Essential Role of Newly Synthesized ATP for Cyclic GMP-Induced Relaxation in α-Toxin Permeabilized Smooth Muscle of Rat Proximal Colon

Tadayoshi TAKEUCHI*,**, Akikazu FUJITA*, Hideaki NISHIO* and Fumiaki HATA*•**

*Department of Veterinary Pharmacology, College of Agriculture and
**Department of Molecular Physiology and Biochemistry, Research Institute for Advanced Science and Technology, Osaka Prefecture University, Sakai 599-8531, Japan

Abstract

The role of newly synthesized ATP in cyclic GMP-induced relaxation was studied in membrane-permeabilized longitudinal muscle preparations of the rat proximal colon. Cyclic GMP and 8-bromo cGMP induced concentration-dependent relaxation of α-toxin permeabilized preparations which were precontracted by 3 μM Ca2+ in the presence of 4 mM ATP and 5 mM phosphocreatine (PC). The relaxation by 8-bromo cGMP was inhibited by Rp-8-pCPT-cGMPS, an inhibitor of cyclic GMP-dependent protein kinase. The relaxation was inhibited by removal of PC from the bathing solution, in spite of the presence of ATP. The relaxation was also inhibited by dinitrofluorobenzene (DNFB), a selective inhibitor of creatine kinase. The removal of PC or treatment with DNFB is known to produce accumulation of ADP within smooth muscle cell, however, ADP did not affect the relaxation. After irreversible inhibition of endogenous creatine kinase by DNFB in β-escin permeabilized preparations, treatment of the preparations with exogenous creatine kinase restored the relaxation. In the presence of ADP and PC but without ATP, 8-bromo cGMP induced the relaxation to the similar extent to that in the presence of ATP and PC. These results suggest that ATP newly synthesized from ADP and PC by creatine kinase is essential for cyclic GMP-induced relaxation of the smooth muscle preparations obtained from the proximal colon of rats.

Key words: Creatine kinase, Cyclic GMP, α-Toxin permeabilized smooth muscle, Rat proximal colon, Newly synthesized ATP

Introduction

Creatine kinase catalyzes the regeneration of ATP from phosphocreatine (PC) and ADP (Wallimann et al., 1992). Functional coupling of creatine kinase system with ATP-requiring processes has been shown in smooth muscle cells (Clark, 1994). In the Triton X100-skinned

Correspondence to: Tadayoshi Takeuchi, Department of Veterinary Pharmacology, College of Agriculture, Osaka Prefecture University, Sakai 599-8531, Japan.
Phone: 0722-54-9479, Fax: 0722-54-9480
preparations of the taenia coli and carotid artery of guinea pigs (Clark et al., 1994; Clark et al., 1992), the addition of Ca\(^{2+}\) into the bathing fluid induced contraction in the presence of PC and ADP instead of ATP, and 2,4-dinitrofluorobenzene (DNFB), a selective inhibitor of creatine kinase, abolished the contraction. The results suggest that newly synthesized ATP from ADP and PC is utilized for contraction of the smooth muscle cells, and that creatine kinase colocalize with myosin and myosin light chain kinase on the contractile protein. We showed that omission of PC from the bathing fluid in spite of the presence of ATP resulted in significant decrease in phasic phase component of Ca\(^{2+}\)-induced contraction in \(\alpha\)-toxin permeabilized smooth muscle of the rat colon (Takeuchi et al., 1995). Furthermore, DNFB inhibited the phasic contraction in the presence of ATP and PC. These results suggest that the activity of creatine kinase as an energy source supplier in smooth muscle cells controls the Ca\(^{2+}\)-induced contraction.

It was shown that regulation of ATP content by creatine kinase in the site close to site of ATP utilization is also important to maintain other cellular functions than contractile system in some tissues. In the skeletal and cardiac muscles, regulation of local ATP/ADP ratio by creatine kinase is important for Ca\(^{2+}\) stimulated ATP-driven pump activity of sarcoplasmic reticulum (Korge and Campbell, 1994; Minajeva, et al., 1996). Functional coupling between H\(^+\)/K\(^+\)-ATPase and creatine kinase is also reported in the parietal cells of the stomach (Sistermans et al., 1995). Thus, ATP regeneration by creatine kinase is necessary for many cell functions.

Increase in an intracellular guanosine 3':5'-cyclic monophosphate (cyclic GMP) induces relaxation of the various smooth muscles. In the intestinal and vascular smooth muscles, for example, an application of sodium nitroprusside or nitric oxide caused an increase in cyclic GMP content and induced relaxation (Makhlouf and Grider 1993; McDaniel et al., 1992; Ward et al., 1992). Cyclic GMP was suggested to decrease intracellular calcium ion concentration ([Ca\(^{2+}\)]\text{,i}) by reducing Ca\(^{2+}\) influx and/or increasing Ca\(^{2+}\) efflux, or sequestration by sarcoplasmic reticulum (Chen and Rembold 1992; Kannan et al., 1997; McDaniel, et al., 1992) and to cause hyperpolarization of membrane potential by activating Ca\(^{2+}\)-activated K\(^+\) channel (Carrier et al., 1997; Yamakage et al., 1996). In skinned preparations of the smooth muscles, exogenous cyclic GMP relaxes the muscles contracted by Ca\(^{2+}\), indicating that cyclic GMP decreases Ca\(^{2+}\) sensitivity of the contractile elements (Nishimura and van Breemen, 1989). These effects of cyclic GMP have been suggested to be associated with phosphorylation of proteins, such as Ca\(^{2+}\) channel, regulatory protein of sarcoplasmic reticulum Ca\(^{2+}\)-ATPase, K\(^+\) channel and contractile elements of the smooth muscle cells, through activating cyclic GMP-dependent protein kinase (PKG) (Butt et al., 1993; Lohmann et al., 1997). Protein phosphorylation is the process by which highly charged phosphate group of ATP is transferred to a serine, threonine or tyrosine residue of the substrate proteins (Kurosawa, 1994). However, origin of ATP used by PKG-mediated phosphorylation is unknown. In this study, the role of newly synthesized ATP in the cyclic GMP-induced relaxation was investigated in \(\alpha\)-toxin permeabilized preparation of the rat proximal colon and discussed in relation to phosphorylation of the contractile elements by PKG.
Materials and methods

Male Wistar rats (250–350 g) were lightly anesthetized with diethyl ether and then stunned by a blow on the head and bled via the carotid arteries. The proximal colon was then removed and placed in Tyrode solution containing (in mM) 127 NaCl, 2.7 KCl, 1.8 CaCl2, 1.05 MgCl2, 11.9 NaHCO3, 0.4 NaH2PO4 and 5.6 glucose. Small strips of longitudinal muscle of the proximal colon were prepared according to the procedures described previously (Takeuchi et al., 1995). In brief, small strips (0.1–0.2×1 mm) of longitudinal muscle were prepared under inverted microscope. The strips were tied with monofilament silk to the fine tips of two tungsten needles, one of which was connected to a force transducer. Then they were placed in a well on a bubble plate (Horiuti, 1988) kept at 25°C by circulating water under it. For change of solutions, an adjacent well was moved to the position of the tissue preparation by sliding the plate. Isometric tension was measured with a force displacement transducer (AE801, SensoNor, Horten, Norway) and recorded with a recorder (LR4110, Yokogawa, Japan) equipped with a preamplifier (EF601G, Nihon Koden, Japan). Preparations were permeabilized by treatment with α-toxin (2,500–5,000 U/ml) for 60 min, or β-escin (40 μM) for 30 min in a relaxing solution containing 111 mM potassium methanesulfonate, 4 mM ATP-2Na, 4 mM magnesium methanesulfonate, 4 mM EGTA, 20 mM Tris maleic acid (pH 6.8), and 5 mM phosphocreatine (Itoh et al., 1986). After equilibration for 10 min in the relaxing solution, responses of the skinned muscle to 3 μM Ca2+ were recorded isometrically in an activating solution, with 10 min intervals between tests. In activating solution containing various concentrations of Ca2+, 4 mM EGTA was used to clamp free Ca2+ concentrations, and an appropriate amount of calcium methanesulfonate was added to give a desired concentration of free Ca2+ ions (Harafuji and Ogawa, 1980). Concentrations of ATP, ADP and phosphocreatine in the relaxing and activating solutions are expressed as total ATP, total ADP and total phosphocreatine concentrations. On changes of the ATP, ADP and phosphocreatine concentrations, the total magnesium methanesulfonate concentration was also changed to keep the Mg2+ concentration at 0.9 mM.

Drugs. S. aureus α-toxin was purchased from Gibco Biological Research Laboratories, MD, USA. [Ethylenebis (oxyethylenenitrilo)] tetraacetetic acid (EGTA) was from Dojindo, Kumamoto, Japan. ATP, creatine kinase, guanosine 3’:5’-cyclic monophosphate (cyclic GMP) and 8-bromo guanosine 3’:5’-cyclic monophosphate (8-bromo cGMP) were from Sigma, St. Louis, USA. ADP, phosphocreatine (PC), 2,4-dinitrofluorobenzene (DNFB) were from Wako Pure Chemicals, Osaka, Japan. 8-(4-chlorophenylthio) guanosine 3’:5’-monophosphorothioate, Rp-isomer (Rp-8-pCPT-cGMPS) was from Biolog, Bremen, Germany. Maleic anhydride, magnesium hydroxide, calcium carbonate and potassium hydroxide were from Koso Co., Tokyo, Japan. All other chemicals used were of analytical grade.

Results

In α-toxin permeabilized longitudinal muscle preparations of the rat proximal colon, Ca2+ induced the phasic-type contraction, consisting of rapid phasic and subsequent tonic contrac-
tion. Since cyclic GMP induced roughly the same magnitude relaxation on the tonic contractions induced by 1–30 \( \mu \text{M} \) \( \text{Ca}^{2+} \) as shown in our previous study (Maehara et al., 1994), 3 \( \mu \text{M} \) \( \text{Ca}^{2+} \) which induced 80% of maximum contraction was used in the present study. After the 3 \( \mu \text{M} \) \( \text{Ca}^{2+} \)-induced contraction reached a plateau level of tonic contraction, cumulative addition of cyclic GMP or 8-bromo cyclic GMP (8-bromo cGMP) relaxed the tonic contraction concentration-dependently (Fig. 1). However, the relaxant effect of 8-bromo cGMP was significantly greater than that of cyclic GMP. The different potency of both drugs may be due to difference in sensitivity of the drugs to phosphodiesterase, because concentration-response curve of cyclic GMP was shifted to the left in the presence of zaprinast, a selective inhibitor of cyclic GMP-phosphodiesterase (data not shown). Next, we examined effect of Rp-8-pCPT-cGMPS, a specific inhibitor of protein kinase G, on the 8-bromo cGMP-induced relaxation. Rp-8-pCPT-

![Fig. 1. Effects of cyclic GMP and 8-bromo cGMP on \( \text{Ca}^{2+} \)-induced contraction in \( \sigma \)-toxin permeabilized longitudinal muscle preparations of rat proximal colon. A: Typical response of \( \sigma \)-toxin permeabilized preparations to cyclic GMP or 8-bromo cGMP. After equilibration in a relaxing solution for 10 min, contraction induced by 3 \( \mu \text{M} \) \( \text{Ca}^{2+} \) was recorded in an activating solution containing 4 mM ATP and 5 mM PC (control condition). Then, after steady-state contraction (tonic phase) developed, the effects of various concentrations of cyclic GMP or 8-bromo cGMP were examined. B: Concentration-dependent inhibitory effects of cyclic GMP (○) and 8-bromo cGMP (●). Values are expressed as a percentage of tonic contraction induced by 3 \( \mu \text{M} \) \( \text{Ca}^{2+} \). All data represent means±S.E.M. of values in 4 experiments.

![Graph showing concentration-response curve of cyclic GMP and 8-bromo cGMP](image-url)
cGMPS itself relaxed slightly the 3 μM Ca^{2+}-induced contraction. However, Rp-8-pCPT-cGMPS at 10 μM and 100 μM concentration-dependently inhibited the relaxant effect of 8-bromo cGMP, resulting in a shift of the concentration curve for 8-bromo cGMP to the right (Fig. 2). These results suggest that 8-bromo cGMP relaxes the α-toxin permeabilized preparations of the rat proximal colon through activation of cyclic GMP-dependent protein kinase.

As shown in the previous report (Takeuchi, et al., 1995), omission of PC from the extracellular bathing solution resulted in significant decrease in the phasic component of Ca^{2+}-induced contraction. Under the conditions, 8-bromo cGMP did not relax the tonic component of Ca^{2+}-induced contraction (Fig. 3). 8-Bromo cGMP even at the concentration 100 times higher than that to induce the maximum effect in the normal bathing solution (in the presence of PC) did not induce the relaxation (Fig. 3). The increase in ATP concentration from 4 mM to 8 mM in the absence of PC slightly increased the phasic contraction, but did not bring about 8-bromo cGMP-induced relaxation (Fig. 3). Thus, essential role of newly synthesized ATP in 8-bromo cGMP-induced relaxation was suggested. We next examined effect of DNFB, a selective inhibitor of creatine kinase, on the relaxation. DNFB inhibited the phasic component of Ca^{2+}-induced contraction significantly. It also inhibited 8-bromo cGMP-induced relaxation of the tonic component of the Ca^{2+}-induced contraction (Fig. 4).

The omission of PC from the bathing solution or the inhibition of creatine kinase cause accumulation of ADP within smooth muscle cells (Nishimura et al., 1991). Therefore, effect of ADP₆ on cyclic GMP-induced relaxation was examined. The addition of ADP₆ to the bathing solution increased the Ca^{2+}-induced contraction as shown by Nishimura et al. (1991). However, ADP₆ at 100 μM did not affect the 8-bromo cGMP relaxation (Fig. 5): EC₅₀ values of 8-bromo cGMP in the absence and presence of ADP₆ were the same, 0.4 μM.

In the next series of experiments using β-escin permeabilized preparations, endogenous
Fig. 3. Effect of removal of phosphocreatine (PC) from the bathing solution on 8-bromo cGMP-induced relaxation in \( \alpha \)-toxin permeabilized preparations. A: Effects of various concentrations of 8-bromo cGMP were examined in the presence of 4 mM ATP without (center) or with (left) 5 mM PC. The effects were also examined in the presence of 8 mM ATP without PC (right). B: Effect of removal of PC on the concentration response curve of 8-bromo cGMP-induced relaxation. Values are expressed as a percentage of tonic contraction induced by 3 \( \mu \)M Ca\(^{2+} \). All data represent means ± S.E.M. of values in 3 experiments.

Creatine kinase was first inhibited by DNFB, and then the effect of the exogenously added enzyme on cyclic GMP-induced relaxation was examined. Cyclic GMP relaxed the tonic component of Ca\(^{2+} \)-induced contraction also in the \( \beta \)-escin permeabilized preparations (Fig. 6). DNFB abolished the effect of cyclic GMP. Since DNFB is known to inhibit creatine kinase irreversibly, creatine kinase was added exogenously after washout of DNFB. The relaxant effect of cyclic GMP developed again. The results also support the role of creatine kinase for the 8-bromo cGMP-induced relaxation.

In our previous report using \( \alpha \)-toxin permeabilized preparations of the rat proximal colon, Ca\(^{2+} \) induced phasic type-contraction in the presence of ADP and PC but without ATP, and DNFB inhibited the contraction. Under the conditions, ATP newly synthesized from ADP and PC by creatine kinase is utilized for the regulation of contractile response. The tonic component of the contraction was inhibited by 8-bromo cGMP in a similar manner to that in normal
Newly synthesized ATP for cGMP relaxation

A) Fig. 4. Inhibitory effect of DNFB on 8-bromo cGMP-induced relaxation in α-toxin permeabilized preparations. A: The effect of DNFB on 8-bromo cGMP-induced relaxation was examined in the presence of 4 mM ATP and 5 mM PC. For further details, see Materials and methods and legend to Figure 3. B: Effect of DNFB on 8-bromo cGMP-induced relaxation. Values are expressed as a percentage of tonic contraction induced by 3 μM Ca^{2+}. All data represent means ± S.E.M. of values in 3 experiments. The relaxations were induced in the presence of 4 mM ATP and 5 mM PC without (○) or with (●) 10 μM DNFB.

condition (4 mM ATP and 5 mM PC) (Fig. 7). EC_{50} value in this condition was the same as in normal condition (4 mM ATP and 5 mM PC).

Discussion

In the presence of ATP and PC, cyclic GMP and 8-bromo cGMP relaxed concentration-dependently Ca^{2+}-induced contraction in α-toxin permeabilized longitudinal muscle of the rat proximal colon. The potency of 8-bromo cGMP in the relaxant effect was about 50 times stronger than that of cyclic GMP. Kawada et al. (1997) showed that 8-bromo cGMP activated cyclic GMP-dependent protein kinase (PKG) more potent than cyclic GMP did. The difference in potency between both drugs may be due to their sensitivity to phosphodiesterase, because the relaxant effect of cyclic GMP was augmented in the presence of a phosphodiesterase inhibitor.
Fig. 5. Effects of ADP$_2$S on 8-bromo cGMP-induced relaxation in $\alpha$-toxin permeabilized preparations. A: Effect of ADP$_2$S was examined on 8-bromo cGMP-induced relaxation in the presence of 4 mM ATP and 5 mM PC. For further details, see Materials and methods and legend to Figure 3. B: Effect of ADP$_2$S on 8-bromo cGMP-induced relaxation. The relaxations were induced in the presence of 4 mM ATP and 5 mM PC without (○) or with (●) 100 μM ADP$_2$S. Values are expressed as a percentage of tonic contraction induced by 3 μM Ca$^{2+}$. All data represent means±S.E.M. of values in 3 experiments.

Omission of PC from the bathing solution abolished the relaxant effects of cyclic GMP or 8-bromo cGMP, in spite of the presence of ATP. An increase in ATP concentration from 4 mM to 8 mM did not reverse the effect of omission of PC. Treatment of the preparations with DNFB, a specific inhibitor of creatine kinase, also resulted in complete inhibition of the relaxant effect of cyclic GMP in the presence of ATP and PC. Previously, we indicated that creatine kinase in this preparation could synthesize ATP from ADP and PC, because Ca$^{2+}$-induced contraction of $\alpha$-toxin permeabilized preparations in the presence of ADP and PC, even in the absence of ATP, and the contraction was abolished by the treatment of DNFB (Takeuchi et al., 1995). Under the conditions, cyclic GMP relaxed the preparation to similar extent to that in the presence of ATP and PC. Also in $\beta$-escin permeabilized preparations in the present study, cyclic GMP–induced relaxation was inhibited by DNFB and creatine kinase added exogenously reversed the inhibition. These results suggest that ATP newly synthesized by creatine kinase rather than exogenously added ATP into the bathing solution has an important role in the relaxation by cyclic GMP.
Preparation permeabilized by β-Escin

![Graph](image)

Fig. 6. Inhibition by DNFB of cyclic GMP-induced relaxation and recovery from its inhibition by exogenously added creatine kinase. Cyclic GMP-induced relaxation was first recorded in the presence of 4 mM ATP and 5 mM PC. Next, the inhibitory effect of DNFB (10 μM) on cyclic GMP-induced relaxation and then, recovery of the relaxation by creatine kinase (CPK, 10 U/ml) were examined. For further details, see Materials and methods and legend to Figure 3.

Omission of PC or addition of DNFB may cause an accumulation of ADP within smooth muscle cells (Clark et al., 1992; Nishimura and Van Breemen, 1991). ADP was shown to enhance the Ca sensitivity of the contractile elements and inactivate myosin light chain phosphatase (Morimoto and Ogawa, 1995; Nishimura and Van Breemen, 1991). These effects of ADP may produce the opposite action on the relaxant effect of cyclic GMP. However, the addition of ADP or ADP₆S in the bathing solution containing ATP and PC did not affect the 8-bromo cGMP-induced relaxation. Therefore, it seems unlikely that lack of the effect of 8-bromo cGMP in the absence of PC is due to accumulation of ADP within the smooth muscle cells.

There is a line of evidence that cyclic GMP induces the smooth muscle relaxation through the phosphorylation of protein by activation of PKG (Francis and Corbin, 1994). In the present study, 8-bromo cGMP-induced relaxation was competitively inhibited by an inhibitor of PKG. In contractile element of smooth muscle, it was suggested that PKG activated by cyclic GMP decreases Ca²⁺-sensitivity of the myofilaments via activation of MLC phosphatase (Lee et al., 1997; Wu et al., 1996). The possibility that omission of PC from the bathing solution inhibits MLC phosphatase seems likely. However, MLC phosphatase regulates the contractile response to Ca²⁺ in the absence of PC, since an inhibitor of MLC phosphatase increased the Ca²⁺-induced contraction (data not shown). It is known that cyclic GMP activates PKG by binding to cyclic GMP binding domains of PKG in a ATP-independent manner (Butt et al., 1993). Therefore, newly synthesized ATP necessary for cyclic GMP-induced relaxation in the present study may be utilized for the protein phosphorylation by PKG.

In the present study, inhibition of creatine kinase also resulted in a significant inhibition in cyclic GMP-induced relaxation, suggesting that newly synthesized ATP by creatine kinase is necessary for the relaxation, probably for the phosphorylation by PKG.
Fig. 7. Relaxant effect of 8-bromo cGMP in the presence of PC and ADP instead of ATP. A: After 8-bromo cGMP-induced relaxation was recorded in the presence of 4 mM ATP and 5 mM PC, ATP was substituted by 1 mM ADP and the relaxations were induced again. For further details, see Materials and methods and legend to Figure 3. B: Effect of substitution of ATP by ADP on 8-bromo cGMP-induced relaxation. Values are expressed as a percentage of tonic contraction induced by 3 μM Ca²⁺. Relaxations were induced in the presence of 4 mM ATP and 5 mM PC (○), or 1 mM ADP and 5 mM PC, (●). All data represent means ± S.E.M. of values in 4 experiments.

Acknowledgment

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

References

Butt, E., Geiger, J., Jarchau, T., Lohmann, S.M. and Walter, U. (1993). The cGMP-dependent protein kinase—gene, protein, and function. Neurochem. Res. 18: 27–42.

Carrier, G.O., Fuchs, L.C., Winecoff, A.P., Giulumian, A.D. and White, R.E. (1997). Nitrovasodilators relax mesenteric microvessels by cGMP-induced stimulation of Ca²⁺-activated K⁺ channels. Am. J. Physiol. 273: H76–H84.

Chen, X.-L. and Rembold, C.M. (1992). Cyclic nucleotide-dependent regulation of Mn²⁺ influx, [Ca²⁺], and arterial smooth muscle relaxation. Am. J. Physiol. 263: C468–C473.
Clark, J.F. (1994). The creatine kinase system in smooth muscle. *Molecu. Cell. Biochem.* **133/134**: 221–232.

Clark, J.F., Khuchua, Z., Boehm, E. and Venture-Clapier, R. (1994). Creatine kinase activity associated with the contractile proteins of the guinea-pig carotid artery. *J. Muscle Res. Cell Motil.* **15**: 432–439.

Clark, J.F., Khuchua, Z. and Ventura-Clapier, R. (1992). Creatine kinase binding and possible role in chemically skinned guinea-pig taenia coli. *Biochim. Biophys. Acta* **1100**: 137–145.

Francis, S.H. and Corbin, J.D. (1994). Structure and function of cyclic nucleotide–dependent protein kinases. *Annu. Rev. Physiol.* **56**: 237–277.

Harafuji, H. and Ogawa, Y. (1980). Re-examination of the apparent binding constant of ethylene-glycol-bis-(β-aminoethyl-ether)-N, N', N'-tetraacetic acid with calcium around neutral pH. *J. Biochem.* **87**: 1305–1312.

Horiuti, K. (1996). Mechanism of contracture on cooling of caffeine-treated frog skeletal muscle fibres. *J. Physiol. (Lond)* **398**: 131–148.

Itoh, T., Kanamura, Y. and Kuriyama, H. (1986). Inorganic phosphate regulates the contraction-relaxation cycle in skinned muscles of the rabbit mesenteric artery. *J. Physiol.* **376**: 231–252.

Kannan, M.S., Prakash, Y.S., Johnson, D.E. and Sieck, G.C. (1997). Nitric oxide inhibits calcium release from sarcoplasmic reticulum of porcine tracheal smooth muscle cells. *Am. J. Physiol.* **272**: L1–L7.

Kawada, T., Toyosato, A., Islam, M.O., Yoshida, Y. and Imai, S. (1997). cGMP-kinase mediates cGMP- and cAMP-induced Ca²⁺ desensitization of skinned rat artery. *Eur. J. Pharmacol.* **323**: 75–82.

Korge, P. and Campbell, K.B. (1994). Local ATP regeneration is important for sarcoplasmic reticulum Ca²⁺ pump function. *Am. J. Physiol.* **267**: C357–C366.

Kurokawa, M. (1994). Phosphorylation and dephosphorylation of protein in regulating cellular function. *J. Pharmacol. Toxicol. Meth.* **31**: 135–139.

Lee, M.R., Li, L. and Kitazawa, T. (1997). Cyclic GMP causes Ca²⁺ desensitization in vascular smooth muscle by activating the myosin light chain phosphatase. *J. Biol. Chem.* **272**: 5063–5068.

Lohmann, S.M., Vaandrager, A.B., Smolenski, A., Walter, U. and De Jonge, H.R. (1997). Distinct and specific functions of cGMP–dependent protein kinases. *TIBS* **22**: 307–312.

Maehara, T., Fujita, A., Suthamnatpong, N., Takeuchi, T. and Hata, F. (1994). Differences in relaxant effects of cyclic GMP on skinned muscle preparations from the proximal and distal colon of rats. *Eur. J. Pharmacol.* **261**: 163–170.

Makhlouf, G.M. and Grider, J.R. (1993). Nonadrenergic noncholinergic inhibitory transmitters of the gut. *NIPS* **8**: 195–199.

McDaniel, N.L., Chen, X.-L., Singer, H.A., Murphy, R.A. and Rembold, C.M. (1992). Nitrovasodilators relax arterial smooth muscle by decreasing [Ca²⁺], and uncoupling stress from myosin phosphorylation. *Am. J. Physiol.* **263**: C461–C467.

Minajeva, A., Ventura-Clapier, R. and Veksler, V. (1996). Ca²⁺ uptake by cardiac sarcoplasmic reticulum ATPase in situ strongly depends on bound creatine kinase. *Pflugers Arch.* **432**: 904–912.

Morimoto, S. and Ogawa, Y. (1995). Ca²⁺-insensitive sustained contraction of skinned smooth muscle after acidic ADP treatment. *Am. J. Physiol.* **268**: C21–C29.

Nishimura, J. and van Breemen, C. (1989). Direct regulation of smooth muscle contractile elements by second messengers. *Biochem. Biophys. Res. Commun.* **163**: 929–935.

Nishimura, J. and Van Breemen, C. (1991). Energetic aspects of the regulation of Ca²⁺ sensitivity of permeabilized rabbit mesenteric artery: Possible involvement of a second Ca²⁺ regulatory system in smooth muscle contraction. *J. Pharmacol. Exp. Therap.* **258**: 397–402.
Sistermans, E.A., Klaassen, C.H.W., Peters, W., Swarts, H.G.P., Jap, P.H.K., De Pont, J.J.H.H.M. and Wieringa, B. (1995). Co-localization and functional coupling of creatine kinase B and gastric H+/K+ -ATPase on the apical membrane and the tubulovesicular system of parietal cells. *Biochem. J.* **311**: 445-451.

Takeuchi, T., Fujita, A., Ishii, T., Nishio, H. and Hata, F. (1995). Necessity of newly synthesized ATP by creatine kinase for contraction of permeabilized longitudinal muscle preparations of rat proximal colon. *J. Pharmacol. Exp. Ther.* **275**: 429-434.

Wallimann, T., Wyss, M., Brdiczka, D., Nicolay, k. and Eppenberger, H.M. (1992). Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissue with high and fluctuating energy demands: the ‘phosphocreatine circuit’ for cellular energy homeostasis. *Biochem. J.* **281**: 21-40.

Ward, S.M., Dalziel, H.H., Bradley, M.E., Buxton, I.L.O., Keef, K., Westfall, D.P. and Sanders, K.M. (1992). Involvement of cyclic GMP in non-adrenergic, non-cholinergic inhibitory neurotransmission in dog proximal colon. *Br. J. Pharmacol.* **107**: 1075-1082.

Wu, X., Somlyo, A.V. and Somlyo, A.P. (1996). Cyclic GMP-dependent stimulation reverses G-protein-coupled inhibition of smooth muscle myosin light chain phosphatase. *Biochem. Biophys. Res. Commun.* **220**: 658-663.

Yamakage, M., Hirshman, C.A. and Croxton, T.L. (1996). Sodium nitroprusside stimulates Ca2+-activated K+ channels in porcine tracheal smooth muscle cells. *Am. J. Physiol.* **270**: L338-L345.

(Received December 1, 1997: Accepted December 19, 1997)