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Short communication

Role of MaxiK channels in vasoactive intestinal peptide-induced relaxation of rat mesenteric artery

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Abstract

We investigated the functional relevance of large conductance voltage-dependent and Ca^{2+}-sensitive K^+(MaxiK) channels in vasoactive intestinal peptide (VIP)-induced relaxation of rat mesenteric artery. VIP, which is known to increase cAMP levels, produced a concentration-dependent relaxation in endothelium-denuded arteries. Iberiotoxin, a MaxiK channel blocker, greatly diminished the VIP-induced relaxation. In a similar manner, a significant portion of the relaxant response to dibutyryl-cAMP (DBcAMP), a membrane-permeable analog of cAMP, was inhibited by iberiotoxin. These results suggest that activation of MaxiK channels significantly contributes to the relaxant response of rat mesenteric artery to VIP, possibly via cAMP-mediated pathways.

Keywords: VIP (vasoactive intestinal peptide); MaxiK channels; Iberiotoxin; cAMP; Mesenteric artery, rat

1. Introduction

Vasoactive intestinal peptide (VIP), a 28-amino acid neuropeptide which was first isolated from porcine small intestine (Said and Mutt, 1970), exerts various biological functions including modulation of neurotransmission, immune response and vascular relaxation. VIP causes an endothelium-independent relaxation in cat cerebral artery (Duckles and Said, 1982; Lee et al., 1984), dog carotid artery (D'Orleans-Juste et al., 1985), bovine (Itoh et al., 1990) and porcine (Kawasaki et al., 1997) coronary arteries and rat mesenteric artery (Tanaka et al., 1997). In contrast, VIP causes an endothelium-dependent relaxation in rat aorta (Davies and Williams, 1984), bovine intrapulmonary artery (Ignarro et al., 1987) and rabbit mesenteric artery (Hattori et al., 1992). Endothelium-independent vascular relaxation produced by VIP is associated with vascular smooth muscle cell membrane hyperpolarization, which is in part attributed to the activation of ATP-sensitive K^+ (K_{ATP}) channels (Standen et al., 1989).

In addition to K_{ATP} channels, large conductance voltage-dependent and Ca^{2+}-sensitive K^+ (MaxiK) channels are present in vascular smooth muscle cells and play an important role in the regulation of their contraction and relaxation (Nelson and Quayle, 1995; Kaczorowski et al., 1996; Standen and Quayle, 1998; Toro et al., 1998). MaxiK channels are abundant invascular smooth muscle and are target proteins for cyclic nucleotide-dependent protein kinases such as cAMP-dependent protein kinase (A-kinase) (Scornik et al., 1993; Kaczorowski et al., 1996; Standen and Quayle, 1998; Toro et al., 1998). Since VIP elevates cytosolic concentrations of cAMP via activation of adenylate cyclase (Edvinsson et al., 1985; Itoh et al., 1985; Ganz et al., 1986; Ignarro et al., 1987; Tanaka et al., 1997), it is possible that activation of MaxiK channels is involved in VIP-induced vascular relaxation. In the present study, we investigated whether MaxiK channel activation contributes to VIP-induced functional relaxation of rat mesenteric artery.

2. Materials and methods

Male Wistar rats were housed under controlled conditions (temperature 21°C–22°C, relative air humidity 50%

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± 5%). Food and water were available ad libitum to all animals. This study was conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of Toho University School of Pharmaceutical Sciences, which is accredited by the Ministry of Education, Science, Sports and Culture, Japan.

2.1. Preparations

Male Wistar rats (200–300 g) were anesthetized with pentobarbital sodium (30 mg/kg, i.v.) and exsanguinated by bleeding from the carotid arteries. A section of intestine between the pylorus and colon and its network of supplying blood vessels were isolated and placed in normal Tyrode's solution of the following composition (mM): NaCl, 158.3; KCl, 4.0; $CaCl_2$, 2.0; $MgCl_2$, 1.05; NaH_2PO_4, 0.42; $NaHCO_3$, 10.0 and glucose, 5.6. A superior mesenteric artery with an average outer diameter of 500–700 μm was isolated. The arteries were cleared of connective tissue under a dissection microscope and cut into ring segments approximately 1-mm long (0.2 mg in wet weight). Endothelium was removed by rubbing the intimal surface gently with cotton strings.

2.2. Tension measurements

Experimental details on contraction and relaxation of rat mesenteric arterial preparations were given in earlier publications (Tanaka et al., 1997). Mesenteric rings were mounted using stainless steel hooks (100 μm in diameter) under an optimal resting tension of 750 mg in a 4-ml organ bath (Micro Tissue Organ Bath: MTOB-1, Labo Support, Suita-City, Japan) containing a bathing solution (Tyrode's solution). Isotonic high KCl (80 mM) Tyrode's solution was prepared by replacing the NaCl by an equimolar amount of KCl. The solution was continuously gassed with a 95% O_2–5% CO_2 mixture and maintained at a pH of 7.35 at 35.0 ± 0.5°C. The mesenteric artery in the organ bath was allowed to equilibrate for 1.5 h under the resting tension before the experiments were started. Isometric tension measured with a force transducer (UL-10GR, Mineber, Tokyo, Japan) was amplified with a minipolygraph (Signal Conditioner: Model MSC-1, Labo Support, Suita-City, Japan) and recorded on a pen-writing recorder (Model SS-250F, Sekonic, Tokyo, Japan). Before the experiments started, the contractility of the artery was assessed by exposing it to 80 mM KCl Tyrode's solution. The absence of endothelial cells was ascertained by the elimination of acetylcholine (10^{-5} M)-induced relaxation in endothelium-denuded rings preconstricted with phenylephrine (3×10^{-6} M). After confirmation of the absence of the endothelium, the bath solution was exchanged with a fresh one, and the rings were left to re-equilibrate for 30 min before the experiments started. Iberiotoxin was applied to the bath solution 10–20 min before the addition of VIP or dibutyryl-cAMP (DBcAMP).

2.3. Drugs

The drugs used were VIP (Peptide Institute, Osaka, Japan), dibutyryl cAMP (DBcAMP) (BioLog life Science Institute, La Jolla, CA, USA), L-phenylephrine hydrochloride (Sigma, St. Louis, MO, USA.), acetylcholine chloride (Daiichi, Tokyo, Japan), phentolamine mesylate (Ciba-Geigy (Japan), Takarazuka-City, Hyogo, Japan), diltiazem hydrochloride, papaverine hydrochloride (Wako, Tokyo, Japan), 1*H*-[1,2,4]-oxadiazolo-[4,3-*a*]-quinoxalin-1-one (ODQ) (Biomol, Plymouth Meeting, PA, USA). Stock solutions, except DBcAMP and ODQ, were prepared in distilled water. DBcAMP was dissolved in 100% ethanol to make a stock solution of 10^{-2} M. ODQ was dissolved in 100% dimethylsulfoxide to make a stock solution of 10^{-2} M.

2.4. Data collection and analysis

Data were collected and analyzed using a MacLab/400™ and Chart™ (Version 3.5) software (ADInstruments Japan, Tokyo, Japan).

The percentage of relaxation was calculated by considering 0% relaxation the maximum tension level obtained with phenylephrine and 100% relaxation the full recovery to basal tension before application of phenylephrine. Data were plotted as a function of drug concentration and fitted to the equation:

$$E = E_{max} \times A^{n_H} / \left(EC_{50}^{n_H} + A^{n_H} \right)$$

where E is the % relaxation at a given drug concentration, E_{max} is the maximal relaxation, A is the concentration of the drug, n_H is the slope function and EC_{50} is the effective drug concentration that produces a 50% response (Parker and Waud, 1971). Curve fitting was carried out using GraphPad Prism™ (version 2.01) (GraphPad Software, San Diego, CA, USA). The EC_{50} values were converted to logarithmic values for statistical analysis.

The data are presented as mean values ± S.E.M. and n refers to the number of experiments. The significance of the difference between mean values was evaluated by unpaired Student's *t*-test and unpaired Student's *t*-test with Welch's correction, if necessary. P values less than 0.05 were considered statistically significant.

3. Results

Phenylephrine at a concentration of 3×10^{-6} M produced a sustained contraction in de-endothelialized rat mesenteric artery. VIP (10^{-9} to 10^{-6} M) produced a concentration-dependent relaxation of the precontracted vessel (Fig. 1a, upper panel). Consistent with a role of cAMP, the VIP-induced relaxation was insensitive to the guanylyl cyclase inhibitor ODQ (10^{-5} M, $n = 4$). VIP did not produce any further relaxation at concentrations over

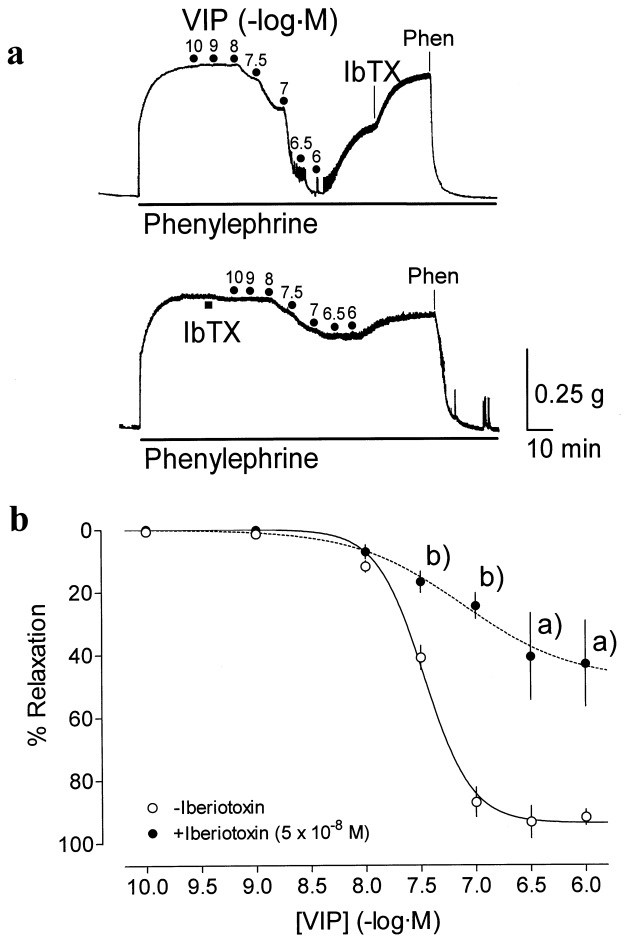

Fig. 1. Inhibitory effects of iberiotoxin on the vasoactive intestinal peptide (VIP)-induced relaxation of rat mesenteric artery. (a) Mesenteric arteries were precontracted with phenylephrine (3×10^{-6} M) (line under traces). VIP was cumulatively applied to the bath solution. Iberiotoxin (5×10^{-8} M) was applied 10–20 min before the addition of VIP (closed square) (lower panel). Iberiotoxin (5×10^{-8} M), when applied during the relaxation induced by VIP, partly restored the peptide-induced relaxation (upper panel). Numbers correspond to the negative logarithm of VIP concentration. IbTX: iberiotoxin (5×10^{-8} M); Phen: phentolamine (3×10^{-6} M). (b) Concentration–response relationships for VIP-induced relaxation in the absence and presence of iberiotoxin. Vascular relaxation is expressed as % inhibition against the phenylephrine-induced contraction. Data are mean values ± S.E.M. ($n=5$). Significant difference from control values: (a) $P<0.05$; (b) $P<0.01$.

10^{-6} M. The relaxation produced by 10^{-6} M VIP gradually recovered after it reached its maximum level. In contrast to the vasorelaxant effect of VIP, acetylcholine (10^{-5} M)-induced relaxation was abolished in this endothelium-denuded preparation (data not shown).

In comparison with the relaxant response in the absence of iberiotoxin, VIP-induced relaxation was reduced in the presence of the toxin (Fig. 1a, lower panel). Iberiotoxin (5×10^{-8} M) was applied to the bathing solution 10–20 min before the cumulative application of VIP (marked with a closed square in Fig. 1a, lower panel). The inhibition of VIP-induced relaxation by iberiotoxin was quantified as the change in the concentration–response relationship in Fig. 1b. In the absence of iberiotoxin (open circles), the estimated maximum relaxation in response to VIP was 93.8 ± 2.3% ($n=5$), whereas in the presence of iberiotoxin (filled circles) it diminished to 48.3 ± 16.6% ($n=5$) ($P<0.05$). The concentration of VIP required to induce 50% relaxation (pEC_{50}) remained practically the same; values were 7.47 ± 0.03 ($n=5$) in the absence and 7.11 ± 0.51 ($n=5$) in the presence of iberiotoxin ($P>0.05$).

The substantial inhibition of mesenteric artery relaxation induced by iberiotoxin was also obtained when the membrane-permeable cAMP analog, dibutyryl cAMP, was used to induce relaxation (Fig. 2a). DBcAMP (10^{-4} M)

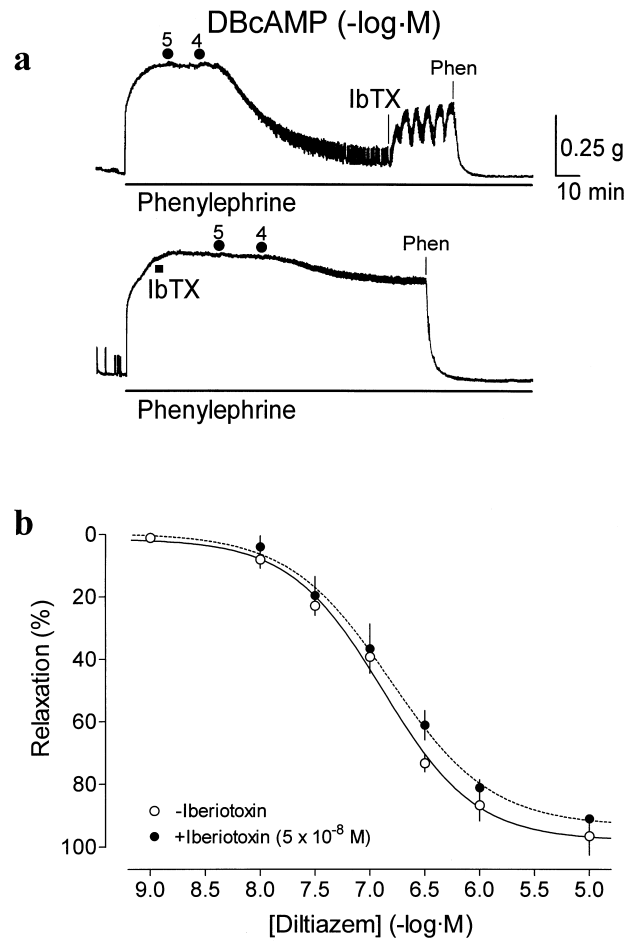

Fig. 2. (a) Typical traces showing the inhibitory effects of iberiotoxin (IbTX) on the relaxant response to DBcAMP in rat mesenteric artery. Arteries were precontracted with phenylephrine (3×10^{-6} M) (line under traces). DBcAMP was applied to the bath solution. Iberiotoxin (5×10^{-8} M) was applied to the bath solution 10–20 min before the addition of DBcAMP (closed square). Iberiotoxin (5×10^{-8} M), when applied during the relaxation induced by DBcAMP, partly restored the relaxation induced by the cAMP analog (upper panel). Numbers are the negative logarithm of DBcAMP concentration. IbTX: iberiotoxin (5×10^{-8} M); Phen: phentolamine (3×10^{-6} M). (b) Concentration–response relationships for diltiazem-induced relaxation of rat mesenteric artery in the absence and presence of iberiotoxin (5×10^{-8} M). Vascular relaxation is expressed as in Fig. 1 as % inhibition against phenylephrine-induced contraction. Data are mean values ± S.E.M. ($n=4$).

relaxed the mesenteric artery precontracted with phenylephrine by 85.2 ± 6.9% ($n = 3$) in the absence of iberiotoxin (Fig. 2a, upper panel). In contrast, the relaxant response to 10^{-4} M DBcAMP was reduced by about 70% to 25.3 ± 1.8% ($n = 3$) ($P < 0.01$) in the presence of iberiotoxin (5×10^{-8} M) (Fig. 2a, lower panel).

Iberiotoxin (5×10^{-8} M) did not affect the relaxation induced by phentolamine, an α-adrenoceptor antagonist (Figs. 1 and 2). In the absence of iberiotoxin, the relaxation of phenylephrine-contracted artery in response to phentolamine (3×10^{-6} M) was 104.3 ± 2.0% ($n = 5$), and it was 103.8 ± 1.5% ($n = 4$) ($P > 0.05$) in the presence of iberiotoxin (5×10^{-8} M). Furthermore, iberiotoxin did not affect the vascular relaxation induced by a calcium channel blocker, diltiazem. This lack of effect is clear from the concentration–response relationships, which were almost identical in the absence (open circles) and presence (filled circles) of iberiotoxin (Fig. 2b). The pEC_{50} value and estimated maximum responses to diltiazem were 6.89 ± 0.07 and 97.9 ± 4.0% ($n = 4$) in the absence iberiotoxin, and 6.82 ± 0.09 and 93.2 ± 5.1% ($n = 4$) in the presence of iberiotoxin ($P > 0.05$ for each).

We also compared VIP-induced relaxation of mesenteric artery in preparations precontracted with phenylephrine (3×10^{-6} M) vs. high (80 mM) KCl solution. In depolarizing solution with high KCl, VIP-induced relaxation was strongly suppressed. For instance, the maximum relaxant response to VIP (10^{-6} M) was inhibited from 93.2 ± 1.2% to 29.6 ± 1.8% ($n = 4$ for each) ($P < 0.01$). These findings indicate that VIP-induced relaxation of rat mesenteric artery is largely mediated via activation of K^+ channels in vascular smooth muscle cells, and are consistent with a significant role of iberiotoxin-sensitive MaxiK channels (Fig. 1).

4. Discussion

Iberiotoxin is thought to be a selective MaxiK channel blocker that does not show any appreciable effects on other K^+ channels (Galvez et al., 1990). Therefore, at present, this toxin is the best pharmacological tool to determine whether vascular relaxations in response to biologically active substances are mediated via activation of toxin-sensitive MaxiK channels. In the present study, we showed that vascular relaxations induced by both phentolamine and diltiazem were not affected by iberiotoxin at a concentration of 5×10^{-8} M, which significantly diminished the relaxant responses to VIP and DBcAMP. These results indicate that the inhibition by iberiotoxin of VIP- and DBcAMP-induced relaxation in the rat mesenteric artery is not attributable to non-specific actions of this toxin, but is due to its ability to block MaxiK channels.

Vascular relaxation induced by VIP has been shown to be accompanied by a decrease in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) in rat mesenteric artery (Tanaka et al., 1997) and porcine coronary artery (Kawasaki et al., 1997). The present findings suggest that the VIP-induced decrease in $[Ca^{2+}]_{cyt}$ can be partly ascribed to the closure of voltage-gated Ca^{2+} channels following the activation of MaxiK channels. VIP-stimulated activation of MaxiK channels would enhance K^+ efflux, leading to smooth muscle membrane hyperpolarization which limits Ca^{2+} influx through voltage-gated Ca^{2+} channels, thus decreasing $[Ca^{2+}]_{cyt}$ and inducing vascular relaxation.

The mechanism by which VIP receptor stimulation causes activation of MaxiK channels still remains to be elucidated. VIP elevates intracellular cAMP levels via activation of adenylate cyclase in a variety of tissues including vascular smooth muscles (Edvinsson et al., 1985; Itoh et al., 1985; Ganz et al., 1986; Ignarro et al., 1987; Tanaka et al., 1997). In rat mesenteric artery, we showed that VIP elevates the cAMP content in a concentration-dependent manner (Tanaka et al., 1997). Intracellular cAMP increased by VIP may activate in turn the protein kinase dependent on this nucleotide (A-kinase). A-kinase has been shown to activate MaxiK channels in non-vascular (Kume et al., 1989; Meera et al., 1995) and vascular (Scornik et al., 1993) smooth muscles through phosphorylation of the channel or closely associated proteins. Thus, cAMP elevation with the subsequent activation of A-kinase may mediate the activation of MaxiK channels, leading to the relaxant response of mesenteric artery to VIP. This idea can be supported by our present finding that dibutyryl cAMP-induced relaxation was largely inhibited by iberiotoxin. Mechanisms independent of A-kinase-mediated channel phosphorylation are also possible for the activation of MaxiK channels by VIP: (a) direct regulation of MaxiK channels by G proteins (Scornik et al., 1993); (b) channel activation due to the A-kinase-triggered increase in Ca^{2+} spark frequency through ryanodine-sensitive Ca^{2+} release channels (Porter et al., 1998); and/or (c) activation of cGMP kinase by cAMP (Lincoln and Cornwell, 1993). To confirm further that the cAMP/A-kinase cascade is responsible for the activation of MaxiK channels by VIP, it would be necessary to perform electrophysiological studies using isolated smooth muscle cells from rat mesenteric artery and specific inhibitors of A-kinase.

The VIP-induced relaxation of rat mesenteric artery recovered spontaneously after reaching its maximum level (see Fig. 1a). A similar recovery phenomenon was also observed when a single dose of VIP was applied (3×10^{-8}–10^{-6} M). Full recovery of VIP-induced relaxation was attained within 20–30 min following the maximum vasorelaxant level. A similar spontaneous recovery of VIP-induced relaxation was also observed in porcine coronary artery (Kawasaki et al., 1997). One possible explanation for this phenomenon may be the degradation of VIP by endogenous proteases in these vascular beds. The following observations support this hypothesis: (1) helodermin, a VIP-related peptide (Hoshino et al., 1984; Vandermeers et al., 1984) with a Pro–Pro–Pro sequence that

stabilizes the active core of the peptide (Naruse et al., 1986) exhibits a relatively sustained vasorelaxant action (Tanaka et al., 1997); (2) because the relaxation induced by DBcAMP was not transient but relatively sustained (Fig. 2a), the spontaneous recovery after VIP-induced relaxation may be attributed to an event before accumulation of intracellular cAMP; and (3) the recovery phenomenon was also observed in the presence of a phosphatase inhibitor, okadaic acid (10^{-8} M) ($n = 4$), ruling out an enhanced phosphatase activity that could reverse A-kinase-dependent phosphorylation of MaxiK channels or other proteins.

Although there is general consensus that VIP induces an increase in cAMP content, Chakder and Rattan (1993) showed that in anal sphincter muscle a high concentration of VIP (10^{-6} M) induced a similar increase in both cAMP and cGMP levels. This seems not to be the case in rat mesenteric artery since ODQ (10^{-5} M), an inhibitor of guanylate cyclase, did not prevent VIP-induced relaxation, whereas it does inhibit NO-induced relaxation that involves cGMP production.

We previously showed that VIP relaxes rat mesenteric artery partly through the activation of K_{ATP} channels (Tanaka et al., 1997). The present findings show that in addition to K_{ATP} channels, iberiotoxin-sensitive MaxiK channels significantly contribute to VIP-induced endothelium-independent relaxation of rat mesenteric artery.

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References

Chakder, S., Rattan, S., 1993. Involvement of cAMP and cGMP in relaxation of internal anal sphincter by neural stimulation, VIP, and NO. Am. J. Physiol. 264, G702–G707.

Davies, J.M., Williams, K.I., 1984. Endothelial-dependent relaxant effects of vaso-active intestinal polypeptide and arachidonic acid in rat aortic strips. Prostaglandins 27, 195–202.

D'Orleans-Juste, P., Dion, S., Mizrahi, J., Regoli, D., 1985. Effects of peptides and non-peptides on isolated arterial smooth muscles: role of endothelium. Eur. J. Pharmacol. 114, 9–21.

Duckles, S.P., Said, S.I., 1982. Vasoactive intestinal peptide as a neurotransmitter in the cerebral circulation. Eur. J. Pharmacol. 78, 371–374.

Edvinsson, L., Fredholm, B.B., Hamel, E., Jansen, I., Verrecchia, C., 1985. Perivascular peptides relax cerebral arteries concomitant with stimulation of cyclic adenosine monophosphate accumulation or release of an endothelium-derived relaxing factor in the cat. Neurosci. Lett. 58, 213–217.

Galvez, A., Gimenez-Gallego, G., Reuben, J.P., Roy-Contancin, L., Feigenbaum, P., Kaczorowski, J., Garcia, M.L., 1990. Purification and characterization of a unique, potent, peptidyl probe for the high conductance calcium-activated potassium channel from venom of the scorpion *Buthus tamulus*. J. Biol. Chem. 265, 11083–11090.

Ganz, P., Sandrock, A.W., Landis, S.C., Leopold, J., Gimbrone, J.M.A., Alexander, R.W., 1986. Vasoactive intestinal peptide: vasodilatation and cyclic AMP generation. Am. J. Physiol. 250, H755–H760.

Hattori, Y., Nagashima, M., Endo, Y., Kanno, M., 1992. Glibenclamide does not block arterial relaxation caused by vasoactive intestinal polypeptide. Eur. J. Pharmacol. 213, 147–150.

Hoshino, M., Yanaihara, C., Hong, Y.-M., Kishida, S., Katsumaru, Y., Vandermeers, A., Vandermeers-Piret, M.-C., Robberecht, P., Christophe, J., Yanaihara, N., 1984. Primary structure of helodermin, a VIP-secretin-like peptide isolated from Gila monster venom. FEBS Lett. 178, 233–239.

Ignarro, L.J., Byrns, R.E., Buga, G.M., Wood, K.S., 1987. Mechanisms of endothelium-dependent vascular smooth muscle relaxation elicited by bradykinin and VIP. Am. J. Physiol. 253, H1074–H1082.

Itoh, T., Sasaguri, T., Makita, Y., Kanmura, Y., Kuriyama, H., 1985. Mechanisms of vasodilation induced by vasoactive intestinal polypeptide in rabbit mesenteric artery. Am. J. Physiol. 249, H231–H240.

Itoh, H., Lederis, K.P., Rorstad, O.P., 1990. Relaxation of isolated bovine coronary arteries by vasoactive intestinal peptide. Eur. J. Pharmacol. 181, 199–205.

Kaczorowski, G.J., Knaus, H.-G., Leonard, R.J., McManus, O.B., Garcia, M.L., 1996. High conductance calcium-activated potassium channels; structure, pharmacology, and function. J. Bioenerg. Biomembr. 28, 255–267.

Kawasaki, J., Kobayashi, S., Miyagi, Y., Nishimura, J., Fujishima, M., Kanaide, H., 1997. The mechanisms of the relaxation induced by vasoactive intestinal peptide in the porcine coronary artery. Br. J. Pharmacol. 121, 977–985.

Kume, H., Takai, A., Tokuno, H., Tomita, T., 1989. Regulation of Ca^{2+}-dependent K^{+}-channel activity in tracheal myocytes by phosphorylation. Nature (London) 341, 152–154.

Lee, T.J.-F., Saito, A., Berezin, I., 1984. Vasoactive intestinal polypeptide-like substance: the potential transmitter for cerebral vasodilation. Science 224, 898–901.

Lincoln, T.M., Cornwell, T.L., 1993. Intracellular cyclic GMP receptor proteins. FASEB J. 7, 328–338.

Meera, P., Anwer, K., Monga, M., Oberti, C., Stefani, E., Toro, L., Sanborn, B.M., 1995. Relaxin stimulates myometrial calcium-activated potassium channel activity via protein kinase A. Am. J. Physiol. 269, C312–C317.

Naruse, S., Yasui, A., Kishida, S., Kadowaki, M., Hoshino, M., Ozaki, T., Robberecht, P., Christophe, J., Yanaihara, C., Yanaihara, N., 1986. Helodermin has a VIP-like effect upon canine blood flow. Peptides 7 (Suppl. I), 237–240.

Nelson, M.T., Quayle, J.M., 1995. Physiological roles and properties of potassium channels in arterial smooth muscle. Am. J. Physiol. 268, C799–C822.

Parker, R.B., Waud, D.R., 1971. Pharmacological estimation of drug-receptor dissociation constants. Statistical evaluation: I. Agonist. J. Pharmacol. Exp. Ther. 177, 1–12.

Porter, V.A., Bonev, A.D., Knot, H.J., Heppner, T.J., Stevenson, A.S., Kleppisch, T., Lederer, W.J., Nelson, M.T., 1998. Frequency modulation of Ca^{2+} sparks is involved in regulation of arterial diameter by cyclic nucleotides. Am. J. Physiol. 274, C1346–C1355.

Said, S.I., Mutt, V., 1970. Polypeptide with broad biological activity: isolation from small intestine. Science 169, 1217–1218.

Scornik, F.S., Codina, J., Birnbaumer, L., Toro, L., 1993. Modulation of coronary smooth muscle K_{Ca} channels by $G_{s\alpha}$ independent of phosphorylation by protein kinase A. Am. J. Physiol. 265, H1460–H1465.

Standen, N.B., Quayle, J.M., 1998. K^{+} channel modulation in arterial smooth muscle. Acta. Physiol. Scand. 164, 549–557.

Standen, N.B., Quayle, J.M., Davies, N.W., Brayden, J.E., Huang, Y.,

Nelson, M.T., 1989. Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. Science 245, 177–180.

Tanaka, Y., Horikawa, N., Ishiro, H., Kataha, K., Nakazawa, T., Watanabe, N., Ishii, K., Nakayama, K., Yanaihara, N., Shigenobu, K., 1997. Glibenclamide-sensitive mechanism is involved in helodermin-produced vasodilation in rat mesenteric artery. Res. Comm. Mol. Pathol. Pharmacol. 98, 141–156.

Toro, L., Wallner, M., Meera, P., Tanaka, Y., 1998. Maxi-K_{Ca}, a unique member of the voltage-gated K channel superfamily. News Physiol. Sci. 13, 112–117.

Vandermeers, A., Vandermeers-Piret, M.-C., Robberecht, P., Waelbroeck, M., Dehaye, J.-P., Winand, J., Christophe, J., 1984. Purification of a novel pancreatic secretory factor (PSF) and a novel peptide with VIP- and secretin-like properties (helodermin) from Gila monster venom. FEBS Lett. 166, 273–276.