voiding function in bladder smooth muscle. Neurogenic bladder is well known as one of the consequence of autonomic dysfunction in diabetic patients. We studied the expression of gap junctional protein, connexin-43 (Cx-43), on smooth muscle of bladder in rat with streptozotocin-induced diabetes.

Methods

The 12 week-old female Wistar rats were divided into three groups: 1) normal controls; 2) diuretic rats with sucrose fed; 3) diabetic rats. Sucrose-treated rats were fed 5% sucrose in their drinking water. Diabetes was induced in rats by intraperitoneal injection of streptozotocin (60mg/kg). Approximately 5 days after the injection, a plasma sample was obtained by tail-vein puncture, and the glucose level was estimated. Animals with plasma glucose levels > 300mg/dl were assumed to be diabetic, and only these rats were used for further experimentation. Two, four and eight weeks later, cystometrical investigation was performed under urethane anesthesia. Maximum detrusor pressure, voided volume interval between micturitions and bladder capacity were measured. After filling cystometry, the entire bladder was excised and small pieces were cut from the initial sample and fixed in 4% paraformaldehyde. The expression of Cx-43 protein on detrusor muscle was examined in the bladder of each three group by immunohistochemical staining. Immunohistochemical analysis of connexin 43 protein expression was performed by using a polyclonal antibody (Rabbit anti-Connexin 43, Zymed Laboratories Inc, San Francisco, CA). Briefly, paraffin-embedded specimens were sectioned and placed on MAS coated glass slides (Matsunami, Osaka, Japan). Deparaffinized sections were then placed in 10 mM citrate buffer (pH 6.0) and treated with autoclave 90 °C, 30min. After washing with phosphate-buffered saline (PBS), sections were incubated. The primary antibody, rabbit anti-Connexin 43, was applied to the sections. These sections were then incubated for overnight at 4 °C temperature. After washing in PBS, the sections were incubated with the second antibody of the anti-rabbit IgG (H+L chain)-Peroxydase for 1 hour at room temperature. After appropriate washings, the sections were incubated in Liquid DAB Substrate Kit (Zymed) for 5 min. Finally, the sections were counterstained with Mayer's hematoxylin.

Results

The weights of bladder in diabetic rats were significantly increased more than those of controls and diuretic rats. According to cystometry, mean bladder capacity of diabetic rats for 8 weeks was significantly increased comparison with the other two groups. However, there were no significant differences in mean voided volume, mean maximum detrusor pressure between the three groups. Histologically, hypertrophy of the detrusor muscle was investigated in diuretic rats and diabetic rats. In immunohistochemical staining, the expression of Cx-43 protein was detected on cell membrane of detrusor muscle of each three group, and there was no significant difference in localization to the Cx-43 protein expression among each three group.

Conclusions

In this study, we investigated gap junctional protein, Cx-43 of detrusor muscle in diabetic rats, for short term, such as 2,4 and 8 weeks. The bladder capacity of detrusor muscle was significantly increased. However, the maximum detrusor pressure and Cx-43 protein

expression were not altered. Increase of bladder capacity might be due to the dysfunction of the afferent sensory innervations of the bladder in early phase of diabetes. It is considered that the Cx-43 protein expression of the detrusor muscle was normally kept in the early diabetic rat, until the normal detrusor pressure was maintained.

References

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