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THE MUSCARINIC RECEPTOR SUBTYPE OF M2 MEDIATES CA2+ SENSITIZATION VIA INDIRECT ACTIVATION OF RHO-KINASE PATHWAY IN HUMAN DETRUSOR SMOOTH MUSCLE.

Hypothesis / aims of study

In urinary bladder, carbachol (CCh) induces contraction through M_2 and mainly M_3 receptor subtypes.[1] However, how the muscarinic receptor subtypes contribute to the Ca^{2+} sensitization, so called, rho-kinase (ROK) pathway and protein kinase C (PKC) pathway are still unknown. In particular, there is absolutely no report regarding the contribution of M_2 receptor subtype on the Ca^{2+} sensitization. However, it is very likely and of our interest that the down-regulation of cyclic adenosine monophosphate (cAMP) via M_2 receptor subtype would an indirect influence on contraction since it has been suggested that cAMP might antagonize ROK pathway.[2] The aim of our present study is to clarify the role and signaling cascades of these M_2 and M_3 receptor subtypes on Ca^{2+} sensitization using alpha-toxin permeabilized human detrusor smooth muscle (DSM).

Study design, materials and methods

The DSM specimens were obtained from urinary bladder of human who underwent radical cystectomy due to urinary bladder cancer.

Alpha-toxin permeabilized human DSM strips were prepared and mounted horizontally for isometric force recording. The role and signalling cascade of M_2 and M_3 receptor subtypes in Ca^{2+} sensitization was studied using selective antagonists of M_2 (AF-DX116) and M_3 (4-DAMP) receptor subtypes. The effects of a selective inhibitor of ROK, Y-27632, and a selective inhibitor of PKC, bisindolylmaleimide I (GF-109203X), were also studied in contraction induced by 10 μM CCh with 100 μM GTP at fixed 1 μM $[Ca^{2+}]_i$ after pre-application with 1 μM AF-DX 116 or with 1 μM 4-DAMP. The effect of a cAMP specific phosphodiesterase (PDE IV) inhibitor Rolipram on Ca^{2+} sensitization was also investigated. Experiment was carried out after pre-treatment with Ca^{2+} store depletor thapsigargin (1 μM) and with cyclopiazonic acid (CPA; 1μM) present in all solutions after permeabilization.

All data and graphs are expressed as the mean ± SEM of n (the number of samples) and N (the number of patients). Student *t*-test was used for statistical analyses to determine any statistical differences of mean values between two variables. A *P* value of less than 0.05 was considered to be statistically significant.

results

As preliminary information, the small inhibitory effects of 5 μM Y-27632 (10.6 ± 2.2%, n = 4) and greater inhibitory effect of 5 μM GF-109203X (39.7 ± 1.5%, n = 4) were observed following contraction induced by only 1 μM Ca^{2+} (Fig. 1A). After the increase of Ca^{2+} sensitization was observed by the application of 10 μM CCh with 100 μM GTP at the fixed concentration of 1 μM Ca^{2+}, 1μM AF-DX116 and 1μM 4-DAMP inhibited the contraction by 13.0 ± 1.5 % and 41.4 ± 2.9 %, respectively (n = 6, Fig. 1B). 5 μM Y-27632 attenuated the increase of Ca^{2+} sensitization by 10 μM CCh with 100 μM GTP at 1 μM Ca^{2+} for 54.7 ± 6.0 % (n = 6). Not only 1μM 4-DAMP but also 1 μM AF-DX 116 remarkably inhibited this attenuation effect of Y-27632 from 54.7 ± 6.0 % to 4.4 ± 1.0% (n = 6) and 14.7 ± 1.1% (n = 6), respectively although the inhibition by 1 μM 4-DAMP was significantly stronger than that by 1 μM AF-DX116 (Fig. 2A). Likewise, 5 μM GF-109203X attenuated the increase of Ca^{2+} sensitization by 10 μM CCh with 100 μM GTP at 1 μM Ca^{2+} for 70.7 ± 3.2 % (n = 6). Both 1 μM AF-DX 116 and 1μM 4-DAMP inhibited this attenuation effect of GF-109203X from 70.7 ± 3.2 % to 16.4 ± 5.8 % (n = 6) and 35.9 ± 2.7 % (n = 6), respectively. The inhibition by 1 μM 4-DAMP was significantly stronger than that by 1 μM AF-DX116 (Fig. 2B). On the contrary to the prediction of ROK pathway, M_2 selective inhibitor AF-DX 116 (1 μM) certainly inhibited the attenuation effect of Y-27632 although AF-DX116 induced little attenuation in Ca^{2+} sensitization activated by 10 μM CCh with 100 μM GTP. Since the indirect effect of M_2 receptor on Ca^{2+} sensitization via down regulation of cAMP was speculated, the effect of PDE IV specific inhibitor Rolipram on the contraction was investigated. 5 μM Rolipram attenuated the Ca^{2+} (1 μM) induced contraction by 35.1 ± 1.0 % (n = 4, N = 3,. This attenuation by Rolipram was significantly relatively decreased from 35.1 ± 1.0 % to 15.5± 1.0 % (n=4) in the condition where Ca^{2+} sensitization was activated by 10 μM CCh with 100 μM GTP (Fig. 3)

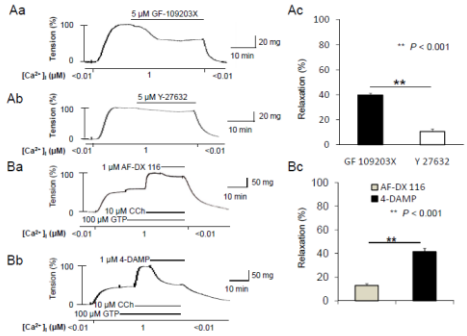

Fig. 1 Effect of GF-109203X and Y-27632 on Ca^{2+}-induced contraction
Effect of AF-DX116 and 4-DAMP on CCh-induced contraction

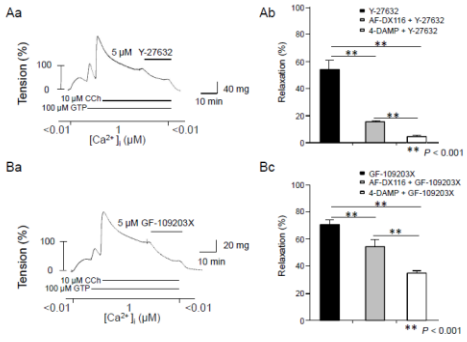

Fig. 2 Effect of Y-27632 and GF-109203X on CCh-induced contraction via M_2 or M_3 receptor

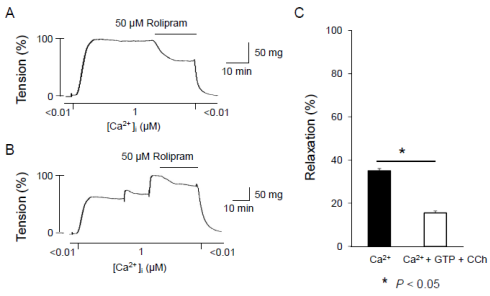

Fig. 3 Effect of Rolipram on Ca^{2+}- or CCh-induced contraction

Interpretation of results

The inhibition by Y-27632 and GF-109203X on only Ca^{2+}-induced contraction indicated that ROK and PKC pathways have already been activated in Ca^{2+}-induced contraction of permeabilized DSM (Fig. 1A). The inhibition by 1 μM 4-DAMP was significantly stronger than that by 1 μM AF-DX116 on the Ca^{2+} sensitization by CCh with GTP indicating that the predominant role of M_3 receptor in Ca^{2+} sensitization of human DSM.

AF-DX 116 and mainly 1μM 4-DAMP inhibited the attenuation effect of GF-109203X on the Ca^{2+} sensitization by CCh with GTP indicating the coherent understanding that M_3 receptor plays a predominant role in PKC pathway. Regarding ROK pathway, from the fact that M_2 selective inhibitor AF-DX 116 certainly inhibited the attenuation effect of Y-27632 although AF-DX116 induced little inhibition in Ca^{2+} sensitization activated by CCh with GTP, the indirect effect of M_2 receptor was suggested. To support this hypothesis, the attenuation by PDE IV inhibitor Rolipram on the contraction just by Ca^{2+} was relatively decreased by the activation of Ca^{2+} sensitization by CCh with GTP. These results suggested that cAMP formation had already been inhibited in advance by muscarinic stimulation via M_2 receptor.

Concluding message

Our present study demonstrated the first evidence in human DSM that both M_2 and M_3 receptor subtypes mediate Ca^{2+} sensitization. Our study also revealed the predominant role of M_3 receptor subtypes to ROK and PKC pathways with comparable contribution which induced Ca^{2+} sensitization. Further, interestingly, the contribution of M_2 receptor subtype is indirectly and preferably to ROK pathway but not to PKC pathway via the down-regulation of cAMP. These findings might be important in the clinical application of highly selective antimuscarinic treatment for lower urinary tract dysfunction.

References

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2. Bolz SS, Vogel L, Sollinger D et.al. Circulation 2003;107:3081 – 3087

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What were the subjects in the study?	**HUMAN**
Was this study approved by an ethics committee?	**Yes**
Specify Name of Ethics Committee	**Kyushu University Hospital Ethical Committee**
Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**