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THE ROLE OF CALCIUM-ACTIVATED POTASSIUM CHANNELS IN THE CONTROL OF BLADDER SMOOTH MUSCLE FUNCTION IN RATS WITH BLADDER OUTLET OBSTRUCTION

Hypothesis / aims of study

Bladder outlet obstruction (BOO) causes various structural and functional changes in the voiding system and causes problematic storage symptoms such as urgency, frequency, and urgency incontinence due to detrusor overactivity (DO). These pathophysiological conditions have been simulated using animal models and various neurogenic and myogenic changes have been reported as a cause of DO in the BOO model. Generally, potassium channels have a role in reducing excitability of membranes of excitable cells and could suppress the instability of detrusor smooth muscle. Thus, there is possibility that alterations of potassium channel expression occur in the bladder of the BOO model. The aim of this study is therefore to investigate the relationship of large conductance (BK) and small conductance (SK) calcium-activated potassium channels and detrusor muscle function in rats with BOO.

Study design, materials and methods

BOO was produced by partial urethral ligation in adult female Sprague Dawley rats. Six weeks after urethral ligation, cystometry was performed in the conscious condition. According to the result of cystometry, BOO rats were divided into two groups with (BOO-c) or without non-voiding contractions (NVC) (BOO-nc) during the storage phase. After cystometry, the whole bladder was removed and prepared for the evaluation of the mRNA expression of a BK channel alpha, beta1 and beta4 subunits and SK1, 2 and 3 channels using real time RT-PCR. In another group of BOO rats with NVC, bladders were also removed and divided into mucosa and muscle layers, and subjected to the RT-PCR evaluation. To investigate functional changes in smooth muscle activity, mucosa-denuded bladder smooth muscle strips were prepared from another group of BOO rats. The effects of iberiotoxin (ITx) and charybdotoxin (CTx), which are blockers of BK channels, and apamin, which is a blocker of SK channels, on spontaneous and carbachol-induced contractions were evaluated with tension measurements of these muscle strips in organ baths filled with 37° C Krebs solution.

Results

1. Cystometry: Bladder weight, premicturition pressure, and baseline pressure were significantly increased in BOO-c rats compared to BOO-nc ($p<0.01$, $p<0.05$, $p<0.05$, respectively) and controls ($p<0.001$, $p<0.01$, $p<0.01$, respectively). Maximum pressure was increased in both groups of BOO rats ($p<0.001$), but was significantly lower in BOO-c compared to BOO-nc ($p<0.05$).
2. RT-PCR: There were no differences in the mRNA expression of the BK alpha subunit when ribosomal protein L13A was used as the internal control, however, the beta1 subunit was significantly increased in both BOO groups compared to control ($p<0.01$ BOO-c, $p<0.05$ BOO-nc). In contrast, both BOO groups showed markedly decreased expression of the beta4 subunit compared to control ($p<0.001$). All SK channels tended to increase in BOO models. Especially, the SK3 channel showed a significant increase in both BOO groups ($p<0.05$). Furthermore, the investigation using the isolated detrusor muscle and mucosa revealed that these alterations in BK and SK channel expression occurred mainly in the muscle layer.
3. Muscle strip study: An application of SK or BK blockers increased amplitude and frequency of spontaneous contractions of bladder muscle strips in control and BOO models. The larger changes in the ratio of post- vs. pre-application were observed in the amplitude of spontaneous contractions in BOO muscle strips ($p<0.01$ in all blockers), but not in the frequency, compared to controls. The responses of muscle strips to the cumulative carbachol applications were also facilitated by the blockers of BK and SK channels to the greater degree in BOO muscle strips, compared to controls. In controls, each blocker did not show the changes of maximal contraction (Emax) to carbachol stimulation compared to a vehicle application. On the other hand, in the BOO model, CTx and ITx elicited an increase of Emax ($p<0.05$.in both blockers). Apamin did not affect the Emax in either group. The EC50 (molar carbachol concentration producing 50% of the maximal contraction response) was also affected by the potassium channel blockers. ITx and CTx decreased the EC50 in both groups compared to a vehicle application ($p<0.05$ ITx in control, $p<0.01$ the others). The degree of the decrements in EC50 was larger in the BOO model than in controls ($p<0.05$ ITx, $p<0.01$ CTx). Furthermore, in the BOO model, this response to CTx was larger than to ITx ($p<0.05$). Apamin also showed the decrease of the EC50 in the BOO model.

Interpretation of results

The BK channel beta1 subunit is known to increase calcium and voltage sensitivity of the alpha subunit, resulting in facilitation of BK channel function (1, 2). On the other hand, the beta 4 subunit decrease voltage sensitivity and slow activation kinetics, so it is known to act as a downregulator of the BK channel (1, 3). Therefore, increased and decreased expression of the BK channel beta1 and beta4 subunits, respectively, and increased SK channel expression suggest upregulated function of both BK and SK channels in the bladder smooth muscle of the BOO model (6 weeks). This assumption is supported by the larger increases of spontaneous activity and contractions induced by muscarinic receptor stimulation by BK or SK channel blockers in muscle strips from the BOO model.

Concluding message

The calcium-activated potassium channels act in a direction in which they suppress the excitability of the smooth muscle in both spontaneous activity and contractile responses to muscarinic receptor stimulation. Thus, BK or SK channels seem unlikely to contribute to the development of DO in the BOO model, but they rather undergo compensatory reactions to counteract the altered property of smooth muscle cells after BOO.

References

1. J Biol Chem (2000) 275; 6453-6461
2. Neuron (1995) 14; 645-650
3. J Neurrosci (2000) 20; 3563-3570

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