73

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THE INFLUENCE OF THE MUCOSA AND BLADDER OUTLET OBSTRUCTION ON THE EFFECT OF CYCLOOXYGENASE INHIBITOR, INDOMETHACIN, ON SPONTANEOUS CONTRACTILE ACTIVITY IN THE RAT BLADDER

Hypothesis / aims of study

Spontaneous contractions (SCs) may evoke afferent nerve firing. The mucosa is now recognized as being involved in the mechanism for the generation of SCs in the bladder. Removal of the mucosa reduces SCs in strips from the bladder body. The inhibitory effect of an ATP-sensitive potassium channel opener, cromakalim, on SCs has been reported to be enhanced by removal of the mucosa [1]. The mechanism underlying the modulation of SCs by the mucosa remains largely to be established. There is evidence that the urothelium releases mediators such as ATP, acetylcholine and prostaglandin (PG) E2 that may be involved in the modulation of SCs by the mucosa. Cyclooxygenase (COX) that is responsible for the synthesis of prostanoids including PGE2 has been demonstrated to be present in urothelial cells as well as in interstitial cells of Cajal (ICCs) in the suburothelium and the detrusor layer, and the intravesical amount of PGE2 probably released from the urothelium has been reported to be increased by bladder outlet obstruction (BOO) [2]. However, little is known about the influences of the mucosa and BOO on the effects of COX inhibitors on SCs. We examined the influence of the mucosa on the effect of the COX inhibitor, indomethacin, on SCs in rats with and without BOO.

Study design, materials and methods

Female Wistar rats (8 weeks old) were used in this study. BOO was induced by incomplete urethral ligation (urethral outer diameter of 1.1 mm) (n=8). Sham-operated rats underwent only the dissection of urethra (n=6). Four weeks following the operation, bladders were removed and weighed. Two pairs of mucosa-intact and denuded strips from each bladder body were created and mounted in tissue baths, equilibrated at 1 g resting tension for at least 1 hour and washed with Krebs solution every 20 minutes. After SCs had developed, the one pair of the mucosa-intact and denuded strips were incubated with indomethacin (1 μM) for 15 minutes and the other pair were incubated with vehicle for 15 minutes. After the incubation with indomethacin or vehicle, cromakalim was added cumulatively to all strips in increasing concentrations. The frequency and amplitude of SCs were recorded for each strip over the entire duration of the experiment. The percentage decreases in the frequency and amplitude following the addition of cromakalim were calculated and used to create concentration-response curves. Data are expressed as mean ± SEM.

Results

The frequency of SCs was not changed by the removal of the mucosa while the amplitude of SCs was significantly reduced by the removal of the mucosa both in BOO rats and in sham-operated rats ($p < 0.05$ for BOO rats: $p < 0.01$ for sham-operated rats). Indomethacin did not significantly change the frequency of SCs in the mucosa-intact and denuded strips irrespective of the presence or absence of BOO while it reduced the amplitude of SCs by 20% in the intact strip and by 52% in the denuded strip in BOO rats ($p < 0.05$ and < 0.01, respectively), and by 29% in the intact strip and by 38% in the denuded strip in sham-operated rats ($p < 0.05$ for both). The percentage reduction in the amplitude of SCs was significantly greater in the denuded strip in BOO rats than in that in sham-operated rats ($p < 0.01$).

The removal of the mucosa or the incubation with indomethacin affected only the amplitude of SCs, so the effect of cromakalim on SCs was analysed for the amplitude of SCs. Cromakalim abolished SCs in all strips. The potency of cromakalim in reducing the amplitude of SCs was expressed as the pEC50, the negative logarithm of the concentration that produces a half-maximal effect. The data are shown in the Table. In the strip pre-incubated with vehicle, the pEC50 was greater in the mucosa-denuded strip than in the intact strip both in BOO rats and in sham-operated rats. Pre-incubation with indomethacin increased the pEC50s in the mucosa-intact and denuded strips in BOO rats and also in the intact strip in sham-operated rats. However, indomethacin did not significantly affect the pEC50 in the denuded strip in sham-operated rats. In the strip pre-incubated with indomethacin, the significant difference in the pEC50 of cromakalim between strips with and without the mucosa was observed again in BOO rats but the pEC50 of cromakalim was similar in the strip with and without the mucosa in sham-operated rats.

Table. The pEC50s of cromakalim for reducing the amplitude of SCs

	BOO rats (n=8)		Sham-operated rats (n=6)	
	Mucosa-intact strips	Mucosa-denuded strips	Mucosa-intact strips	Mucosa-denuded strips
Strips pre-incubated with vehicle	6.14 ± 0.19	6.86 ± 0.12**	6.25 ± 0.10	6.66 ± 0.15*
Strips pre–incubated with indomethacin	6.79 ± 0.14††	7.64 ± 0.14**,††	6.93 ± 0.08†	7.01 ± 0.16

* $p < 0.05$, ** $p < 0.01$ compared to mucosa-intact strips. † $p < 0.05$, †† $p < 0.01$ compared to strips pre-incubated with vehicle.

Interpretation of results

The removal of the mucosa reduced the amplitude of SCs and increased the potency of cromakalim in suppression of SCs in the strip pre-incubated with vehicle both in BOO rats and in sham-operated rats, so the modulation of SCs by the mucosa was confirmed to be present in rats with BOO like in those without BOO. Indomethacin could reduce the amplitude of SCs irrespective of the presence of the mucosa and BOO. The percentage reduction in the amplitude of SCs by indomethacin in the mucosa-denuded strip was greater in BOO rats than in sham-operated rats, indicating that the machinery for generating SCs in the detrusor is more sensitive to indomethacin in rats with BOO compared to those without BOO. This finding implies that the

obstruction upregulates the expression of COX in the detrusor and increases the synthesis of prostanoids including PGE2 that is related to the generation of SCs. This idea may be supported by a previously reported finding that ICCs in the detrusor are increased in number by BOO [3]. In BOO rats the removal of the mucosa increased again the potency of cromakalim in the strip pre-incubated with indomethacin, suggesting that the modulation of SCs by the mucosa is not inhibited by indomethacin in BOO. The inhibition of COX in the detrusor by indomethacin may directly suppress the generation of SCs in BOO. In sham-operated rats, the removal of the mucosa failed to increase the potency of cromakalim in the strip pre-incubated with indomethacin and indomethacin did not significantly increase the potency of cromakalim in the mucosa-denuded strip. These findings suggest that in rats without BOO indomethacin inhibits the modulation of SCs by the mucosa and consequently suppresses SCs probably due to a decreased synthesis of prostanoids including PGE2 in the mucosa and that the contribution of prostanoids in the detrusor to the generation of SCs is small in rats without BOO.

Concluding message

The COX inhibitor indomethacin acts to suppress SCs in the rat bladder irrespective of the presence or absence of BOO. However, the mechanism underlying the suppressive effect of indomethacin on SCs may be different between rats with and without BOO. In rats with BOO the inhibition of COX in the detrusor by indomethacin may directly suppress the generation of SCs in the detrusor. In rats without BOO the inhibition of COX in the mucosa by indomethacin may inhibit the modulation of SCs by the mucosa and consequently suppress the generation of SCs in the detrusor.

References

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Disclosures

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