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Regulation of Arterial Tone by Calcium-Dependent K^+ Channels and ATP-Sensitive K^+ Channels

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Summary. **Resistance arteries depolarize and constrict to elevations in intravascular pressure. However, many of the molecular aspects of this phenomenon are not known. We present evidence that large conductance calcium-dependent potassium (K_{Ca}) channels, which are activated by intracellular calcium and membrane depolarization, play a fundamental role in regulating the degree of intravascular pressure-induced, myogenic tone. We found that blockers of K_{Ca} channels, charybdotoxin (CTX, <100 nM) and TEA^+ (<0.5 mM), further depolarized pressurized arteries by as much as 12 mV and decreased diameter by up to 40%. CTX blocked K_{Ca} channels in outside-out patches from arterial smooth muscles with half-block constant of 10 nM and external TEA^+ caused a flickery block, with a half-block constant of 200 μM. We propose that K_{Ca} channels serve as a negative feedback pathway to limit the degree of membrane depolarization and hence vasoconstriction to pressure. In contrast, CTX and TEA^+ (<1 mM) were without effect on membrane hyperpolarization and dilation to a wide variety of synthetic (cromakalim, pinacidil, diazoxide, minoxidil sulfate) and endogenous agents [calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide, an endothelial-derived hyperpolarizing factor]. Glibenclamide and low concentrations of external barium that inhibit ATP-sensitive potassium (K_{ATP}) channels, however, blocked the hyperpolarizations and dilations to these substances. We have identified K_{ATP} channels as well as high-affinity glibenclamide binding sites in arterial smooth muscle. These channels are activated by cromakalim and CGRP, and are blocked by glibenclamide. Further, the existence of K_{ATP} channels in arterial smooth muscle suggests the possibility that compromising cellular metabolism through metabolic poisons, hypoxia, or alterations in glucose may open K_{ATP} channels and lead to vasodilation. Indeed, other workers have provided evidence that metabolic poisons and hypoxia lead to an increase in glibenclamide-sensitive potassium efflux and vasodilation. We have found that replacement of external glucose by deoxyglucose caused glibenclamide-sensitive coronary artery dilation, membrane hyperpolarization, and activation of K_{ATP} channels. We conclude that both K_{ATP} and K_{Ca} channels serve important functions in the regulation of arterial tone.**

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Small arteries operate in a contracted state (tone) from which they can constrict or dilate depending on need. Intravascular pressure causes many resistance arteries to develop (myogenic) tone, which is further modulated by constricting and dilating substances. Myogenic tone is a significant contributor to basal arterial tone as well as to autoregulation, which is responsible for maintaining constant blood flow in the brain during blood pressure changes. Myogenic tone of cerebral arteries is dependent on external Ca, blocked by Ca channel antagonists, and appears to be regulated by membrane potential [1].

The development of tone in response to increases in transmural pressure in vitro is associated with a depolarization of smooth muscle cells [2, 3]. For example, physiological pressures depolarized feline middle cerebral arteries from about −60 to −45 mV [2]. Resistance-sized arteries that exhibit myogenic tone under physiological pressures have membrane potentials similar to those measured in vivo [4]. Conversely, membrane hyperpolarization of these arteries at constant pressure causes vasodilation [2]. In fact, synthetic agents (e.g., cromakalim, pinacidil) that hyperpolarize arterial smooth muscle are potent vasodilators in vitro and in vivo [5, 6], and hold promise as a new class of smooth muscle relaxants. Thus, membrane potential appears to play a critical role in tone development and regulation. In this manuscript, we summarize some of our recent work on K_{Ca} and K_{ATP} channels.

Calcium-Activated Potassium Channels

Large conductance calcium-activated K^+ channels [7] exist in a wide variety of smooth muscle cell types [1]. K_{Ca} channels are activated by submicromolar Ca^{2+} and membrane depolarization, and are blocked by relatively low concentrations of external TEA^+ (K_d about

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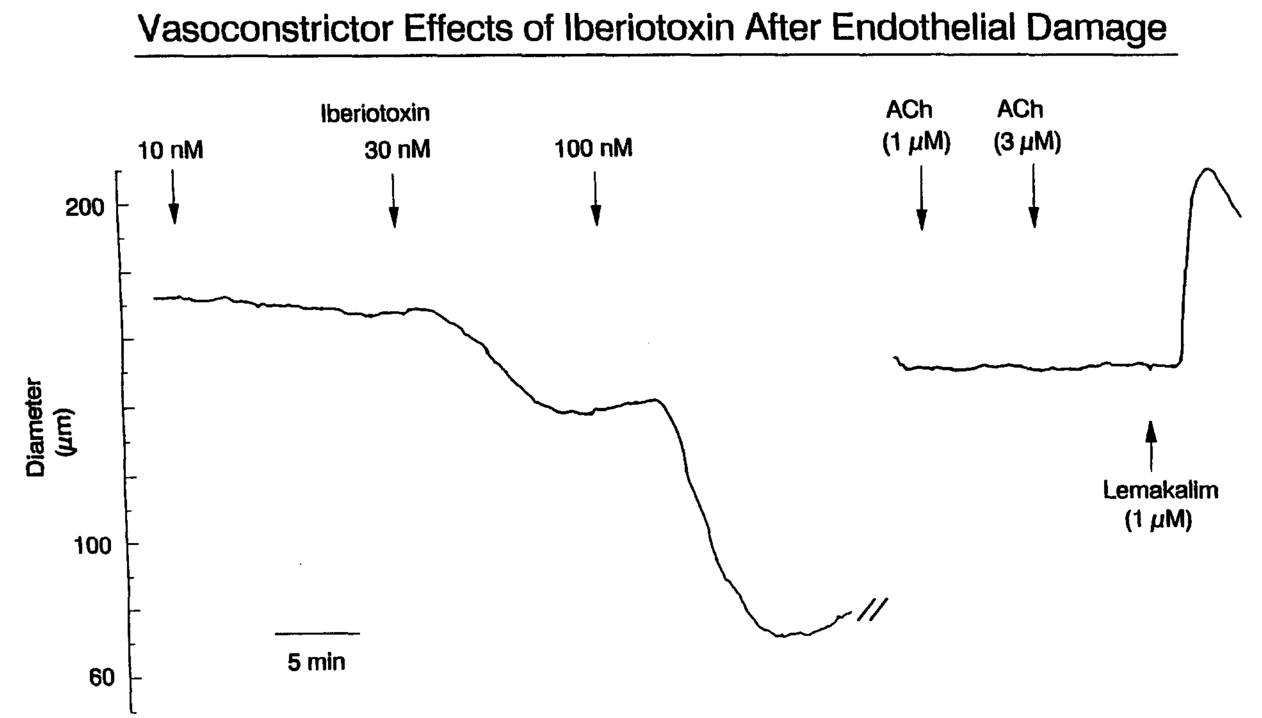

Fig. 1. Myogenic tone developed in response to an increase in transmural pressure to 80 mmHg and iberiotoxin constricted an artery after the endothelium was removed. Prior to mounting the artery, the endothelium was disrupted by placing an air bubble in the lumen for 1 minute and then perfusing the lumen with distilled water for 30 seconds. Damage to the endothelium was verified by the absence of a dilator response to acetylcholine.

0.2 mM) [8] and by the toxin, charybdotoxin (K_d about 10 nM) [9, 10]. Despite the fact that these channels are widepsread in nature, there has been a general lack of functional evidence supporting a physiological role for these channels. Here we summarize our evidence that these channels may be involved in controlling the myogenic tone of cerebral arteries.

Charybdotoxin, a peptide produced by the scorpion *Leirus quinquestriatus*, blocks K_{Ca} channels in smooth muscle with a high affinity with a half-block constant of 16 nM at 0 mV [10]. Iberiotoxin from the venom of the scorpion *Buthus tamulus* also blocks K_{Ca} channels in arterial smooth muscle and is thought be highly selective for K_{Ca} channels [11]. Block of ion channels has a number of appearances, depending on the rate of block. If the block is of high affinity, as is the case with charybdotoxin, then the fraction of time the channel is open would decrease without an effect on the mean current through a single channel. In fact, when charybdotoxin or iberiotoxin block a K_{Ca} channel, they stay bound for seconds, i.e., the off rate is very slow. In contrast, external TEA^+, which blocks K_{Ca} channels with much lower affinity than charybdotoxin, blocks and unblocks the pore so rapidly (mean block time about 70 μsec) that this block can only be partially resolved by the recording equipment [8].

Brayden and Nelson [10] proposed that pressure-induced membrane depolarization and increases in intracellular calcium activate K_{Ca} channels. Activation of K_{Ca} channels would increase potassium efflux, which would counteract the depolarization and constriction caused by pressure and vasoconstrictors. This mechanism predicts that blockers of K_{Ca} channels will depolarize and constrict arteries with tone. This is, indeed, the case in that TEA^+, charybdotoxin, and iberiotoxin depolarized and constricted myogenic cerebral and coronary arteries [10]. Figure 1 shows the effect of iberiotoxin on the diameter of a pressurized cerebral artery. Iberiotoxin at 10 nM caused a small vasoconstriction. Increasing the iberiotoxin concentration to 30 nM caused an additional 30 μm constriction, and 100 nM iberiotoxin decreased the diameter by about 100 μm. This artery was also mechanically stripped of its endothelium. As can be seen in the right side of Figure 1, acetylcholine had no effect on this artery. Acetylcholine at this concentration would ordinarily cause full dilation by stimulating the release of relaxing factors from the endothelium. Lemakalim, a direct activator of K_{ATP} channels in smooth muscle, dilated this artery. This experiment suggests that K_{Ca} channels are involved in the regulation of cerebral artery diameter and that blocking these channels can cause a prolonged vasoconstriction [10]. It also indicates that the action of iberiotoxin is independent of the endothelium.

These results suggest that lowering intravascular pressure or blocking calcium channels, which would decrease intracellular calcium and thus decrease active tone, should decrease the effects of K_{Ca} channel blockers on membrane potential and arterial tone [10]. Table 1 shows that charybdotoxin and TEA^+ depolarize arteries that have tone (i.e., that are depolarized and have elevated intracellular Ca^{2+}). In contrast, charybdotoxin is without effect on membrane potential when active tone is reduced either by lowering intravascular pressure or by blocking calcium channels with nimodipine (1 nM, Table 1).

ATP-Sensitive Potassium Channels

Arterial tone is regulated by a number of substances, including neurotransmitters, neuropeptides, endoethelial-derived factors, metabolites, and pharmacological agents that appear to act in part through activation of potassium channels (Fig. 2). We next summarize recent evidence suggesting that a wide variety of vasodilators act through activation of a common target, the adenosine 5′-triphosphate (ATP)-sensitive potassium (K_{ATP}) channel.

Table 1. *Effects of CTX and TEA^+ on membrane potential*

Blocker	Conditions	Depolarization (mV)
CTX (100 nM)	Myogenic tone	7 ± 1 (n = 11)
CTX (100 nM)	No myogenic tone (low pressure)	<1 (n = 2)
CTX (100 nM)	Nimodipine (minimal tone) (1 nM)	<1 (n = 4)
TEA^+ (1 mM)	Myogenic tone	4 ± 1 (n = 5)

Potassium channels closed by intracellular ATP were first identified in cardiac muscle by Noma in 1983 [12]. Since then they have been identified in pancreatic beta cells, skeletal muscle, neurons, and smooth muscle [1,13,14]. ATP-sensitive K^+ channels are blocked by the sulphonylurea drugs, such as tolbutamide and glibenclamide (also called glyburide), and by external barium, with a K_d for external barium ions of about 100 μM at −62 mV in skeletal muscle [15]. Block by the sulphonylureas appears to be specific [14]; tolbutamide and glibenclamide do not affect the inward rectifier in heart or skeletal muscle or the delayed rectifier potassium channel, the voltage-dependent calcium channel, or the calcium-activated potassium channel in beta cells. Glibenclamide also inhibits arterial smooth muscle ATP-sensitive potassium channels [13,16–18] and does not inhibit arterial smooth muscle Ca^{2+}-activated potassium channels [8].

A number of experiments have shown that the vascular actions of hyperpolarizing vasodilators are inhibited by known blockers of K_{ATP} channels (such as glibenclamide). For instance, ATP-sensitive potassium

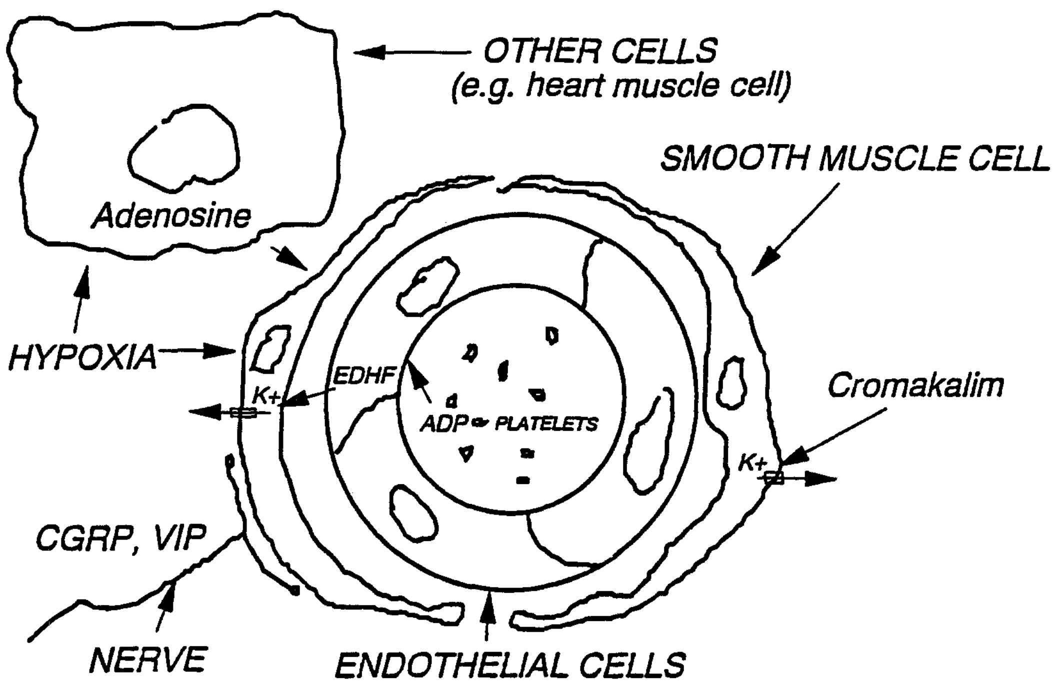

Fig. 2. *Proposed actions of hyperpolarizing vasodilators. This figure summarizes recent data that a number of endogenous substances from neurons (CGRP, VIP), from the endothelium (EDHF, release stimulated by ADP and ACh), from cardiac myocytes (adenosine), as well as synthetic substances (e.g., cromakalim) appear to act on a common target, namely the ATP-sensitive K^+ channel. (CGRP = calcitonin gene-related peptide; VIP = vasoactive intestinal peptide).*

channel blockers (glibenclamide, tolbutamide, and low concentrations (<100 μM) of barium reverse the vasorelaxing and hyperpolarizing actions of cromakalim, pinacidil, diazoxide, minoxidil sulfate, and RP 49356 in several vascular tissues [1,5,13,19]). In contrast, the blockers of small and large conductance Ca^{2+}-activated K^+ channels, apamin and charybdotoxin, respectively, appear to be without effect on relaxations produced by hyperpolarizing vasodilators [13,19]. TEA^+ at concentrations (0.5 mM) that would significantly block the Ca^{2+}-activated K^+ channel had no effect on vasorelaxation to cromakalim [5,8,13].

The antagonism of the effects of hyperpolarizing vasodilators by specific sulphonylurea blockers of ATP-sensitive K^+ channels, together with the pattern of action of other K^+ channel blockers, provides strong support for the idea that opening of ATP-senstitive K^+ channels forms a major route for the action of these hyperpolarizing vasodilators [5]. A functional action on Ca^{2+}-dependent K^+ channels is not supported, however, by reports of the lack of antagonism by specific blockers of these channels, apamin and charybdotoxin, outlined above, and by the lack of effect low [TEA^+]. Further, hyperpolarizing vasodilators such as cromakalim have no effect on single Ca^{2+}-activated potassium channels in membrane patches from several different types of smooth muscle [8,13,19].

In several tissues, hyperpolarizing vasodilators have been shown to activate ATP-sensitive K^+ channels directly. Diazoxide activates K_{ATP} channels in pancreatic beta cell lines [20]. In these studies activation by diazoxide occurs against a background of channel inhibition by ATP. Cromakalim activates ATP-sensitive K^+ current in cardiac myocytes [21,22]. The hyperpolarizing vasodilator RP 49356 activates single ATP-sensitive K^+ channels in inside-out patches from cardiac myocytes [22]. Pinacidil, another hyperpolarizing vasodilator, activates ATP-sensitive K^+ currents in cardiac muscle [23]. Recent evidence suggests that the K^+ channel openers RP49356, cromakalim [24], and pinacidil reduce the apparent affinity of ATP for inhibition of K_{ATP} channels in cardiac muscle.

ATP-sensitive K^+ channels have been identified in arterial smooth muscle [13,16,25]. Cromakalim activates single K_{ATP} channels in excised, inside-out patches from single smooth muscle cells of the mesenteric artery [13], in portal vein [16], and in planar lipid bilayers [25]. Glibenclamide inhibits single K_{ATP} channels in arteries [13,16]. Additional support for the existence for the K_{ATP} channels in arterial smooth muscle has come from potassium efflux in intact arteries and whole-cell current experiments. Metabolic poisons increased potassium efflux from mesenteric arteries [26]. This K^+ efflux could be blocked by glibenclamide and was most prominent in low external calcium, a condition that minimized K_{Ca} channel activity. Recently, Clapp and Gurney [17] demonstrated that ATP inhibits the glibenclamide-sensitive whole-cell potassium current in smooth muscle cells isolated from pulmonary arteries. These studies, in combination with those mentioned above, provide strong evidence for the existence of K_{ATP} channels in arterial smooth muscle function.

There appears to be substantial diversity in single-channel conductances of K_{ATP} channels in smooth muscle, with the values basically falling into two groups. Small conductance (10–30 pS in high K^+) K_{ATP} channels have been identified in the cell membranes of smooth muscle cells from portal vein [16] and urinary bladder [18]. Large conductance K_{ATP} channels (130 pS in high K^+) have been found in smooth muscle cells from mesenteric arteries [13] and canine aorta [25]. At present, the significance of this heterogeneity of single-channel conductances is unclear.

Acetylcholine (ACh) stimulates the secretion of one or more factors from the endothelium that cause vasodilation [27,28]. Endothelial-derived hyperpolarizing factor(s) (EDHF), which appears to be distinct from endothelial-derived relaxing factor (EDRF), hyperpolarizes and dilates arterial smooth muscle [2]. The precise mechanism of action of EDHF is not known; however, hyperpolarization of rabbit cerebral arteries to ACh is blocked by the ATP-sensitive potassium channel blockers, glibenclamide, and low concentrations of barium [13]. In addition, vasorelaxations to ACh are partially reversed by glibenclamide and low concentrations of barium [29]. Thus, it appears that an EDHF opens ATP-sensitive potassium channels.

A number of peptides are very potent vasodilators that appear to act directly on smooth muscle [30]. Vasoactive intestinal peptide (VIP) is a hypotensive agent in many vascular beds. Calcitonin gene-related peptide (CGRP) has been observed in perivascular nerves that distribute to a number of vascular beds and is a potent vasodilator [6,31]. VIP and CGRP both increase cAMP levels and cause sustained hyperpolarizations of cerebral arterial smooth muscle. VIP and CGRP appear to open K_{ATP} channels, since VIP- and CGRP-induced hyperpolarizations are blocked by glibenclamide and barium [6,13]. Further, CGRP activates single K^+ channels in on-cell patches of arterial smooth muscle [6].

A number of arteries (e.g., coronary arteries) dilate when the intravascular oxygen tension decreases. Since K_{ATP} channels open under conditions of compromised oxygen (e.g., during cardiac ischemia) or glucose (e.g., in pancreatic β cells) supply, K_{ATP} channels in arterial smooth muscle may play a role in hypoxia-induced vasodilation [1,13]. Hypoxic vasodilation of coronary arteries can be abolished by glibenclamide, a blocker of K_{ATP} channels, and can be mimicked by cromakalim and metabolic poisons, activators of K_{ATP} channels [32]. Activation of K^+ channels in arterial smooth muscle may be a cause of hypoxic and post-ischemic vasodilation in the heart, and K^+ channel openers could be useful drugs for the treatment of coronary heart disease.

Conclusions

K_{Ca} channels in the membranes of arterial smooth muscle cells respond to changes in intracellular calcium to regulate membrane potential. K_{Ca} channels appear to play a fundamental role in regulating the degree of intrinsic tone in resistance arteries. These channels help regulate arterial responses to pressure [10] (Fig. 1) and vasoconstrictors. Inhibition of K_{Ca} channels should contribute to vasoconstriction, whereas activation of K_{Ca} channels would tend to cause vasodilation. Defects in K_{Ca} channels could lead to or contribute to pathological conditions that are characterized by highly constricted arteries (vasospasm). K_{ATP} channels in smooth muscle cell membranes respond to the metabolic state of the cell. K_{ATP} channels appear to be the targets of variety of synthetic (diazoxide, cromakalim, pinacidil, nicorandil, monoxidil sulfate) and endogenous (CGRP, VIP, adenosine, EDHF) vasodilators (Fig. 2).

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References

1. Nelson MT, Patlak JB, Worley, JF, Standen NB. Calcium channels, potassium channels and the voltage dependence of arterial smooth muscle tone. *Am J Physiol* 1990;259: C3–C18.
2. Brayden JE, Wellman GC. Endothelium-dependent dilation of feline cerebral arteries: Role of membrane potential and cyclic nucleotides. *J Cereb Blood Flow Metab* 1989;9: 256–263.
3. Harder DR, Gilbert R, Lombard JH. Vascular muscle cell depolarization and activation in renal arteries on elevation of transmural pressure. *Am J Physiol* 1987;253:F778–F781.
4. Hirst GDS, Edwards FR. Sympathetic neuroeffector transmission in arteries and arterioles. *Physiol Rev* 1989;69: 546–604.
5. Quast U, Cook NS. Moving together: K^+ channel openers and ATP-sensitive K^+ channels. *Trends Pharmacol Sci* 1989;10:431–435.
6. Nelson MT, Huang Y, Brayden JE, et al. Activation of K^+ channels is involved in arterial dilations to calcitonin gene-related peptide. *Nature* 1990;344:770–773.
7. Blatz AL, Magleby KL. Calcium-activated potassium channels. *Trends Neurosci* 1987;10:463–467.
8. Langton PD, Nelson MT, Huang Y, Standen NB. Block of calcium-activated potassium channels in mammalian arterial myocytes by tetraethylammonium ions. *Am J Physiol* 1991;260:H927–H934.
9. Miller CE, Moczydlowski E, Latorre R, Phillips M. Charybdotoxin, a protein inhibitor of single Ca^{2+}-activated K^+ channels from mammalian skeletal muscle. *Nature* 1985;313:316–318.
10. Brayden JE, Nelson MT. Regulation of arterial tone by activation of calcium-dependent potassium channels. *Science* 1992;256:532–535.
11. Galvez A, Gimenez-Gallego G, Reuben JP, et al. 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* 1990;265:11083–11090.
12. Noma A. ATP-regulated K+ channels in cardiac muscle. *Nature* 1983;305:147–148.
13. Standen NB, Quayle JM, Davies NW, et al. Hyperpolarising vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. *Science* 1989;245:177–180.
14. Ashcroft SJH, Ashcroft FM. Properties and functions of ATP-sensitive K-channels. *Cell Sig* 1990;2:197–214.
15. Quayle JM, Standen NB, Stanfield PR. The voltage-dependent block of ATP-sensitive potassium channels of frog skeletal muscle by caesium and barium ions. *J Physiol* 1988;405:677–697.
16. Kajioka S, Kitamura K, Kuriyama H. Guanosine diphosphate activates an adenosine-5′-triphosphate-sensitive K^+ channel in the rabbit portal vein. *J Physiol* 1991;444: 397–418.
17. Clapp LH, Gurney AM. ATP-sensitive K^+ channels regulate resting potential of pulmonary arterial smooth muscle cells. *Am J Physiol* 1992;262:H916–H920.
18. Bonev A, Nelson MT. ATP-sensitive potassium channels in urinary bladder smooth muscle (abstr). *Gastroenterology* 1992; in press.
19. Winquist RJ, Heaney LA, Wallace AA, et al. Glyburide blocks the relaxation response to BRL 34915 (cromakalim), minoxidil sulfate and diazoxide in vascular smooth muscle. *J Pharm Exp Ther* 1989;248:149–156.
20. Trube G, Rorsman P, Ohno-Shosaku T. Opposite effects of tolbutamide and diazoxide on the ATP-dependent K^+ channel in mouse pancreatic β-cells. *Pflügers Arch* 1987;407: 493–499.
21. Sanguinetti MC, Scott AL, Zingaro GJ, Siegl PKS. BRL 34915 (cromakalim) activates ATP-sensitive K^+ current in cardiac muscle. *Proc Nat Acad Sci USA* 1988;85:8360–8364.
22. Escande D, Thuringer D, Leguern S, et al. Potassium channel openers act through an activation of ATP-sensitive K^+ channels in guinea-pig cardiac myocytes. *Pflügers Arch* 1989;414:669–675.
23. Arena JP, Kass RS. Enhancement of potassium-sensitive current in heart cells by pinacidil: Evidence for modulation of the ATP-sensitive potassium channel. *Circ Res* 1989;65: 436–445.
24. Ripoll C, Lederer WJ, Nichols CG. Cromakalim and RP49356 in modulation of ATP-sensitivity of cardiac K_{ATP} (abstr). *Biophy J* 1990;57:114.
25. Kovacs R, Nelson MT. ATP-sensitive K^+ channels from aortic smooth muscle incorporated into planar lipid bilayers. *Am J Physiol* 1991;261:H604–H609.
26. Post JM, Jones AW. Stimulation of arterial ^{42}K efflux by ATP depletion and cromakalim is antagonized by glyburide. *Am J Physiol* 1991;260:H848–H854.
27. Furchgott RF. Role of endothelium in responses of vascular smooth muscle. *Circ Res* 1983;53:557–573.
28. Taylor SG, Weston AH. Endothelium-derived hyperpolarizing factor: A new endogenous inhibitor from the vascular endothelium. *Trends Pharm Sci* 1988;9:272–274.
29. Brayden JE. Membrane hyperpolarization is a mechanism of endothelium-dependent cerebral vasodilation. *Am J Physiol* 1990;259:H668–H673.

30. Edvinsson L, Fredholm B, Hamel E, et al. 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* 1985;58:213–217.
31. Brain SD, Williams TJ, Tippins J, et al. Calcitonin gene-related peptide is a potent vasodilator. *Nature* 1985; 313:54–56.
32. Daut J, Maier-Rudolph W, von Beckerath N, et al. Hypoxic dilation of coronary arteries is mediated by ATP-sensitive potassium channels. *Science* 1990;247:1341–1344.