Smooth Muscle Regulation of PGE$_1$ and Forskolin in Rabbit Cavernosal Tissue by Cyclic GMP- and Cyclic AMP-Dependent Mechanisms

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Abstract

Prostaglandin E1 (PGE$_1$) is widely used for treating erectile dysfunction, but involvement of its molecular action in penile erection is still unknown. To elucidate the paracrine mechanism of PGE$_1$ and forskolin inducing relaxation of rabbit corpus cavernosum, we performed in vitro experiments using KT-5720, a novel adenosine 3',5'-cyclic monophosphate (cyclic AMP)-dependent protein kinase inhibitor, and KT-5823 and KT-5926, both novel guanosine 3',5'-cyclic monophosphate (cyclic GMP)-dependent protein kinase inhibitors. Prior to contraction with phenylephrine $1 \times 10^{-4}$ M, treated isolated strips of rabbit corpus cavernosum were treated for 30 min either with KT-5720 $1 \times 10^{-5}$ M, KT-5823 $1 \times 10^{-5}$ M, or KT-5926 ($1 \times 10^{-5}$ M). Control strips did not receive protein kinase inhibitors. Following cavernous smooth muscle maximal contraction by phenylephrine, both treated and control strips received forskolin or PGE$_1$ to induce relaxation. The group treated with protein kinase inhibitor was compared to the control group.

KT-5926 inhibited PGE$_1$ ($1 \times 10^{-5}$ M: final dose) induced relaxation of rabbit corpus cavernosum (control 104.1±8.0 vs 80.6±11.4%). Neither KT-5720 nor KT-5823 inhibited PGE$_1$ induced relaxation. KT-5720 and KT-5823 inhibited forskolin ($1 \times 10^{-5}$ M: final dose) induced relaxation of rabbit corpus cavernosum (control 139.6±6.3 vs 107.3±11.0 vs 96.7±4.6%). KT-5926 had no significant effect on forskolin induced relaxation.

Our findings suggest that the relaxation of rabbit corpus cavernosum smooth muscle inducing by forskolin is mediated by the cyclic AMP- and cyclic GMP-dependent pathway, and that induced by PGE$_1$ is mediated by the cyclic GMP-dependent pathway.

Key words
Prostaglandin E$_1$, forskolin, cyclic GMP, cyclic AMP, protein kinase inhibitor

Introduction

Prostaglandin E$_1$ (PGE$_1$) is widely used for diagnosis and treatment of erectile dysfunction$^{1-4}$. Forskolin is a United States Food and Drug Administration nonapproved vasoactive agent that acts in synergism with PGE$_1$ to induce smooth muscle relaxation. However, its mechanism is still unknown. Cyclic nucleotide-dependent kinases are useful probes for the study of cyclic nucleotide-dependent vasorelaxation. Many studies suggested cyclic nucleotides to be mediators of relaxation in visceral and vascular smooth muscle by virtue of their ability to activate distinct cyclic AMP-dependent and cyclic GMP-dependent protein kinase$^{5-9}$). By using protein kinase inhibitors, KT-5720 (cyclic AMP-dependent)$^{10}$, KT-5823 (cyclic...
cyclic GMP-dependent) and KT-5926 (cyclic GMP-dependent), we performed in vitro experiments to elucidate the mechanism of both PGE$_1$ and forskolin on rabbit smooth muscle relaxation.

**Materials and Methods**

**Animal preparation**

Immediately after death by sanguination, the penises were removed from mature New Zealand white rabbits (body weight 3.7–4.6 kg) and dissected to obtain a strip of corpus cavernosum (8×3×4 mm). These strips were mounted in organ baths (30 ml), with one end fixed to a J-shaped metal holder and the other to an isometric transducer (Radnoti glass technology TRN001) by means of small metal hooks. The strips were kept in fresh Kreb’s solution made in the following composition (mM): NaCl 118, KCl 4.7, MgSO$_4$ 1.18, KH$_2$PO$_4$ 1.17, Glucose 11.1, NHCO$_3$ 24.9, CaCl$_2$ 2.5. The buffer solution was maintained at 37°C and aerated continuously with a mixture of 5% CO$_2$ and 95% O$_2$ maintaining a pH of approximately 7.4. First, the strips were set at a 2g tension and equilibrated for 1 hour. After equilibration, the drugs were added. Changes in tension were monitored using the isometric transducer and recorded with a pen recorder (Gould electronics the 5900 Signal Conditioner Frame).

**Organ chamber studies**

The following drugs were used: l-phenylephrine hydrochloride (Sigma Chemical Co.), guanethidine monosulfate (Sigma Chemical Co.), atropine (Sigma Chemical Co.), forskolin (Sigma Chemical Co.) and prostaglandin E$_1$ (Upjohn Co. Ltd. USA). We also used three kind of protein kinase inhibitor, KT-5720, KT-5823 and KT-5926 (Kamiya Biomedical Co.). All drug concentrations shown are expressed as the final concentration in the organ bath. Each protein kinase inhibitor was added in the Kreb’s solution, and after thirty minutes waiting, and phenylephrine, guanethidine (an adrenergic neuronal blocker), atropine (a muscarinic receptor blocker) was used to contract smooth muscle. Control strips did not receive a protein kinase inhibitor. When the strip was maximally contracted, either PGE$_1$ or forskolin was added to induce relaxation of the cavernous smooth muscle. The strips teated with several protein kinase inhibitors were compared with the controls. The magnitudes of relaxation are expressed as percentage of phenylephrine-induced contraction.

**Statistical analysis**

Data was compared with the non-parametric Mann-Whitney U tests for unpaired samples with Statview 4.02 software (SAS Institute Inc., Cary, North Carolina).

Each data represent the MEAN±SE of at least 4 strips each from 5 rabbits.

P < 0.05 was considered significant.

**Results**

**PGE$_1$ study**

Treatment with KT-5926 (1×10$^{-7}$ M) for 30 minutes before addition of phenylephrine (1×10$^{-4}$ M) inhibited PGE$_1$ (1×10$^{-3}$ M) induced relaxation of rabbit corpus cavernosum. From its contracted state, untreated tissue relaxed 104.1±8.0% while the treated tissue demonstrated relaxation of only 80.6±11.4%. The relaxation ratio of treated tissue was significantly lower than that of control tissue (p < 0.05). Neither KT-5720 nor KT-5823 inhibited PGE$_1$-induced relaxation (111.6±18.2% and 93.6±9.5%, respectively) when compared with controls (Fig. 1).

**Forskolin study**

Treatment with KT-5720 (1×10$^{-3}$ M) and KT-5823 (1×10$^{-3}$ M) for 30 minutes before addition of

![Fig. 1. Effect of protein kinase inhibitors on relaxation of PGE$_1$.](image)
phenylephrine (1 × 10^{-4} M) exerted inhibitory effect on rabbit corpus cavernosum. Control strips relaxed 139.6 ± 6.3% from their maximally contracted state while tissue treated with KT-5720 and KT-5823 relaxed 107.3 ± 11.0% and 96.7 ± 4.6%, respectively. The relaxation ratio of treated tissue was significantly lower than that of control tissue (p < 0.05). KT-5926 had no significant effect (125.7 ± 10.9%) on forskolin-induced relaxation (Fig. 2).

Discussion

The clinical efficacy of PGE_1 for the treatment of erectile dysfunction has been well demonstrated over the past several years^{16-18}. However there are several questions on its pharmacological basis that remain unanswered.

To study the molecular basis of the pharmacology of PGE_1-induced penile erection, we performed in vitro experiments using rabbits corpus cavernosum. Our recent in vivo studies in the rat, dog, and monkey (unpublish data) have confirmed that cyclic GMP-dependent pathway may be predominant for erections induced by electrostimulation or intracavernous injection of sodium nitroprusside (a nitric oxide releasing substance). However, it is not clear whether PGE_1 activates relaxation of rabbit cavernosal tissue on this pathway.

The protein phosphorylation plays a key role in regulating relaxation of smooth muscle. Both cyclic AMP and cyclic GMP act as second messengers, and enhance smooth muscle relaxation. The biological effects of cyclic AMP and cyclic GMP are mediated through activation of cyclic nucleotide-dependent protein kinases. Identification of cyclic nucleotide-dependent pathways was made possible by the use of inhibitors in smooth muscle cells that exhibited distinctive effects on production of cyclic AMP, cyclic GMP and on activation of protein kinase A (PKA), protein kinase G (PKG)^{19-22}. Nitric oxide acting through the cyclic-GMP pathway was recently been found to play an important role in erection, possibly as the major non-adrenergic non-cholinergic neurotransmitter^{23-25}. Recent studies done in human and rat models have shown opposite interaction between the cyclic AMP- and cyclic GMP-dependent pathways^{26}. Synergistic inhibitions was demonstrated in platelet aggregation but additive effects were seen on smooth muscle relaxation^{27-28}. These opposite effects were the basis of our exploration of the PGE_1 and forskolin molecular action in the cavernous smooth muscle.

Selective protein kinase inhibitors were synthetically derived from a microbial metabolite of Norcardiopsis species. KT-5720 is a selective inhibitor of cyclic AMP-dependent protein kinase. Recent data has demonstrated that KT-5720, the 9'-n-hexyl ester derivative, selectively inhibits PKA^{41}. KT-5823 is an excellent but not specific cyclic GMP-dependent protein kinase inhibitor. KT-5926 inhibits myosin light chain kinase by interacting with the ATP-binding site in cyclic GMP-dependent pathway and has some inhibitory effect on cyclic GMP-dependent protein kinase^{29}.

Many studies have been done on the molecular mechanism of PGE_1 in other tissue than the penis^{30-34}. McCarthy et al.^{30} reported that in cultured human fibroblasts, PGE_1 induces an increase in intracellular cyclic AMP that could be blocked by an adenylcyclase inhibitor. Simmons^{32} reported in a canine renal epithelial cell line that PGE_1 and forskolin induce an intracellular cyclic AMP accumulation, suggesting that their actions are mediated via a cyclic AMP-dependent mechanism.

Although these studies have indicated that PGE_1 acts through cyclic AMP-dependent pathway, it appears to be different in the corpus cavernosum smooth muscle. The relaxation mediated by PGE_1 on the rabbit cavernous smooth muscle was dimin-
ished by KT-5926, an inhibitor of the cyclic GMP-dependent pathway, and not by KT5720 and KT-5823. KT5720 is a specific cyclic AMP inhibitor, and KT-5823 is a non-specific inhibitor of PKG compared with KT-5926 from the view point of ki value. KT-5823 was needed the consideration of the influence of cyclic AMP and myosin light chain, too. So, this results implies that PGE\textsubscript{i} induced penile smooth muscle relaxation mainly occurs via the cyclic GMP-dependent pathway through activation of cyclic GMP protein kinase. However, our experiment has been performed with a limited number of inhibitors and further studies on this pathway are needed before one can unequivocally arrive at these conclusions. Furthermore, it is important to note that the relaxation was not completely inhibited and that there may be several factors involved in blockage of relaxation of the cavernous smooth muscle tissue. These results, however, emphasize the fact that the molecular mechanism of erection induced by PGE\textsubscript{i} is complex and might involve more than one pathway.

Forskolin mediated relaxation was attenuated by KT-5720 and KT-5823. KT-5720 specifically inhibits cyclic AMP protein kinase, and KT-5823 is a non-specific inhibitor of PKG compared with KT-5926. So this experiment suggested that forskolin action is mainly via cyclic AMP-dependent pathway. As Jin et al. have reported in their study of isolated gastric muscle cells, these pathways can be activated separately and mediated independently thus emphasizing the interaction of both pathways.

The actions of cyclic AMP and cyclic GMP appears similar. It is not known whether these pathways elicit the same or different cellular processes. Further studies of the effects of [Ca\textsuperscript{2+}] will be helpful in elucidating these mechanisms.

**Conclusion**

We concluded that PGE\textsubscript{i} induced relaxation of rabbit cavernosum smooth muscle by activation of the cyclic GMP-dependent pathway. Forskolin action appears to be via activation of both cyclic AMP- and cyclic GMP-dependent pathways. Our results clinically highlighted the complexity of the mechanism in the treatment of erectile dysfunction.

**References**


ウサギ陰茎海綿体に対するプロスタグラディン E1 およびフォルスコリンの作用における cyclic GMP, cyclic AMP の関与

抄 録

プロスタグラディン E1 は勃起障害患者の治療に広く使われているが、その作用機序についての研究はまだ多く報告されていなかった。そこで新しい cyclic AMP 作動型プロテインキナーゼ A 阻害剤である KT-5720 と cyclic GMP 作動型プロテインキナーゼ A 阻害剤である KT-5823 と KT-5926 を用いて、プロスタグラディン E1 およびフォルスコリンによるウサギ陰茎海綿体平滑筋に対する弛緩反応について検討した。KT-5720, KT-5823, KT-5926 をそれぞれ添加し、30 分後にフェニレフリンにより完全に収縮させたウサギ陰茎海綿体にプロスタグラディン E1 またはフォルスコリンを滴下し、弛緩の抑制効果を評価した。その結果、KT-5926 は PGE_{1} (1×10^{-3} M: 最終濃度) の弛緩反応を抑制した。また KT-5720 と KT-5823 はフォルスコリン (1×10^{-5} M: 最終濃度) の弛緩反応を抑制した。この結果より、フォルスコリンは cyclic AMP と cyclic GMP の経路を介して、PGE_{1} は cyclic GMP の経路を介して、ウサギ陰茎海綿体平滑筋を弛緩させることが示唆された。