

THE ROLE OF CYCLIC NUCLEOTIDES IN THE REGULATION OF PIG URETHRAL SMOOTH MUSCLE.

Hypothesis / aims of study

In urethral smooth muscle (USM), NO-mediated relaxation via cGMP formation is dominant. On the other hand, for bladder relaxation, cAMP formation seems to be more important. To clarify their precise roles, we have investigated the effect of cyclic nucleotides on the α -toxin permeabilized pig detrusor smooth muscle. Since this α -toxin permeabilization preserves receptor-effector pathways independent of membrane transporters involving ion channels and Ca^{2+} stores, we have revealed that both cAMP and cGMP induced relaxation in detrusor smooth muscle contraction via the activation of myosin light chain phosphatase. However, the relaxation effect of cAMP was stronger and this relaxation was attenuated by carbachol-induced Ca^{2+} sensitization. Thus, our present study was designed to clarify the mechanisms underlying relaxation by cyclic nucleotides on Ca^{2+} -dependent or Ca^{2+} -independent pathway (Ca^{2+} sensitization) in contraction of urethral smooth muscle.

Study design, materials and methods

The USM specimen were cut out transversely from the proximal (3-4 cm distal to the bladder neck) part of urethra obtained from female pig. The small strips (2 - 3 mm in length and 300 - 400 μm in diameter) of circular muscle were prepared after gently removing the mucosa and connective tissues under dissecting microscope. As preliminary experiments, the effect of cyclic nucleotides on intact urethral smooth muscle strips were investigated using membrane permeable 8-Br cAMP and 8-Br-cGMP. Accordingly, permeabilization was performed with 5,000 U/ml α -toxin for 60 minutes. The roles of cAMP and cGMP were studied on the contraction induced by 1 μM $[\text{Ca}^{2+}]_i$ or 1 μM $[\text{Ca}^{2+}]_i$ with 100 μM GTP and 10 μM phenylephrine. The experiment using permeabilized tissue was carried out after pre-application with 1 μM thapsigargin and 100 nM xestospongine C for 30 minutes and with cyclopiazonic acid (CPA; 1 μM) present in all solutions after permeabilization at room temperature to exclude the involvement of Ca^{2+} stores. All data are expressed as mean \pm S.E.M. of the number of samples (n). Student *t*-test was used for statistical analyses.

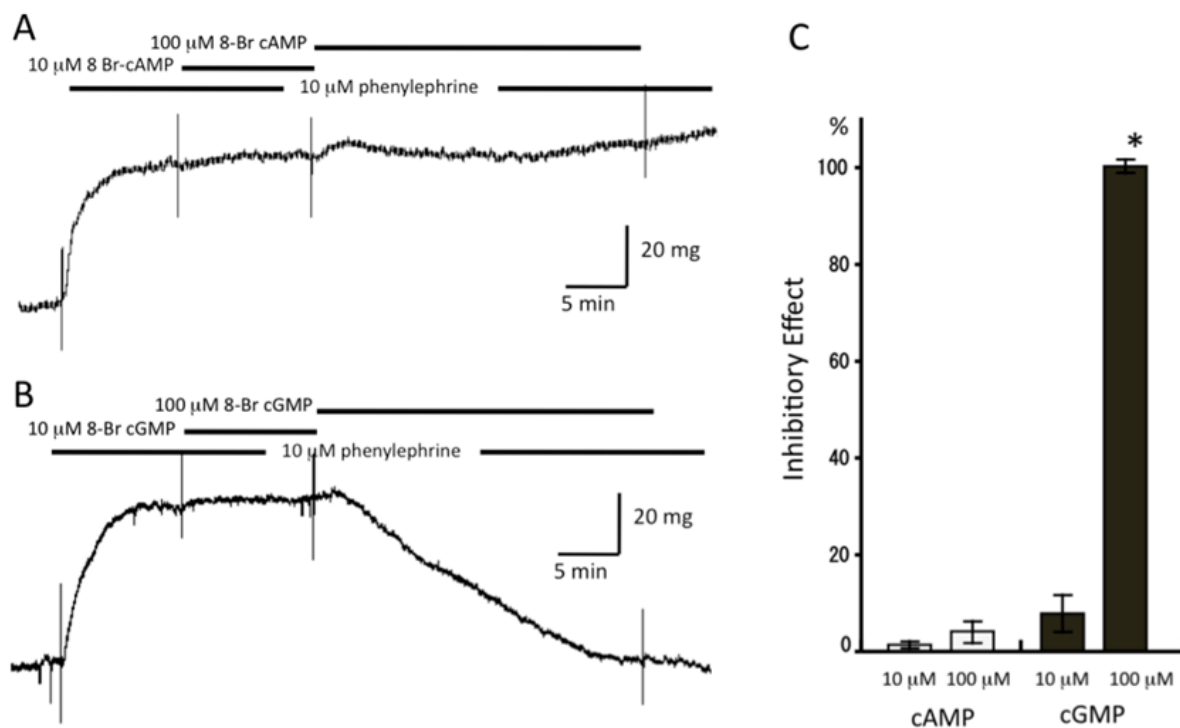


Figure 1. The effect of 8-Br cAMP and 8-Br cGMP on pig urethral smooth muscle. A&B: Representative traces demonstrate the effect of 8 Br-cAMP (A: 10 μM & 100 μM) and 8 Br-cGMP (B: 10 μM & 100 μM) on phenylephrine (10 μM)-induced contraction of intact pig urethral smooth muscle. C: The relative inhibitory effects by cyclic nucleotides are summarized with the tension by 10 μM phenylephrine normalized as 100 %. Values are means \pm S.E.M. An asterisk indicates a significant difference ($P < 0.001$; $n = 6$).

Results

At first, the effect of membrane permeable cyclic nucleotides, 8-Br cAMP and 8 Br-cGMP on intact USM strips were investigated. Predictably, 8 Br-cGMP (100 mM) well inhibited the force development induced by phenylephrine (10 mM) (98.1 ± 1.44 %, $n = 5$). On the other hand, 8-Br cAMP (100 mM) induced little change on phenylephrine-induced contraction (3.7 ± 2.6 %, $n = 5$) (Fig. 1). Next, the effects of cyclic nucleotides on permeabilized strips were investigated using α -toxin permeabilized USM strips. However, in comparison with the results using intact USM, the relaxation effect of cAMP (100 mM) was remarkably potentiated (59.9 ± 14.4 %, $n = 5$) but that of cGMP (100 mM) was reduced in contraction induced by 1 μM Ca^{2+} (37.7 ± 7.2 %, $n = 5$).

n = 5). The relaxation effect of cAMP was stronger than that of cGMP in permeabilized USM. The relaxation effect of cAMP (100 mM) in contraction induced by 1 μ M Ca^{2+} was decreased by 10 μ M phenylephrine in the presence of 100 μ M GTP (31.3 ± 5.5 %, n = 5). However, the relaxation effect of cGMP was not affected by phenylephrine (10 mM) (36.6 ± 6.4 %, n = 5).

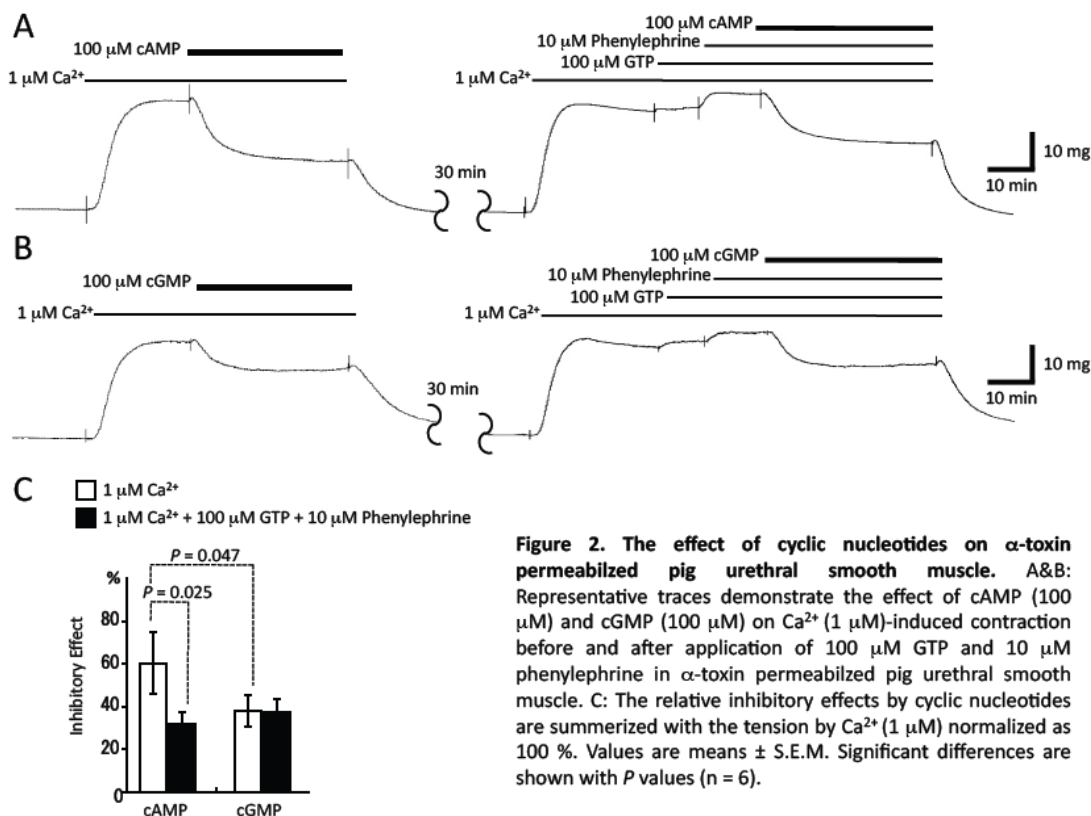


Figure 2. The effect of cyclic nucleotides on α -toxin permeabilized pig urethral smooth muscle. A&B: Representative traces demonstrate the effect of cAMP (100 μ M) and cGMP (100 μ M) on Ca^{2+} (1 μ M)-induced contraction before and after application of 100 μ M GTP and 10 μ M phenylephrine in α -toxin permeabilized pig urethral smooth muscle. C: The relative inhibitory effects by cyclic nucleotides are summarized with the tension by Ca^{2+} (1 μ M) normalized as 100 %. Values are means \pm S.E.M. Significant differences are shown with P values (n = 6).

Interpretation of results

The results of the study using intact USM strips indicated that cGMP but not cAMP plays a dominant role in relaxation of USM in good agreement with previous reports. However, in α -toxin permeabilized USM strips, the relaxation effect of cAMP was potentiated but that of cGMP was attenuated on contraction induced by 1 μ M Ca^{2+} . This potentiation of cAMP was decreased by the activation of α -adrenergic receptor. On the other hand, the relaxation effect of cGMP in the presence of 1 μ M Ca^{2+} was not modulated by the activation α -adrenergic receptor.

Concluding message

This is the first study of the effect of cyclic nucleotides on pig USM using α -toxin permeabilization. The results of the studies were quite unique.

We have demonstrated that the results using intact USM indicated the predominant role of cGMP. The deficient relaxation by 8 Br-cAMP in intact USM tissue is at present unclear, however the direct relaxation by cAMP was recognised in α -toxin permeabilized USM. The potentiated relaxation by cAMP in α -toxin permeabilized USM was reduced by the activation of α -adrenergic receptor in comparison with unchanged result of cGMP indicating that cAMP but not cGMP is modulated by Ca^{2+} sensitization in USM although it is unlikely that cAMP plays a major role in the relaxation of USM. The relaxation effect by cGMP in intact tissue was attenuated in α -toxin permeabilized USM indicating that cGMP predominantly acts on plasma membrane and/or sarcoplasmic reticulum Ca^{2+} -ATPase.

Disclosures

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