

Vasoconstrictors Inhibit ATP-sensitive K⁺ Channels in Arterial Smooth Muscle through Protein Kinase C

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ABSTRACT The effects of vasoconstrictor-receptor (neuropeptide Y, α -adrenergic, serotonergic, histaminergic) stimulation on currents through ATP-sensitive potassium (K_{ATP}) channels in arterial smooth muscle cells were examined. Whole-cell K_{ATP} currents, activated by the synthetic K_{ATP} channel opener pinacidil or by the endogenous vasodilator, calcitonin gene-related peptide, which acts through protein kinase A, were measured in smooth muscle cells isolated from mesenteric arteries of rabbit. Stimulation of NPY-, α_1 -, serotonin (5-HT₂)-, and histamine (H₁)-receptors inhibited K_{ATP} currents by 40–56%. The signal transduction pathway that links these receptors to K_{ATP} channels was investigated. An inhibitor of phospholipase C (D609) and of protein kinase C (GF 109203X) reduced the inhibitory effect of these vasoconstrictors on K_{ATP} currents from 40–56% to 11–23%. Activators of protein kinase C, a diacylglycerol analogue and phorbol 12-myristate 13-acetate (PMA), inhibited K_{ATP} currents by 87.3 and 84.2%, respectively. K_{ATP} currents, activated by calcitonin gene-related peptide, were also inhibited (47–87%) by serotonin, phenylephrine, and PMA. We propose that K_{ATP} channels in these arterial myocytes are subject to dual modulation by protein kinase C (inhibition) and protein kinase A (activation).

KEY WORDS: norepinephrine • neuropeptide Y • serotonin • pinacidil • calcitonin gene-related peptide

INTRODUCTION

Potassium channels closed by intracellular adenosine 5'-triphosphate (referred to as ATP-sensitive potassium [K_{ATP}]¹ channels) and inhibited by oral hypoglycemic drugs, such as glibenclamide, have been identified in cardiac muscle, pancreatic beta cells, skeletal muscle, neurons, vascular smooth muscle, and nonvascular smooth muscle (Ashcroft and Ashcroft, 1990; Nelson and Quayle, 1995). The activity or open state probability (P_o) of K_{ATP} channels in smooth muscle cells with physiological levels of intracellular ATP is low (Nelson and Quayle, 1995). However, this low level of activity can be sufficient to regulate smooth muscle membrane potential (Nelson and Quayle, 1995). Activation of K_{ATP} channels in arterial smooth muscle leads to membrane potential hyperpolarization, which causes vasodilation through closing voltage-dependent calcium channels, decreasing Ca²⁺ entry and thereby intracellular Ca²⁺ (Nelson et al., 1990b). Inhibition of K_{ATP} channels by glibenclamide can lead to membrane potential depolarization and vasoconstriction in some, but not all,

preparations (Nelson et al., 1990a; Nelson et al., 1990b; Murphy and Brayden, 1995b; Nelson and Quayle, 1995).

Understanding the regulation of the P_o of K_{ATP} channels should provide important insights into their role in the control of smooth muscle function. The effects of intracellular ATP (inhibition) and ADP (activation) as well as other nucleotide diphosphates on channel P_o have been well documented (Ashcroft and Ashcroft, 1990; Nelson and Quayle, 1995). Recent evidence indicates that PKA is a potent activator of K_{ATP} channels in intact smooth muscle cells (Quayle et al., 1994; Zhang et al., 1994a; Zhang et al., 1994b; Kleppisch and Nelson, 1995b; Nelson and Quayle, 1995). Indeed, calcitonin gene-related peptide (CGRP) and adenosine, which cause glibenclamide-sensitive vasodilations, have been shown to activate K_{ATP} channels through cAMP and PKA (Quayle et al., 1994; Kleppisch and Nelson, 1995b; Nelson and Quayle, 1995; see also Zhang et al., 1994a; Zhang et al., 1994b).

In contrast to PKA activation of K_{ATP} channels in some types of smooth muscle, muscarinic-receptor stimulation has been shown to inhibit K_{ATP} channels in nonvascular smooth muscle (urinary bladder [Bonev and Nelson, 1993]) and esophageal (Hatakeyama et al., 1995) through activation of PKC. Recently, the vasoconstrictors, serotonin and histamine, have been shown to inhibit K_{ATP} channels in myocytes from cerebral arteries (Kleppisch and Nelson, 1995a), although the receptor subtype and signal transduction pathways were not determined. These results suggested the possibility that K_{ATP} channels in vascular smooth muscle may be

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¹Abbreviations used in this paper: CGRP, calcitonin gene-related peptide; DOG, *sn*-1,2-dioctanoyl-glycerol; GLIB, glibenclamide; K_{ATP} channels, ATP-sensitive potassium channels; NPY, neuropeptide Y; PLC, phospholipase C; 5-HT, serotonin.

subject to dual modulation, i.e., vasodilators activating K_{ATP} channels through PKA and vasoconstrictors inhibiting K_{ATP} channels through PKC. We, therefore, explored the possibility that the vasoconstrictors, neuropeptide Y (NPY), phenylephrine, serotonin, and histamine can inhibit K_{ATP} channels in arterial smooth muscle. Further, the signal transduction pathways linking these vasoconstrictor receptors to the K_{ATP} channels were examined. We provide evidence that this inhibition is through activation of PKC. Since vasodilator (e.g., CGRP, adenosine) activation of K_{ATP} channels appears to be an important mechanism of vasodilation, the effects of vasoconstrictors and PKC activators on K_{ATP} currents induced by CGRP (i.e., protein kinase A) were examined. These results indicate that K_{ATP} channels in smooth muscle cells of mesenteric artery are modulated by both vasoconstrictors and vasodilators and that the activity of these channels will be set by metabolic regulation and the opposing effects of PKA and PKC.

METHODS

Cell Preparation

New Zealand white rabbits were anesthetized with sodium pentobarbital and exsanguinated. A branch of the mesenteric artery was dissected in physiological saline solution (PSS) containing (in mM): 137 NaCl, 5.4 KCl, 0.44 NaH_2PO_4 , 0.42 Na_2HPO_4 , 4.17 NaHCO_3 , 1 MgCl_2 , 2.6 CaCl_2 , 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), pH adjusted to 7.4 with NaOH. The artery was cut into ~2-mm long pieces and placed in PSS containing 0.1 mM CaCl_2 . The tissue was incubated at 35°C in 0.1 mM Ca PSS containing 17 U/ml papain and 1.0 mg/ml dithioerythritol for 30 min and then for a further 10 min in solution containing 2 U/ml collagenase H and 330 U/ml hyaluronidase. Single cells were released by trituration through a pasteur pipette, stored in 0.1 mM Ca PSS at 4°C, and used on the day of preparation.

Solutions

The pipette (internal) solution contained (in mM): 107 KCl, 33 KOH, 10 ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), 1 MgCl_2 , 1 CaCl_2 , 10 HEPES, pH 7.2. Na_2ATP (0.1 mM) and NaADP (0.1 mM) were added to the pipette solution on the day of the experiment, and the pH re-adjusted to 7.2 with NaOH. Free Ca^{2+} was calculated to be 19.8 nM. The extracellular solution contained (in mM): 60 KCl, 80 NaCl, 1 MgCl_2 , 0.1 CaCl_2 , 10 HEPES, pH adjusted to 7.4 with NaOH. Glibenclamide, pinacidil, D609, and GF 109203X were made as stock solutions in DMSO. The highest concentration of DMSO was 0.05%, which, by itself, did not have any effect on membrane currents. Chemicals were obtained from Calbiochem-Novabiochem Corp. (La Jolla, CA), LC Laboratories (Woburn, MA), RBI (Natick, MA), Sigma Chemical Co. (St. Louis, MO), and Worthington Biochemical Corp. (Freehold, New Jersey).

Data Recording

The whole cell configuration of the patch clamp technique was used to record potassium currents through ATP-sensitive potas-

sium channels (Quayle et al., 1994; Quayle et al., 1995). Pipettes were fabricated from borosilicate glass (outer diameter 1.5 mm, inner diameter 1.17 mm; Sutter Instrument Co., Novato, CA) and coated with wax to reduce capacitance. Whole-cell currents were amplified by an Axopatch 200A amplifier, and recorded on video tape (filtered at 10 kHz) at 44.1 kHz and on computer, usually filtered at 2 Hz and digitized at 10 Hz. Currents were digitized and analyzed using an Axon Instruments TL-1 interface and PCLAMP or AXOTAPE software (Axon Instruments, Foster City, CA).

Data Analysis

Recordings were made from 139 cells with a mean cell capacitance of 12.7 ± 0.2 pF. Inward currents through the K_{ATP} channels were recorded at a steady membrane potential of -70 mV. The contribution of calcium-activated potassium channels to measured K^+ currents was minimized by the negative membrane potential, by buffering $[\text{Ca}^{2+}]_i$ to <20 nM with 10 mM EGTA, and by lowering $[\text{Ca}^{2+}]_o$ to 100 μM (Quayle et al., 1994). The negative holding potential also minimized activity of voltage-dependent K^+ (delayed rectifier) channels. We have previously characterized whole cell K_{ATP} channel currents in smooth muscle cells from rabbit mesenteric artery (Quayle et al., 1994; Quayle et al., 1995; Kleppisch and Nelson, 1995b). K_{ATP} channel currents in this preparation are activated by lowering intracellular ATP (Nelson et al., 1990b; Quayle et al., 1994; Quayle et al., 1995) and by the synthetic K^+ channel openers, pinacidil, cromakalim and diazoxide at half-activation concentrations of 0.6, 1.9, and 37.1 μM , respectively (Quayle et al., 1995). The endogenous vasodilators, CGRP (Quayle et al., 1994) and adenosine (via A_2 receptors) (Kleppisch and Nelson, 1995b) also activate K_{ATP} currents in this preparation through protein kinase A. K_{ATP} channels in mesenteric artery were inhibited by glibenclamide, tolbutamide, and tetraethylammonium, with half-inhibition concentrations of 0.1 μM , 351 μM and 6.2 mM, respectively (Quayle et al., 1995). The experimental conditions (0.1 mM pipette ATP, 60 mM external and 140 mM pipette potassium, holding potential -70 mV) used in the present study were identical to those used in the previous studies (Quayle et al., 1994; Kleppisch and Nelson, 1995b; Quayle et al., 1995). Under these conditions, glibenclamide (10 μM) inhibited the entire pinacidil- or CGRP-stimulated current as well as the steady-state K_{ATP} channel current in the absence of these agents. The ATP concentration (0.1 mM) in the pipette (internal) solution is more than sufficient to support PKC activity (Kikkawa et al., 1983; Wilkinson et al., 1993).

Experiments were conducted at room temperature (20–22°C). Results are expressed as the mean \pm SEM of *n* cells.

RESULTS

NPY, Phenylephrine, Serotonin (5-HT), and Histamine Inhibit K_{ATP} Currents

To examine inhibition of K_{ATP} channels in smooth muscle cells from mesenteric artery, whole-cell currents through ATP-sensitive K^+ channels were elevated by lowering intracellular ATP to 0.1 mM and by the synthetic K_{ATP} channel opener pinacidil (5 μM) (Fig. 1). To increase the driving force for K^+ movement and to minimize the activity of voltage-dependent K^+ channels, whole K^+ currents were investigated at -70 mV with 60 mM external and 140 mM internal potassium

(K^+ equilibrium potential, -20 mV). Therefore, K^+ currents were inward. Pinacidil ($5 \mu\text{M}$) elevated K_{ATP} channel currents to 124.6 ± 6.0 pA ($n = 119$). Since, in rabbit mesenteric artery, α_1 -receptors are responsible for norepinephrine contraction (Bülbring and Tomita, 1987), we used the agonist of α_1 -receptor phenylephrine. In the experiments shown in Fig. 1, A–D, pinacidil ($5 \mu\text{M}$) increased K^+ currents to 207, 121, 129, and 229 pA, respectively. NPY (30 nM), phenylephrine ($1 \mu\text{M}$), serotonin ($1 \mu\text{M}$), and histamine ($1 \mu\text{M}$) reduced this current by 95, 64, 59, and 118 pA, respectively. Glibenclamide (GLIB) ($10 \mu\text{M}$), an inhibitor of K_{ATP} channels (Ashcroft and Ashcroft, 1990; Nelson and Quayle, 1995; Quayle et al., 1995), reduced the currents further. On average, NPY, phenylephrine, serotonin, and histamine inhibited GLIB-sensitive currents in the presence of pinacidil by $43.4 \pm 1.0\%$ ($n = 7$), $48.3 \pm 3.9\%$ ($n = 8$), $39.4 \pm 1.9\%$ ($n = 10$), and $55.7 \pm 3.1\%$ ($n = 10$), respectively (see Figs. 2 and 7). NPY at 100 nM ($48.2 \pm 6.0\%$, $n = 3$) did not inhibit >30 nM NPY ($43.4 \pm 1.0\%$, $n = 7$). Similarly, histamine at $10 \mu\text{M}$ ($61.2 \pm 2.8\%$, $n = 8$) did not inhibit $>1 \mu\text{M}$ histamine ($55.7 \pm 3.1\%$, $n = 10$). Under these conditions, the vasoconstrictors were without effect on membrane current, when K_{ATP} channels were inhibited by glibenclamide ($n = 12$).

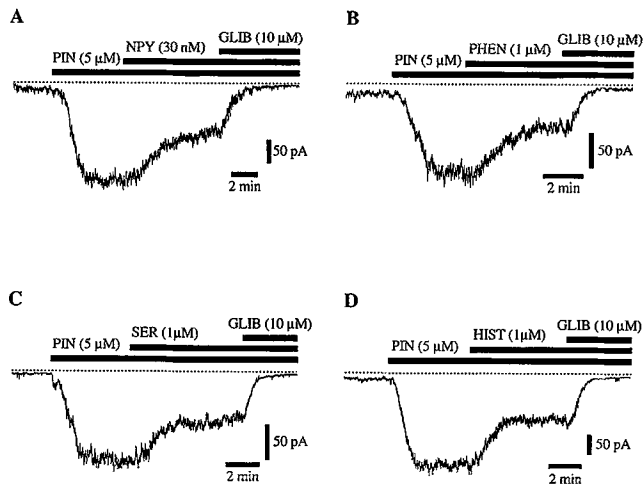


FIGURE 1. Vasoconstrictors inhibit K_{ATP} currents in smooth muscle cells from rabbit mesenteric artery. Original records demonstrating the effect of neuropeptide Y (NPY) (A), phenylephrine (PHEN) (B), serotonin (SER) (C), and histamine (HIST) (D) on whole-cell K_{ATP} currents elevated by pinacidil (PIN) in smooth muscle cells from rabbit mesenteric artery. In these experiments, neuropeptide Y, phenylephrine, serotonin, and histamine inhibited K_{ATP} currents by 46, 53, 46, and 48%, respectively. Glibenclamide (GLIB) inhibited the remainder of the pinacidil-induced current. Dashed line indicates the zero current level. External potassium $[K^+]_o$, 60 mM; internal potassium $[K^+]_i$, 140 mM; holding potential, -70 mV.

Antagonists of α_1 , 5-HT₂, and H₁-receptors Block Phenylephrine, Serotonin, and Histamine Inhibition of K_{ATP} Currents

Specific antagonists of α_1 -receptors (prazosin, $10 \mu\text{M}$), 5-HT₂-receptors (ketanserin, $10 \mu\text{M}$), and H₁-receptors (triprolidine, $1 \mu\text{M}$) nearly abolished the inhibitory effects of phenylephrine, serotonin, and histamine, respectively. Phenylephrine ($1 \mu\text{M}$), serotonin ($1 \mu\text{M}$), and histamine ($1 \mu\text{M}$) reduced GLIB-sensitive currents in the presence of pinacidil ($5 \mu\text{M}$) by $7.9 \pm 1.2\%$ ($n = 5$), $9.3 \pm 2.6\%$ ($n = 5$), and $5.0 \pm 2.7\%$ ($n = 5$), in contrast to 48, 39, and 56% inhibitions in the controls (Fig. 2).

Phospholipase C Inhibitor, D609, Reduces Vasoconstrictor-induced Inhibition of K_{ATP} Currents

Stimulation of NPY-, α_1 -, 5-HT₂-, and H₁-receptors leads to activation of phospholipase C (PLC). This enhances the formation of diacylglycerol (Hill, 1991; Lomasney et al., 1991; Nishizuka, 1992; Zifa and Fillion, 1992; Vila et al., 1993; Lee and Severson, 1994), which results in stimulation of protein kinase C. To test whether the PLC-diacylglycerol-PKC cascade is involved in the inhibition of K_{ATP} currents, the effects of a specific blocker of phosphatidylcholine-PLC, the xanthogenate derivative D609, were tested on vasoconstrictor-induced inhibition of GLIB-sensitive currents (see also Sohn et al., 1995). D609 ($200 \mu\text{M}$), which was added to the bathing solution, should inhibit diacylglycerol formation by $\sim 80\%$ (Schütze et al., 1992). In the presence of D609, NPY, phenylephrine, serotonin, and histamine inhib-

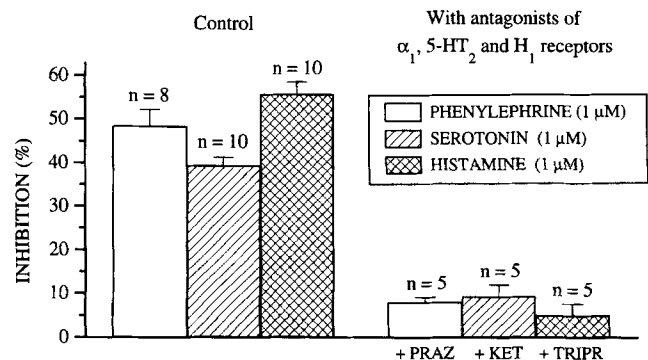


FIGURE 2. Antagonists of α_1 , 5-HT₂, and H₁ receptors inhibit the effects of phenylephrine, serotonin, and histamine on K_{ATP} currents. Summarized data of the mean inhibition of whole-cell K_{ATP} currents by phenylephrine ($1 \mu\text{M}$), serotonin ($1 \mu\text{M}$), and histamine ($1 \mu\text{M}$) without (Control, left bars), and with antagonists of α_1 -receptors (prazosin [PRAZ], $10 \mu\text{M}$), 5-HT₂ receptors (ketanserin [KET], $10 \mu\text{M}$) and H₁ receptors (triprolidine [TRIPR], $1 \mu\text{M}$), respectively (right bars). K_{ATP} currents were elevated by pinacidil ($5 \mu\text{M}$). External potassium $[K^+]_o$, 60 mM; internal potassium $[K^+]_i$, 140 mM; holding potential, -70 mV.

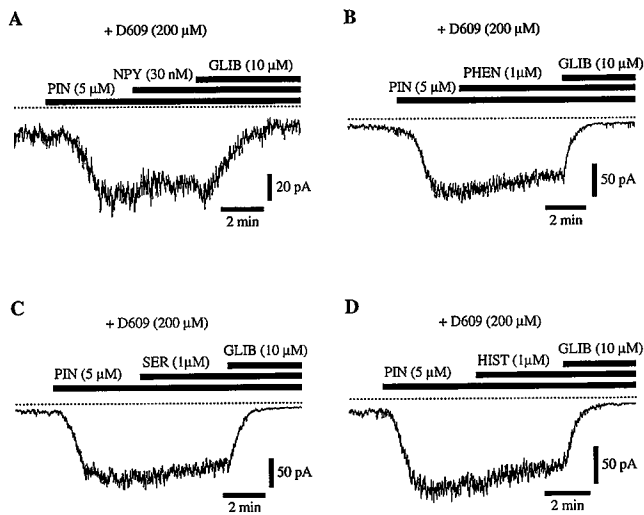


FIGURE 3. Phospholipase C inhibitor, D609, reduces vasoconstrictor inhibition of K_{ATP} currents. Original records of the effects of neuropeptide Y (NPY) (A), phenylephrine (PHEN) (B), serotonin (SER) (C), and histamine (HIST) (D) on K_{ATP} currents elevated by pinacidil (PIN) in the presence of D609 (200 μ M). D609 was applied to the bathing solution 5 min before exposure to pinacidil. In these experiments, neuropeptide Y, phenylephrine, serotonin, and histamine inhibited K_{ATP} currents by 16, 21, 19, and 21%, respectively. Dashed line indicates the zero current level. External potassium $[K^+]_o$, 60 mM; internal potassium $[K^+]_i$, 140 mM; holding potential, -70 mV.

ited GLIB-sensitive currents by only $16.4 \pm 6.5\%$ ($n = 5$), $17.6 \pm 2.6\%$ ($n = 5$), $15.9 \pm 4.7\%$ ($n = 6$), and $18.7 \pm 3.5\%$ ($n = 5$), as compared to the control values of 43, 48, 39, and 56%, respectively, (see Figs. 3 and 7). These results suggest possible involvement of phospholipase C and protein kinase C in K_{ATP} channel inhibition by NPY, phenylephrine, serotonin, and histamine.

Diacylglycerol Analogue and PMA Inhibit K_{ATP} Currents

To test the hypothesis that PLC stimulation inhibits K_{ATP} channels through diacylglycerol and subsequent stimulation of PKC, the effect of the membrane permeable analogue of diacylglycerol, *sn*-1,2-dioctanoyl-glycerol (DOG) (Davis et al., 1985) on GLIB-sensitive currents was examined. In the experiment shown in Fig. 4 A, DOG (1 μ M) inhibited GLIB-sensitive currents by 87%. DOG inhibited GLIB-sensitive currents in six cells by $87.3 \pm 1.3\%$.

DOG can activate PKC which could lead to inhibition of K_{ATP} currents. To test this possibility further, the effects of the PKC activator, PMA, on GLIB-sensitive currents were examined. In the experiment shown in Fig. 4 B, PMA (100 nM) inhibited GLIB-sensitive currents by 86%. PMA (100 nM) inhibited GLIB-sensitive currents in five cells by $84.2 \pm 2.7\%$.

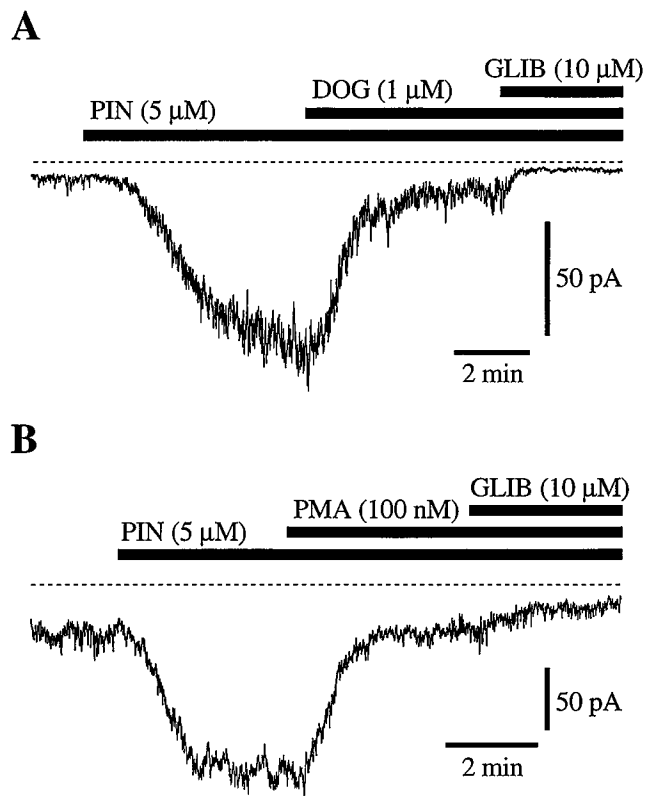


FIGURE 4. Protein kinase C activators inhibit K_{ATP} currents. Diacylglycerol analogue (DOG) (1 μ M) (A) and phorbol 12-myristate 13-acetate (PMA) (100 nM) (B) inhibition of K_{ATP} currents. K_{ATP} currents were increased by pinacidil (PIN) (5 μ M). In these cells, DOG and PMA inhibited K_{ATP} currents by 87 and 86%, respectively. Dashed line indicates the zero current level. External potassium $[K^+]_o$, 60 mM; internal potassium $[K^+]_i$, 140 mM; holding potential, -70 mV.

Protein Kinase C Inhibitor Reduces Vasoconstrictor-induced Inhibitions of K_{ATP} Currents

To examine further that PKC stimulation is involved in the inhibition of K_{ATP} currents, the effect of a specific blocker of PKC, bisindolylmaleimide derivative GF 109203X, was tested on vasoconstrictor-inhibition of GLIB-sensitive currents. GF 109203X is a membrane permeable, competitive inhibitor of PKC with respect to ATP, with a reported half-maximal inhibitory concentration (IC_{50}) in the range of 10 nM in the presence of 10 μ M ATP (Toullec et al., 1991). We used GF 109203X at a concentration of 300 nM. Higher concentrations were not used so as to minimize nonspecific effects of this compound. Since the pipette solution contained 100 μ M ATP, the IC_{50} should be ~ 100 nM. Therefore, the expected inhibition of PKC by 300 nM GF 109203X would be $\sim 75\%$. In the presence of GF 109203X (300 nM in the bathing solution), PMA (100

nM) inhibited K_{ATP} currents by only $24.7 \pm 3.4\%$ ($n = 5$), as compared to 84% inhibition in the absence of GF 109203X. Under the same conditions with GF 109203X (300 nM) present, NPY, phenylephrine, serotonin, and histamine reduced GLIB-sensitive currents by only $16.7 \pm 2.0\%$ ($n = 8$), $11.4 \pm 4.0\%$ ($n = 9$), $19.4 \pm 2.9\%$ ($n = 11$), and $23.3 \pm 1.3\%$ ($n = 5$), as compared to the control values of 43, 48, 39, and 56%, respectively (see Figs. 5 and 7). These results support the idea that vasoconstrictor-induced inhibition of K_{ATP} currents involves the activation of PKC.

The effects of GF 109203X on vasoconstrictor-induced inhibition of K_{ATP} currents were also examined in the same cell. Fig. 6 A shows that histamine (1 μ M) inhibits 19% of the glibenclamide-sensitive current in the presence of the PKC inhibitor GF 109203X. After washout (13 min) of GF 109203X and histamine, re-application of histamine inhibited glibenclamide-sensitive currents by 43%. The reverse order was tested using serotonin (Fig. 6 B). The first application of serotonin (1 μ M) inhibited the glibenclamide-sensitive K^+ currents by 42%. Increasing serotonin to 10 μ M caused a slightly greater inhibition (55%). The second application of 1 μ M serotonin in the presence of GF 109203X caused only a 21% inhibition. Serotonin (1 μ M) in five cells caused $39.2 \pm 4.0\%$ inhibition in the absence of GF 109203X,

and, in the same cells, serotonin caused a $19.0 \pm 2.8\%$ inhibition in the presence of GF 109203X. Similarly, phenylephrine (1 μ M) caused a $46.1 \pm 4.0\%$ inhibition, and, in the same cells in the presence of GF 109203X, phenylephrine (1 μ M) caused $15.7 \pm 7.0\%$ inhibition ($n = 5$). These results provide additional support for the idea that vasoconstrictors act through PKC.

CGRP-induced K_{ATP} Currents Are Also Inhibited by Vasoconstrictors and PMA

A number of endogenous vasodilators including adenosine (Kleppisch and Nelson, 1995b) and calcitonin gene-related peptide act (Quayle et al., 1994), in part, through stimulation of K_{ATP} channels (Nelson and Quayle, 1995). Therefore, it is conceivable that vasoconstrictor-induced inhibition of K_{ATP} channels may oppose vasodilator-induced activation. To address the question of whether the stimulation of PKC could inhibit K_{ATP} currents activated by endogenous vasodilators, we examined the effects of activators of PKC and vasoconstrictors on K_{ATP} channel currents that were activated by calcitonin gene-related peptide. CGRP has been implicated in the control of blood flow in a number of vascular beds (Holzer, 1992) and has been shown to activate K_{ATP} channels in mesenteric artery through stimulation of protein kinase A (Quayle et al., 1994). In this study, CGRP (10 nM) increased GLIB-sensitive cur-

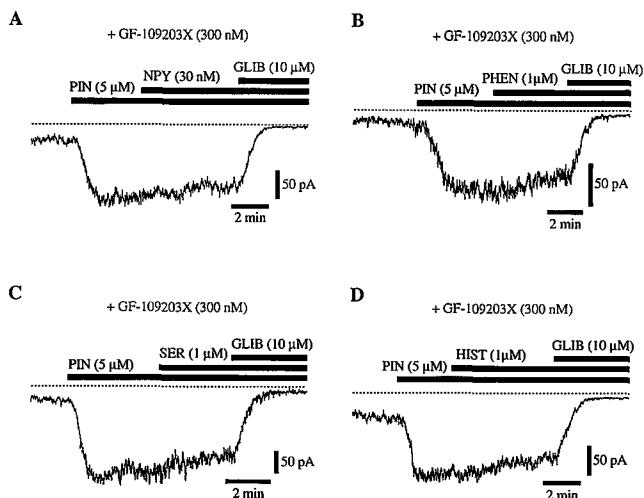


FIGURE 5. Protein kinase C inhibitor, GF 109203X, reduces vasoconstrictor-inhibition of K_{ATP} currents. Original records of the effects of neuropeptide Y (NPY) (A), phenylephrine (PHEN) (B), serotonin (SER) (C), and histamine (HIST) (D) on K_{ATP} currents elevated by pinacidil (PIN) in the presence of GF 109203X (300 nM). GF 109203X was applied to the bathing solution 5 minutes before exposure to pinacidil. In these experiments, neuropeptide Y, phenylephrine, serotonin, and histamine inhibited K_{ATP} currents by 17, 19, 21, and 22%, respectively. Dashed line indicates the zero current level. External potassium $[K^+]_o$, 60 mM; internal potassium $[K^+]_i$, 140 mM; holding potential, -70 mV.

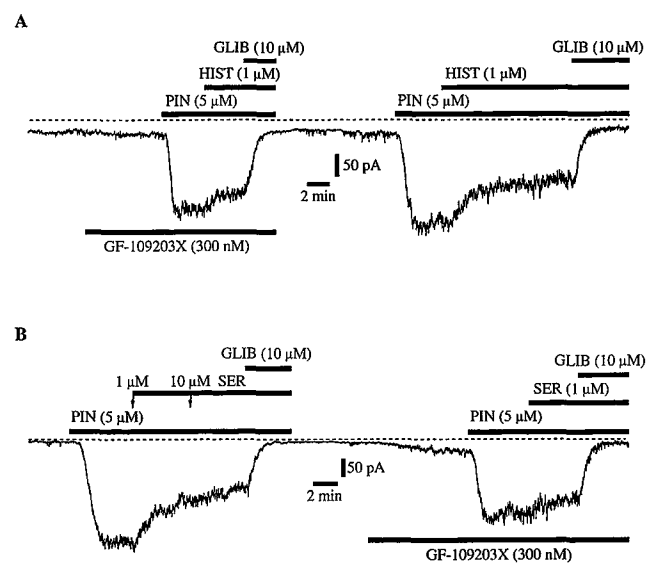


FIGURE 6. GF 109203X inhibition of histamine (A) and serotonin (B) effects in the same cells. Original records of the effects of histamine (HIST) (A) and serotonin (SER) (B) on K_{ATP} currents elevated by pinacidil (PIN) in the presence and absence of GF 109203X (300 nM). Dashed line indicates the zero current level. External potassium $[K^+]_o$, 60 mM; internal potassium $[K^+]_i$, 140 mM; holding potential, -70 mV.

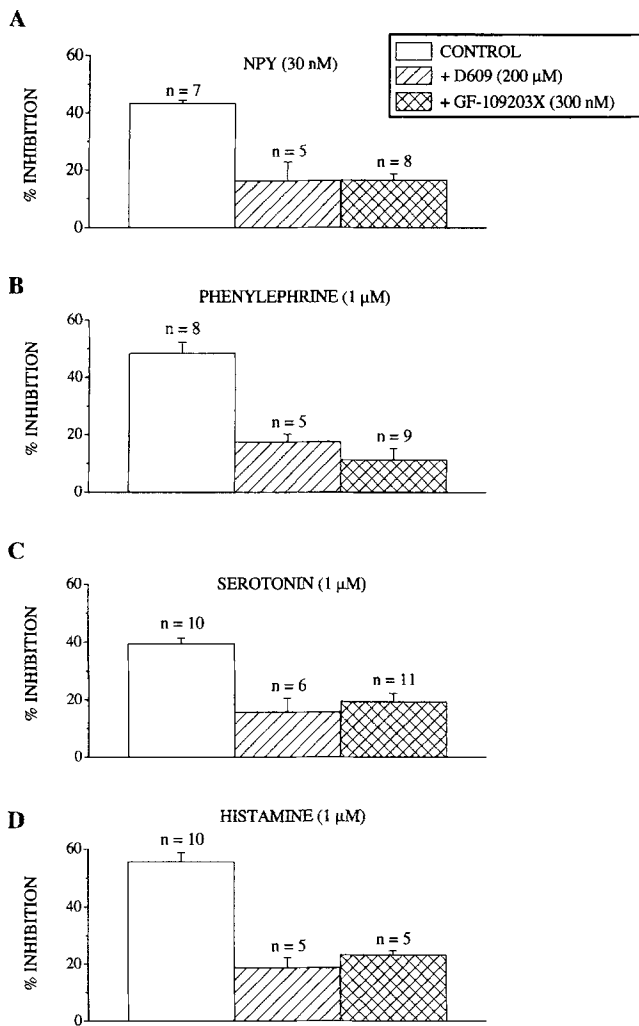


FIGURE 7. Comparison of the inhibition of K_{ATP} currents by vasoconstrictors in the absence and in the presence of PLC and PKC inhibitors. Summarized data of the mean inhibition of K_{ATP} currents by NPY (30 nM), phenylephrine (1 μ M), serotonin (1 μ M), and histamine (1 μ M) without (CONTROL) and with blockers of PLC and PKC, as indicated. K_{ATP} currents were elevated by pinacidil (5 μ M). External potassium $[K^+]_o$, 60 mM; internal potassium $[K^+]_i$, 140 mM; holding potential, -70 mV.

rent to 57.2 ± 6.5 pA ($n = 20$). In the experiment shown in Fig 8 A, PMA (100 nM) reduced the GLIB-sensitive current in the presence of CGRP by 96%. In five cells, PMA inhibited K_{ATP} currents in the presence of CGRP by $87.0 \pm 2.4\%$. This value is very close to the value of 84% inhibition obtained in the presence of pinacidil. In contrast, inactive phorbol ester 4 α -phorbol, 12,13-didecanoate reduced K_{ATP} currents by only $13.7 \pm 1.8\%$ ($n = 4$). Serotonin (1 μ M) inhibited CGRP-induced K_{ATP} currents by $70.4 \pm 1.8\%$ ($n = 8$) (Fig. 8 B). Phenylephrine (1 μ M) inhibited CGRP-stimulated K_{ATP} currents by $47.2 \pm 2.5\%$ ($n = 3$) (not shown), which was comparable to 48% inhibition observed in the presence

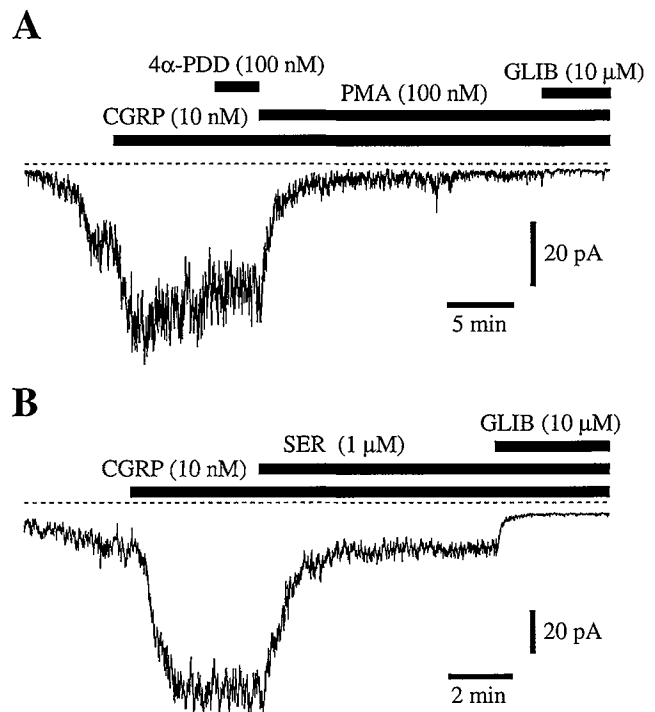


FIGURE 8. PMA and serotonin inhibition of CGRP-induced K_{ATP} currents. Original records of the effect of nonactive analogue of phorbol ester 4 α -phorbol, 12,13-didecanoate (4 α -PDD) (100 nM) and PMA (100 nM) (A) and serotonin (SER) (B) on whole-cell K_{ATP} currents elevated by CGRP (10 nM). In this experiment, 4 α -PDD and PMA inhibited K_{ATP} currents by 9 and 96%, respectively. Serotonin-inhibition was 79%. Dashed line indicates the zero current level. External potassium $[K^+]_o$, 60 mM; internal potassium $[K^+]_i$, 140 mM; holding potential, -70 mV.

ence of pinacidil. These results indicate that activation of PKC can inhibit K_{ATP} currents that were activated by the endogenous vasodilator, calcitonin gene-related peptide.

DISCUSSION

These results are consistent with the following scheme (Fig. 9): (a) Stimulation of neuropeptide Y, α_1 -adrenergic, serotonin (5-HT₂), and histamine (H₁) receptors leads to activation of phospholipase C and subsequent production of diacylglycerol. (b) Diacylglycerol then activates protein kinase C. (c) Protein kinase C inhibits K_{ATP} channels. Neither PMA nor receptor stimulation completely inhibited K_{ATP} currents activated by pinacidil or CGRP. Under our conditions, the concentrations of NPY (30 nM), serotonin (1 μ M), and histamine (1 μ M) that we used appeared to evoke almost maximal inhibitory responses (see RESULTS and Fig 6 B). Presumably, the degree of inhibition of K_{ATP} channels by PKC is set by the balance between the phosphorylation rate of PKC and the dephosphorylation rate of the phosphatases.

Indeed, membrane-associated phosphatase 2A has been shown to reverse PKC-induced inhibition of cardiac K_{ATP} channels (Light et al., 1995). Alternatively, incomplete inhibition by PMA or the vasoconstrictors may reflect a population of K_{ATP} channels that are not regulated by PKC.

Here, we demonstrate that stimulation of NPY-, α_1 -adrenergic, 5-HT₂-, and H₁-receptors inhibits K_{ATP} channels in smooth muscle cells from rabbit mesenteric arteries. Serotonin and histamine have been shown to inhibit K_{ATP} currents in smooth muscle cells isolated from rabbit cerebral (basilar) arteries (Kleppisch and Nelson, 1995a), although the receptor subtype and signal transduction pathways were not elucidated. Muscarinic (M₃) receptor stimulation through activation of PKC has been shown to inhibit K_{ATP} currents in smooth muscle cells isolated from guinea pig urinary bladder (Bonev and Nelson, 1993) and from rabbit esophagus (Hatakeyama et al., 1995). Finally, Tateishi and Faber (1995) provided evidence that activation of α_{2D} -adrenoceptors inhibited the functional response to openers of K_{ATP} channels in rat skeletal muscle arterioles. We found that α_1 -adrenergic receptor stimulation was involved in the inhibition of K_{ATP} channels in rabbit mesenteric artery. Our results suggest that K_{ATP} channel openers such as pinacidil would be less effective as smooth muscle relaxants in tissues that have high levels of receptor-mediated PKC activity, i.e., under conditions where K_{ATP} channels are already heavily inhibited. It should be noted that inhibition of K_{ATP} channels was examined in cells dialyzed with 0.1 mM ATP to facilitate the examination of the inhibitory effects of vasoconstrictors and PKC activators. It is conceivable that these inhibitory effects may be altered in intact cells with physiological levels of ATP. This possibility remains to be explored.

Inhibition of K_{ATP} channels by PKC activation may be a more general feature of K_{ATP} channels in smooth muscle, since phorbol esters inhibit K_{ATP} channels in smooth muscle cells from mesenteric artery (this study), urinary bladder (Bonev and Nelson, 1993), esophagus (Hatakeyama et al., 1995), and cerebral arteries (Kleppisch and Nelson, 1995a). ATP-sensitive K⁺-channels in kidney cells and in *Xenopus* oocyte follicular cells are also subject to dual modulation by PKA and PKC (Wang and Giebisch, 1991; Guillemare et al., 1995). In cardiac muscle and insulin-secreting cells, PKC stimulation has been shown to inhibit (Wollheim et al., 1988; Light et al., 1995) and activate K_{ATP} channels (Ribalet et al., 1988; DeWeille et al., 1989; Hu et al., 1996; Liu et al., 1996). Inhibition of K_{ATP} channels in insulin-secreting cells by PKC has also been shown to be transient, followed by activation (Dunne, 1994). Inhibition of K_{ATP} channels in smooth muscle was maintained during exposure to PMA.

Previous studies have shown that CGRP and adenosine, which elevate cAMP in intact smooth muscle, can activate K_{ATP} channels in single smooth muscle cells through stimulation of protein kinase A (Quayle et al., 1994; Zhang et al., 1994a; Kleppisch and Nelson, 1995a, b) (see also Fig. 8). Consistent with the single cell studies, part of vasodilation of intact arteries to CGRP and adenosine can be blocked by glibenclamide (for review see Nelson and Quayle, 1995). Using the same preparation that we used (rabbit mesenteric artery), Murphy & Brayden (1995a) demonstrated that forskolin causes a glibenclamide-sensitive membrane potential hyperpolarization (~15 mV) of smooth mus-

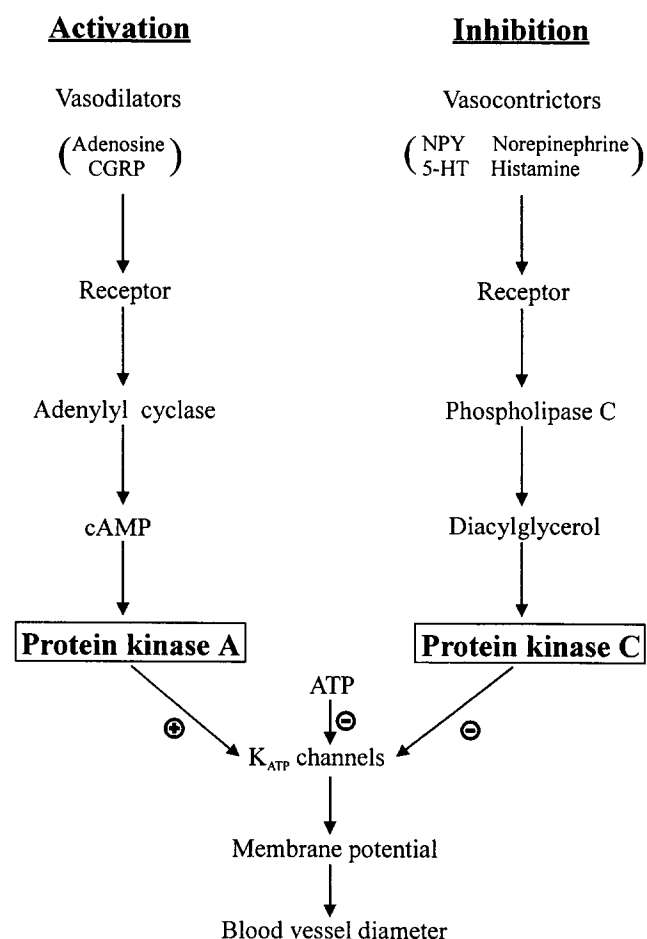


FIGURE 9. Proposed mechanism for dual modulation of K_{ATP} channels by some vasodilators and vasoconstrictors. Adenosine (via A₂ receptors) (Kleppisch and Nelson, 1995b) and CGRP (Quayle et al., 1994; see also Zhang et al., 1994a, 1994b) (see also Fig. 8) have been previously shown to activate K_{ATP} channels in mesenteric arterial myocytes through stimulation of protein kinase A. In this study, we provide evidence that neuropeptide Y, phenylephrine, serotonin, and histamine, acting through different receptors, can inhibit K_{ATP} channels through stimulation of protein kinase C. These results suggest that the final level of K_{ATP} channel activity will be set by opposing PKA activation and PKC inhibition as well as by ATP inhibition and ADP activation.

cle cells in the intact artery, consistent with our single cell data (Quayle et al., 1994) (see Zhang et al., 1994b for similar results of forskolin on intact gallbladder smooth muscle). Vasoconstrictors such as serotonin and phenylephrine (cf., Cheng et al., 1991; Clark and Garland, 1991) have been shown to act in part on denuded vessels through activation of protein kinase C. A recent study on rat skeletal muscle arterioles has shown that K_{ATP} channels activated by synthetic openers or by hypoxia can be inhibited by activation of α_{2D} -adrenoceptors (Tateishi and Faber, 1995). These studies support the idea that dual modulation of K_{ATP} channels by vasodilators and vasoconstrictors also occurs in intact smooth muscle preparations.

The contribution of PKC-mediated inhibition of K_{ATP} channels to the membrane depolarization caused by NPY, phenylephrine, serotonin, and histamine in intact arterial smooth muscle remains to be determined. Glibenclamide constricts and depolarizes smooth muscle cells in intact mesenteric artery by 5 mV (Nelson et al., 1990a; Murphy and Brayden, 1995b), suggesting that inhibition of K_{ATP} channels can have a significant functional effect in mesenteric artery (see also Nelson and

Quayle, 1995). However, vasoconstrictors such as norepinephrine can depolarize as much as 25 mV (Bülbring and Tomita, 1987), suggesting that other ionic mechanisms contribute to the membrane depolarization. These results suggest that in smooth muscle tissues where K_{ATP} channels play an important tonic role (for example in the coronary circulation [Samaha et al., 1992]), agonist stimulation of PKC may lead to membrane depolarization in part through K_{ATP} channel inhibition, and this should contribute to smooth muscle contraction. Further, this inhibitory response of K_{ATP} channels may be particularly prominent under conditions of elevated K_{ATP} channel activity by endogenous vasodilators such as hypoxia (Nelson and Quayle, 1995; Tateishi and Faber, 1995), adenosine (Kleppisch and Nelson, 1995b), and calcitonin gene-related peptide (Quayle et al., 1994). In conclusion, we propose that the physiological activity of K_{ATP} channels in some types of smooth muscle is determined by metabolic regulation (ATP, ADP, and the opposing actions of vasodilator-mediated (PKA) activation and vasoconstrictor-mediated (PKC) inhibition (Fig. 9).

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