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Perspective

Potassium Channel Modulators: Scientific Applications and Therapeutic Promise

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The study of potassium ion channel biochemistry, physiology, pharmacology, and medicinal chemistry has fluorished in recent decades. The reasons for this are manifold: (1) ion channels, including sodium (Na), potassium (K), and calcium (Ca) channels, are present in all mammalian cells and play pivotal roles in the control of a variety of physiological processes; (2) because they are ubiquitous and are integrally involved in normal cellular homeostasis, it is probable that derangements in K channel function are involved in a variety of disease states; (3) our ability to probe and understand ion channel structure and function at the molecular level has increased dramatically with the development of sophisticated electrophysiologic techniques; and (4) a variety of synthetic and naturally derived K channel modulators have been discovered, enabling K channel function to be altered in defined ways.

We have determined the number of papers related to Na, K, and Ca channels that were cited in *Biological Abstracts* per year since 1970, and the data are plotted in Figure 1. Interest in ion channel research began increasing in the mid 1970s, and during this decade Na channels received the greatest attention. One of the reasons for their dominant role was the availability of clinically successful drugs that block Na channels, including the antiarrhythmics procainamide and lidocaine and the local anesthetic drugs procaine and xylocaine. In the early to mid 1980s selective Ca channel blockers such as diltiazem, nifedipine, and verapamil became available throughout the world for treating a variety of cardiovascular diseases, and this was accompanied by a nearly exponential rise in the per annum citations involving Ca channels. The K channel citations began increasing dramatically in 1984, and first exceeded those for Na channels in 1987. It is notable that the number of selective K channel modulators has increased substantially during the decade of the 1980s, and this probably helped stimulate interest in studying these channels. Moreover, the growth rate of interest in K channels should continue unabated as chemical probes become even more sophisticated and potent and as selective K channel modulators begin to reach the market.

Because of the burgeoning interest in K channels, our purpose in this Perspective will be to review the general properties of K channels and describe the medicinal chemistry and pharmacology of agents that selectively alter their function. Some of the literature has been recently summarized in review form.[1-3]

Potassium Channel Biology

Potassium ions are selectively concentrated in the interior of cells, and concentrations of K in the extra- and intracellular fluid are approximately 4 and 150 mM, respectively.[4] Potassium ions play a dominant role in controlling the resting membrane potential in most excitable cells and maintain the transmembrane voltage near the K equilibrium potential (E_k) of about -90 mV. During depolarization, Na ion influx causes the transmembrane potential to become more positive relative to E_k; repolarization causes the cell to return toward E_k (become more negative) and is mediated in large part by the efflux of K ions down their concentration and electrical gradients. Agents that block K channels tend to produce membrane depolarization; i.e., they shift the transmembrane potential in a positive direction away from E_k. Compounds which open K channels tend to produce membrane hyperpolar-

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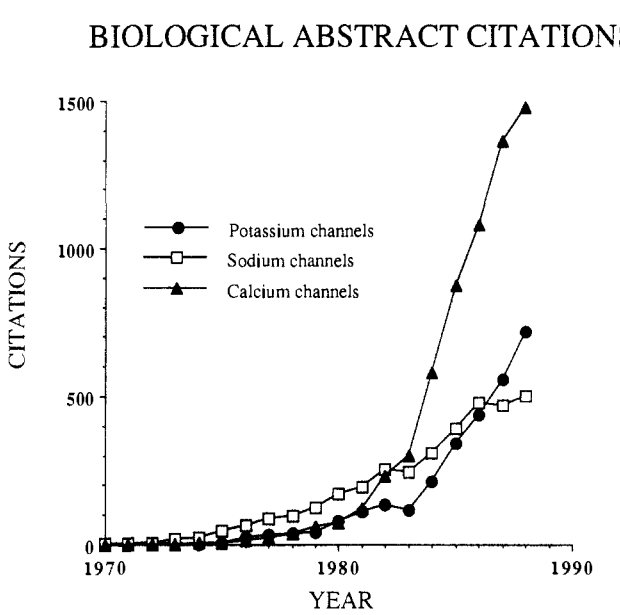

Figure 1. The number of citations in Biological Abstracts for the word channel adjacent to the terms K or potassium, Na or sodium, Ca or Calcium. The data for 1988 were compiled in June and July 1989, and repetitive analyses suggested that the 1988 entries were essentially complete.

ization and shift the resting membrane potential toward E_K.[5]

Potassium Channel Subtypes. Potassium channels are ubiquitous and exist as several subtypes, with multiple subtypes often being present on a single cell.[6,7] Control of these channels is regulated (often termed "gated") physiologically through several means, and K channels are thus often categorized according to their general gating mechanisms. One class of K channels are "G protein gated." A variety of neurotransmitters and hormones such as adenosine, serotonin (5HT), norepinephrine, growth hormone, and acetylcholine can exert their physiological effects by altering K channel function via activation of their specific receptors which are coupled to G proteins; these effects are sensitive to pertussis toxin.[8] For example, the well-characterized negative chronotropic and dromotropic effects of acetylcholine and adenosine on cardiac muscle are produced via opening of K channels following activation of their receptors.[9] A second class of K channels are primarily "voltage-gated." These K channels are opened and closed on the basis of alterations in the transmembrane voltage field. Three well-known subtypes of this class are the delayed rectifier, the inward rectifier, and the transient outward K channels. The delayed rectifier channel carries outward K currents with time constants on the order of hundreds of milliseconds. It is activated at depolarized potentials and is partially responsible for the late repolarization phase of the action potential in cardiac tissues. The transient outward channel carries rapidly activating and inactivating currents possibly responsible for the rapid repolarization phase in cardiac tissues. A third class of K channels are sometimes termed "ligand-gated". These K channels are modulated without G protein coupling by ions such as Ca, nucleotides such as ATP, or neurotransmitters such as 5HT. For example, vascular smooth muscle contains a variety of ligand-gated K channels, with a high conductance Ca-dependent K channel appearing to be particularly important, although low and intermediate conductance K channels are present also.[10-12] Recent discoveries involving ligand-gated K channels include a novel K channel in smooth and cardiac muscle cells that is opened by arachidonic acid and other fatty acids,[13,14] a high conductance Ca-activated K channel in vascular tissue that is activated by intracellular GMP,[15] and the possibility that the $5HT_3$ receptor is a K channel or is tightly coupled to K channels.[16] While K channels are often categorized according to these three gating mechanisms, other biochemical or physiological classifications have been described.[3]

Molecular and Structural Biology. Our understanding of the structure and function of K channels has advanced greatly due to application of recombinant DNA techniques. Most work has centered on the so-called "*Shaker*" gene complex in *Drosophila* that encodes a family of K channels resembling transient outward K channels in excitable tissues. These channels have been cloned, expressed, and studied functionally in *Xenopus* oocytes.[17-19] More recently, a rat hippocampal K channel protein that is highly homologous to *Shaker* K channels was cloned. When expressed in *Xenopus* oocytes, this rat K channel protein of 495 amino acids (54.6 kDa) behaved electrophysiologically and pharmacologically like the transient outward K channel.[20] With use of a *Shaker* cDNA K channel probe, a homologous cDNA from rat cerebral cortices was isolated and expressed in oocytes. The time courses of the K currents mediated by these channels and their pharmacological behavior suggest that they closely resemble delayed rectifier K channels in rat neuronal tissues.[21] Another K channel gene has been expressed in *Xenopus* oocytes which also has the characteristics of delayed rectifier K channels, but it is derived from the *Drosophila Shab* gene complex, rather than the *Shaker* complex.[22] With use of the polymerase chain reaction, six K channel gene sequences have been isolated from humans, suggesting that K channel gene diversity will be as extensive in man as it is in animals.[23]

The regulation of K channels is also being studied at the molecular level. Opening of Ca-dependent K channels in smooth muscle cells isolated from rabbit trachea was

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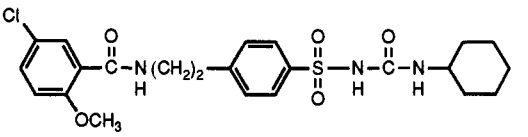
shown to be dependent upon phosphorylation,[24] and it will be of interest to determine if phosphorylation/dephosphorylation is a general physiological motif used for regulation of K channel function. A specific G protein known as G_k, complexed with GTP, appears to mediate the increase in K conductance induced by various chemical signals acting on G protein coupled K channels. To what extent different subunits (α, β/γ) of the G protein/GTP complex are involved in signal transduction is an area of intense investigation.[13,25,26] Recombinantly derived α subunits of the G_i protein have been shown to activate pertussis toxin sensitive muscarinic K channels in cardiac tissues, albeit with less affinity than native α subunits.[27] The β/γ subunit can also activate a cardiac muscarinic K channel via end products of the PLA_2–lipoxygenase pathway, including 5-HPETE, 12-HETE, LTB_4, and LTC_4; this pathway is not sensitive to pertussis toxin.[28]

These molecular biology studies to date suggest that K channels have a conserved core domain and that differences in function derive from alterations in amino acids close to the 3′- and 5′-ends of the protein. Such core areas may become useful in identification of other, as yet unknown, K channel proteins. There is considerable homology between K and Na channels and among K channels derived from different species. As is occurring in most other fields, the number of structural varients of K channels will undoubtedly increase as recombinant DNA techniques are used to probe them, and it will be imperative to determine if these mutants are *functionally* distinct from known classes of K channels using electrophysiologic and pharmacologic criteria. The nomenclature and classification of K channels need to be further codified, with predetermined biochemical, pharmacological, and structural criteria. This will be particularly important as new K channel subtypes are discovered.

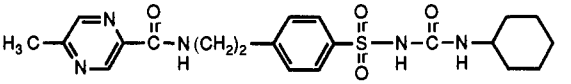
Potassium Channel Antagonists

Naturally Occurring Toxins. Several animal-derived toxins have been discovered and characterized that block certain subtypes of K channels.[29] Transient outward K channels are blocked selectively by the snake venom peptide dendrotoxin.[30] Small-conductance Ca-activated K channels in smooth muscle, neuroblastoma cells, and hepatocytes are blocked by the bee venom peptide apamin.[31] However, in guinea pig ventricular papillary muscles, apamin shortens APD and hyperpolarizes resting cell membrane potential, an effect that would accompany *opening* (vide infra) of K channels.[32] Finally, high-conductance, Ca-activated K channels (sometimes called "maxi" K channels) are antagonized by charybdotoxin, a compound isolated from scorpion venom. The sequence of charybdotoxin has been elucidated, and in radiolabeled form it has been used as a biochemical probe of K channels.[33]

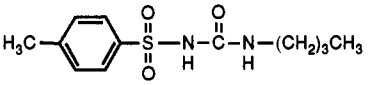
Chart I. Antagonists of ATP-Dependent K Channels



Glyburide (1)



Glipizide (2)



Tolbutamide (3)

Synthetic Compounds. Numerous nonselective antagonists have been important in the characterization of K channels, and two of the more widely studied compounds are 4-aminopyridine and 3,4-diaminopyridine.[6] These compounds are often said to block the transient outward K current, but they are impotent and lack selectivity so that results using them must be interpreted cautiously. The tetraethylammonium ion (TEA) is widely utilized as a K channel blocker for mechanistic studies.[6] This compound is used as a probe of the delayed-rectifier K channel and the ATP-inhibited channel,[34] but like the aminopyridines, it lacks both potency and specificity. In addition to their poor selectivity among K channel subtypes, both TEA and 4-aminopyridine block receptors for a wide variety of neurotransmitters, including muscarinic, D_2, α_1, α_2, $5HT_{1A}$, and $5HT_2$ receptors.[35]

A variety of marketed drugs, whose mechanisms of actions are still being elucidated, appear to function as relatively selective K channel blockers. Among the best-characterized are the antidiabetic sulfonylureas,[36] and the structures of some of the more important members of this class are shown in Chart I. In the physiologic control of pancreatic β-cell function, increases in intracellular glucose or amino acids elevate ATP concentrations and produce closure of ATP-sensitive K channels, leading to membrane depolarization. This enhances the opening of voltage-dependent Ca channels, and the resulting increases in intracellular Ca concentrations lead to exocytotic secretion of insulin.[37] Antidiabetic sulfonylureas appear to stimulate insulin secretion by blocking the pancreatic ATP-sensitive K channel,[38] and these agents, in radiolabeled form, bind avidly and specifically to pancreatic insulinoma

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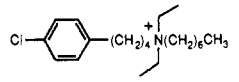
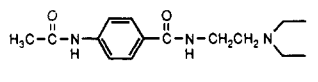
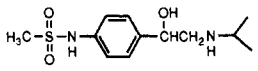
Chart II. Examples of Class 3 Antiarrhythmic Drugs



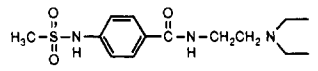
Clofilium (4)



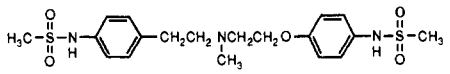
NAPA (5)



Solalol (6)



Sematilide (7)



UK-68798 (8)

cells.[39] Moreover, the abilities of tolbutamide, meglitinide, glipizide, glyburide, and related compounds to stimulate insulin release, block potassium efflux, and to bind to islet cell membranes were highly correlated.[40,41] This mechanistic pathway for the pharmacological effects of sulfonylureas has been confirmed in human islet cells,[42] and now that their mechanism of action is better understood, the antidiabetic sulfonylureas have become invaluable probes of K channels in both β-cells and nonpancreatic cells.

Therapeutic Applications. A proven medical use of K channel antagonists is treatment of type II diabetes using sulfonylureas, and these widely utilized compounds are relatively safe and effective. The sulfonylureas alter K channel flux in a variety of nonpancreatic tissues, and it will be of interest to determine if they can be exploited in additional diseases.

Selective K channel blockers are showing promise as antiarrhythmic agents. The older class 1 antiarrhythmic drugs such as lidocaine, procainamide, phenytoin, etc., function by blocking Na channels and thereby stabilizing cell membranes.[43] However, the clinical utility of class 1 antiarrhythmic drugs, particularly the potent class 1C agents such as flecainide and encainide, has been called into question. Recently reported results from the cardiac arrhythmia suppression trial (CAST) suggest that treatment of mild postmyocardial infarction arrhythmias with class 1C drugs leads to a significantly larger risk of death from arrhythmias or cardiac arrest, compared to placebo control.[44] While certain aspects of the study design have been criticized severely, results of the CAST study will undoubtedly stimulate interest in mechanistically distinct antiarrhythmic drugs, including K channel modulators.

The potency of TEA as a K channel blocker was markedly enhanced via structure–activity relationship (SAR) studies, and one of the resulting analogues, clofilium (Chart II), was the first specifically designed selective class 3 antiarrhythmic.[45] This drug blocks cardiac delayed rectifier K channels and thus lengthens the APD of cardiac cells in a selective manner.[46] Class 3 antiarrhythmics have been extensively studied in experimental animals, and some have been studied in man.[47] In contrast to class 1 agents, which are cardiac depressants and may increase the threshold to defibrillation, class 3 drugs do not depress cardiac function and may actually decrease the threshold to defibrillation.[48]

N-Acetylprocainamide (NAPA) is an electrophysiologically active metabolite of procainamide, and in contrast to procainamide it is a selective, albeit impotent, class 3 antiarrhythmic drug. Exogenously prepared NAPA has been studied clinically and may be effective in treating atrial flutter.[49] Sotalol, a well-studied β-blocker, is a mixed class 2/3 antiarrhythmic, and the class 3 effects appear to reside in the *d* stereoisomer, the enantiomer which does not interact with β receptors. Both *d,l*- and *d*-sotalol have been studied extensively in man as antiarrhythmics. In recently reported clinical studies, orally administered *d*-sotalol was highly effective in suppressing refractory supraventricular tachyarrhythmias[50] and appeared to have efficacy in treating life-threatening, refractory ventricular arrhythmias.[51] Sematilide, a K channel blocker in early clinical trials as an antiarrhythmic, is a hybrid of sotalol and NAPA.[52] UK-68,798 is a potent class 3 antiarrhythmic and K channel blocker in clinical development. This well-designed drug is an antagonist of the delayed rectifier K channel and increases canine Purkinje fiber APD at concentrations as low as 5×10^{-9} M. It is well-absorbed in man and has an elimination half-life of 9.5 h.[53] Finally, two additional class 3 antiarrhythmics have been described recently. E-4031 is a benzenesulfonamide derivative which blocks a cardiac K channel resembling, but not identical with, the delayed rectifier.[54] The benzopyran derivative RP 58866 prolongs APD by specifically blocking the inward rectifying K channel.[55]

In conditions of cardiac hypoxia, K channels appear to open, leading to efflux of K ions and sometimes dramatic

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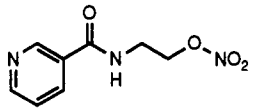
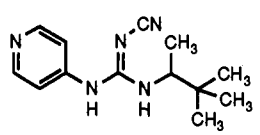
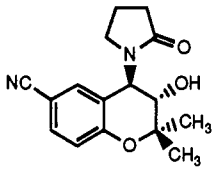
Chart III. Prototypical Potassium Channel Openers



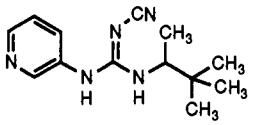
Nicorandil (**9**)



Pinacidil (**10**)



Cromakalim (**11**)



LY222675 (**12**)

shortening of APD.[56,57] The ATP-inhibited K channel seems to be the primary mediator of these electrophysiological effects.[58] Moreover, in rat atrial cells, a non-ATP-dependent K channel which opens when exposed to arachidonic acid and certain phospholipids has been identified, and these channels may be involved in ischemia-induced K efflux.[59] Antidiabetic sulfonylureas, by blocking cardiac ATP-dependent K channels, block some hypoxia-induced electrophysiologic abnormalities.[60] For example, glyburide reverses the hypoxia-induced shortening of APD in ferret and guinea pig papillary muscles.[61] Decreases in time to ventricular fibrillation produced by global ischemia of isolated rat hearts was attenuated by glyburide.[62] In vivo, rabbit monophasic APDs were reduced by acute LAD occlusion, an effect prevented by glyburide.[63] Since arrhythmias are hallmarks of acute myocardial infarction, selective K channel blockers may have a role in treating acute infarctions in man.

Potassium Channel Openers

One of the reasons for the increased interest in K channels was the discovery of compounds which potently and selectively "open" potassium channels. Three compounds have figured prominantly in the discovery and characterization of this class of compounds: nicorandil, pinacidil, and cromakalim (Chart III). Nicorandil is a nitrate-containing nicotinamide derivative, designed to activate guanylate cyclase and produce vasodilation via the resulting increases in intracellular cGMP concentrations. In early studies, the drug was shown to also produce cardiac membrane hyperpolarization by increasing K permeability, suggesting that two mechanisms are involved in the action of this drug.[64,65] Nicorandil has been extensively studied in animals and man, and its pharmacology has been reviewed recently.[66]

Pinacidil and cromakalim (BRL-34915) were discovered and developed as potent "nonspecific" vasodilators. Weston and co-workers demonstrated that cromakalim, like nicorandil, produced membrane hyperpolarization, suggesting a similar mechanism involving opening of K channels. Evidence for alterations in K flux in vascular tissue can be demonstrated by preloading blood vessels with ^{86}Rb (a potassium surrogate) or ^{42}K, and agents that selectively increase K ion permeability lead to enhanced efflux of these radioactive tracers from vascular tissue. In pivotal studies Weston and colleagues demonstrated that cromakalim stimulated ^{86}Rb efflux from rat portal veins.[67,68] Through use of similar techniques, pinacidil was also demonstrated to relax blood vessels via stimulation of K ion efflux and membrane hyperpolarization, and the phrase "K channel opener" was applied to this general class of drugs.[69-71] Although the terms K channel activator or agonist are used sometimes, K channel opener is preferred by many workers; activator implies an opening of inactivated K channels, whereas these drugs may inhibit channel inactivation, thereby keeping the channels permeable to K ions for a longer time frame.[72]

Cromakalim relaxes vascular smooth muscle or inhibits its spontaneous activity at concentrations below those required to stimulate ^{86}Rb efflux, an enigmatic result if the drug exerts its effects predominantly via K channel opening. However, in various tissues cromakalim was more effective at stimulating ^{42}K than ^{86}Rb efflux, suggesting that use of ^{42}K as a tracer minimizes, but does not eliminate, the discrepancy.[73,74] Similar data have appeared with pinacidil.[70,75] Of interest is the finding that in some tissues, such as the guinea pig bladder, only K but not Rb efflux is stimulated by cromakalim.[76] Morever, in strips of isolated rat uterus, cromakalim relaxes contractions induced by oxytocin in the absence of demonstrable increases in ^{42}K flux, suggesting that the drug may have a selective action on pacemaker K channels.[77] Despite the apparently slower kinetics of Rb versus K efflux, ^{86}Rb is probably a useful qualitative marker for effects of K channel openers since classical nitrate-containing vasodilators are generally inactive in stimulating K efflux.[78]

Mechanistic and Pharmacological Studies. As previously described, increases in membrane K permea-

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bility induced by K channel openers cause the transmembrane potential to become more negative (toward E_k). This "hyperpolarization" in vascular smooth muscle leads to vasorelaxation since the cells become resistant to the depolarizing influences of vasoconstrictor neurotransmitters or hormones. The prototypic K channel openers do not interact with cholinergic, histaminergic, serotonergic, or adrenergic receptors, nor do they block Ca channels.[79-83] Moreover, elevation of intracellular cyclic nucleotide concentrations or release of endogenous vasodilators are not involved in their mechanism of action.[81,84,85] These data, taken together with the effects of the drugs on ^{86}Rb efflux and membrane hyperpolarization, strongly suggest that these compounds act as selective K channel openers.

Although they stimulate K efflux, the subtype of vascular smooth muscle K channels affected by these drugs has not been conclusively established. Using patch-clamp recording of whole cell currents from rat veins, some investigators have concluded that pinacidil causes vasodilation by opening the high conductance (200 pS) Ca-dependent K channel;[86] similar data have appeared with cromakalim.[87] These Ca-dependent K channels have been incorporated into planar lipid bilayers, and a low concentration of cromakalim (50 nM) increased their open probability; moreover, cromakalim shifts the voltage/activation relationship in a hyperpolarized direction.[88] Normally these channels are closed at resting potential, but this voltage shift, coupled with their high density and unit conductance, suggest that they might contribute to the pharmacological effects of K channel openers.[88] It would be of great interest to determine if the enantiomers of pinacidil or cromakalim (vide infra) demonstrate stereospecificity in opening this class of K channels; this would provide greater evidence as to the pharmacological relevance of this electrophysiologic phenomenon.

Numerous K channel blockers have been used in attempts to antagonize effects of K channel openers and to determine the subtype of K channels most affected by these drugs. TEA and procainamide produce concentration-dependent antagonism of pinacidil's effects in the rat portal vein, but these data do not reveal the K channel subtype involved because of the aforementioned poor specificity of these K channel antagonists.[89] However, apamin or charybdotoxin do not block effects of K channel openers, suggesting that neither the small-conductance nor the high-conductance Ca-dependent K channel is the target for vasoactive K channel openers.[89,90] Some electrophysiologic evidence suggests that this class of vasodilators acts via a Ca-independent K current.[91] Moreover, a variety of pharmacological data indicates that pinacidil and cromakalim relax vascular smooth muscle through a K channel resembling the ATP-dependent channel present in pancreatic β-cells and cardiac tissue. In portal vein and aortic tissues isolated from rats, glyburide was able to inhibit pharmacological effects of cromakalim in a concentration-dependent fashion,[92] but this K channel blocker did not antagonize the vasorelaxant effects of diltiazem or nitrendipine.[79,90] In conscious rats, the hypotensive effects of cromakalim could be dose-dependently blocked by iv administration of glyburide or glipizide.[79,93] The abilities of the antidiabetic sulfonylureas to inhibit the actions of diazoxide, RP-49356, minoxidil sulfate, cromakalim, and pinacidil have been noted independently by other laboratories.[90,94-97] In addition to vascular tissue, effects of K channel openers on other smooth muscle, such as rabbit vas deferens, can be modulated by sulfonylureas.[97]

This compelling body of data, that sulfonylureas can antagonize the vasorelaxant effects of K channel openers, suggests that at least a portion of their vascular effects are mediated through previously unrecognized ATP-dependent K channels resembling those found in pancreatic tissue; electrophysiological evidence for the existence of such channels in vascular smooth muscle at the single-channel level has been described recently.[98] If these data are confirmed, they may have important implications regarding control of resting membrane potential in blood vessels and the mechanism of action of K channel openers. Thus, the precise K channel(s) mediating the vascular effects of cromakalim, pinacidil, or other K channel openers has not been established with any degree of certainty and will be the subject of intense investigation over the next several years. Moreover, it is worth noting that even in completely depolarized tissues (127 mM K) pinacidil can still dose-dependently relax vascular smooth muscle, suggesting yet additional mechanisms of action.[99]

Regardless of their subcellular mechanism(s) of action, cromakalim and pinacidil potently relax smooth muscle derived from virtually all vascular beds and block the effects of a variety of spasmogens, including endothelin, angiotensin II, phenylephrine, thromboxane A_2, and serotonin.[81,100] Both the phasic[91] and the sustained phase[101] of norepinephrine contractions are antagonized by cromakalim in isolated rabbit blood vessels. In pithed rats, K channel openers inhibit the pressure effects of angiotensin II, but they are less effective as antagonists of cira-

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zoline-, UK-14,304-, or norepinephrine-induced pressor responses.[102] One of the hallmarks of K channel openers is their ability to block contractions induced by K concentrations less than 20–30 mM, but not by K concentrations in excess of 80 mM. At higher K concentrations membrane potential and E_k are nearly identical, rendering K channel openers ineffective. Moreover, at high K concentrations voltage-operated Ca channels are opened, leading to an influx of Ca that can be blocked only with Ca channel antagonists.[91,103,104]

Potassium channel openers and Ca channel blockers both produce vasodilation via reductions in intracellular Ca ions,[84] but the means by which this occurs are fundamentally different. Rather than blocking the voltage-dependent Ca channel, K channel openers, via membrane hyperpolarization, inhibit the influx of Ca through receptor-operated channels and possibly inhibit release of Ca from intracellular stores.[101,105,106] This mechanistic difference is the probable reason why K channel openers relax smooth muscle contracted with low K but not high K concentrations, whereas nifedipine relaxes both. Cromakalim has been extensively compared to nifedipine in antihypertensive models, and the former drug was 10–30 times more potent in spontaneously hypertensive rats and in renal hypertensive cats and dogs.[107] Nifedipine and cromakalim were equipotent after iv administration to spontaneously hypertensive and normotensive rats (ED_{30} values were 207 and 182 μg/kg, respectively), but in contrast, cromakalim was 8.8 times more potent in spontaneously hypertensive than in normotensive rats (ED_{30} values were 13.8 and 123 μg/kg, respectively).[108] Thus, in addition to being considerably more potent than nifedipine in hypertensive rats, effects of cromakalim seemed to be dependent on the intrinsic vascular tone of the animal.

Aside from potency considerations, there are qualitative differences between cromakalim and nifedipine regarding regional blood vessel selectivity. For example, in anesthetized cats and rabbits cromakalim appears to relax renal and gastrointestinal blood vessels and has a less pronounced effect on vascular beds in skeletal muscle.[109] In contrast nifedipine causes robust relaxation of skeletal muscle blood vessels.[110]

Potassium channel openers relax isolated human arteries and veins, and the selectivity of cromakalim for different vascular beds has been studied in healthy human volunteers. The drug was infused directly into the brachial artery (located in the forearm) and into norepinephrine-constricted dorsal veins (located in the hand). Cromakalim dose-dependently dilated the brachial artery, but was without effect in the vein; the responsivity of the dorsal vein was examined by infusion of nitroglycerin, and pronounced vasodilation resulted. This arterioselective vasodilation of cromakalim may be due to differences in the physiology of arteries and veins.[111] The arteriolar selectivity of cromakalim in man has been confirmed by additional investigators in both normal human subjects and in patients with ischemic heart disease.[112,113] It will be important for these studies to be repeated with different K channel openers and surveying various vascular beds so that definitive conclusions can be reached.

Chemistry and SAR. The bioisosteric equivalence of thioureas and cyanoquanidines was conclusively demonstrated with the seminal cimetidine work of Ganellin and co-workers.[114] Pinacidil was discovered by independently converting a series of thiourea vasodilators to the corresponding cyanoquanidines, followed by systematic variation of the branched alkyl moiety.[115] Although the early SAR was conducted with whole-animal antihypertensive end points, many of the conclusions have been confirmed using in vitro methods for determining K channel opening potencies of the analogues.[116,117] The two stereoisomers of pinacidil have been examined, and the *l* enantiomer is the more potent. Eudismic ratios of approximately 22 for the enantiomers of pinacidil have been described in isolated canine cephalic veins, in rat vascular beds, and in ^{86}Rb efflux experiments by multiple laboratories.[89,94,95]

The disposition of pinacidil has been examined in man, and the metabolic fate and rate of elimination of the two enantiomers is comparable. Pinacidil appears to be inactivated[118] by metabolism to the *N*-oxide derivative, but the enzyme which catalyzes this transformation is different from the well-characterized oxidases.[119,120] Although this metabolite is considerably less potent than pinacidil, it appears to have a slightly longer half-life than parent drug and could accumulate upon chronic dosing, particularly in the elderly or patients with impaired renal function.[121] Under these conditions, plasma concentrations of the metabolite may be sufficiently high to be pharmacologically meaningful.

The 3-pyridyl isomer of pinacidil (LY222675, Chart III) has been examined,[94,122] and it was comparable to racemic pinacidil in relaxing phenylephrine-constricted canine cephalic veins (EC_{50} values were 0.56 and 0.76 μM, respectively). However, 3-pyridyl isomer 12 was over 6 times more potent than pinacidil in shortening APD of isolated canine Purkinje fibers (EC_{50} values were 0.50 and 3.00 μM, respectively). Whether this increased potency of 12 on Purkinje fibers represents differences between K channels in the heart and blood vessels, or physicochemical dif-

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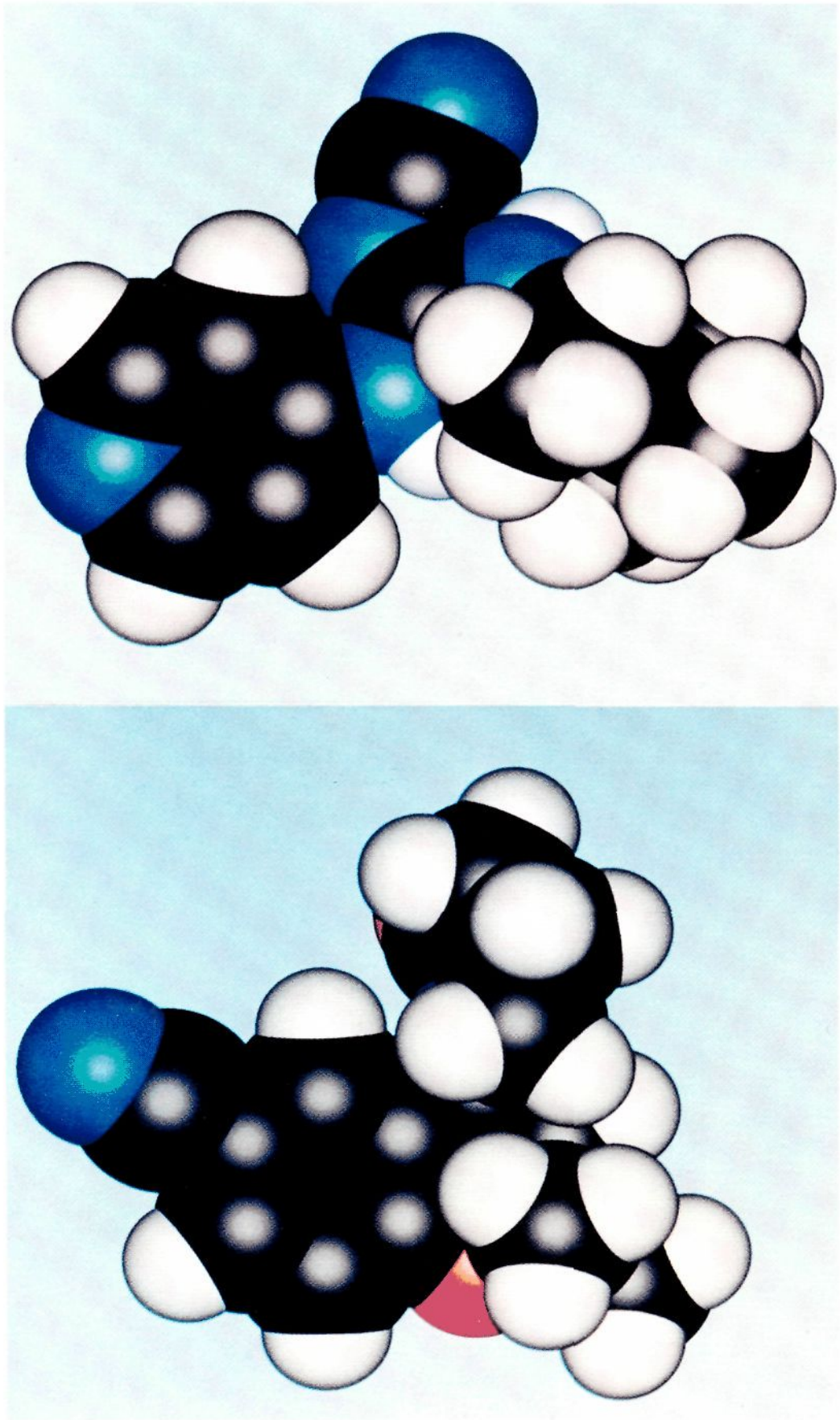

Figure 2. Energy-minimized conformations of cromakalim (lower panel) and pinacidil (upper panel). Calculations were performed with the software program MacroModel and displayed using an Evans and Sutherland PS-390 terminal. The resulting conformation of cromakalim is essentially indistinguishable from that reported by Cassidy et al., in ref 128.

ferences between the two drugs, is not known. The two enantiomers of **12** were prepared, and the eudismic ratio was 71 in veins and 53 in Purkinje fibers, with the (−)-enantiomer being more potent.[95,122]

The antihypertensive SAR of cromakalim has been elegantly explored by Evans and co-workers.[123–125] The lactam carbonyl is pivotal, and its removal leads to a 10-fold decrease in potency; the thiocarbonyl isostere is equipotent, but it has a shorter duration of action in vivo. The presence of geminal methyl substituents at position 2 is also important. Until recently, the presence of a nitrile or other powerful electron-withdrawing subsitutents at position 6 was considered important, but the 6-ethyl derivative surprisingly retains about $^1/_3$ of the potency of cromakalim, suggesting that the σ value of the substituent is not crucial.[126] The pyrrolidinone ring of cromakalim may be replaced with acyclic amides while potent antihypertensive activity is retained.[127] The conformation of cromakalim has been studied with X-ray crystallography

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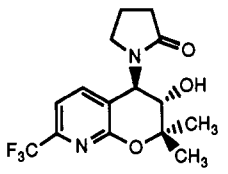
Chart IV. Cromakalim Analogues



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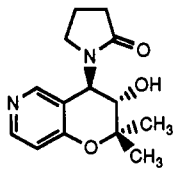

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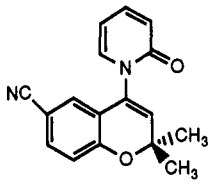

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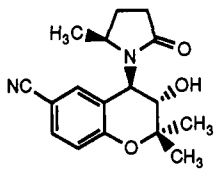

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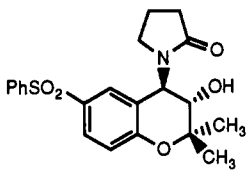

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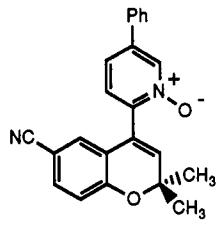
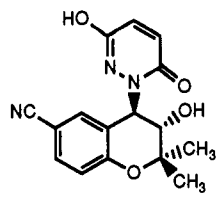
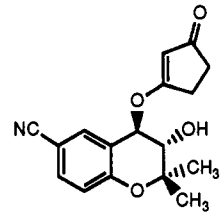


(18)

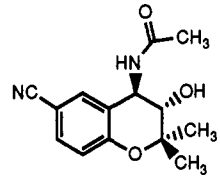
Chart V. Additional Cromakalim Analogues



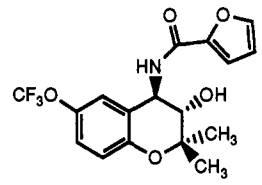
(19)



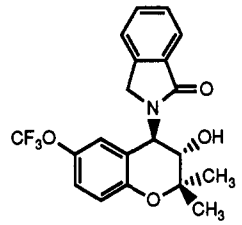
(20)



(21)



(22)



(23)

and NMR techniques. In both the solid phase and in solution, the pyrrolidinone ring is oriented orthogonally to the plane defined by the benzopyran nucleus, and the lactam carbonyl is on the same side of the benzopyran ring as the C-3 hydroxy group.[128] The energy-minimized conformations of cromakalim and pinacidil are depicted in Figure 2.

The enantiomers of cromakalim have been compared in vivo and in vitro, and the (−)-enantiomer is approximately 100–200 times more potent than the (+)-enantiomer as a K channel opener.[109,125] The absolute configuration of (−)-cromakalim has been reported and is 3*S*,4*R*, as depicted in Chart III.[126] The pharmacokinetics of the individual enantiomers of cromakalim in man have been studied.[129]

In the late 1970s and the 1980s, nifedipine served as the prototype of Ca channel blockers, and a tremendous number of nifedipine mimics appeared in the patent and scientific literature. Similarly, cromakalim is stimulating an enormous synthetic effort, and representative examples from the literature are depicted in Charts IV and V. Compound **13**, a pyridine isostere of cromakalim, appears to offer no advantage relative to the parent drug, and homologation of the geminal methyl substituents into a spirocyclopentane ring produces a decrease in potency, suggesting steric intolerance at this position of the molecule.[130] Evans and co-workers have prepared all four regioisomers of these pyranopyridines, and [3,2-*c*] isomer **14** is substantially more potent than the other three regioisomers.[131,132]

Compound **15** (EMD 52,692) demonstrates that homologation and aromatization of the pyrrolidinone ring of cromakalim and dehydration of the alcohol lead to maintenance of potency.[133] In dogs subjected to acute coronary artery occlusion, **15** increased blood flow to collateral-dependent myocardium, suggesting that K channel openers may be effective in treating myocardial ischemia.[134] Placement of a methyl substituent in the pyrrolidinone ring of cromakalim and manipulation of its stereochemistry have been claimed to alter the tissue selectivity of the drug. The (−)-3*R*,4*S*,5′*R* diastereomer of **16**, or its corresponding racemate, were reported to have a more pronounced effect on urinary tract smooth muscle than cromakalim, whereas hypotensive effects were produced by the 3*S*,4*R*,5′*R* diastereomer.[135] Compound **17** was slightly more potent than cromakalim in a guinea pig Langendorff heart preparation and demonstrates that the bulky lipophilic phenylsulfonyl moiety can be used to replace the C-6 nitrile.[136] Pyridine *N*-oxide derivative **18** is a potent K channel opener, and this molecule suggests that carbon–carbon bonds are acceptable at C-4 and that the *N*-oxide is a suitable surrogate for the lactam carbonyl of cromakalim; the desphenyl analogue, designated Ro-31-6930, was 10-fold more potent than cromakalim in relaxing guinea pig trachea and is in development.[137] Compounds **19** and **20** (Chart V) demonstrate the wide range of groups that may be present at

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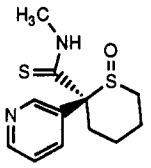
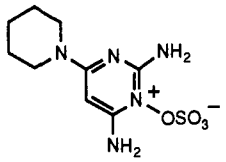
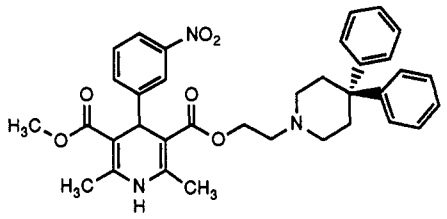
Chart VI. Structurally Distinct Potassium Channel Openers



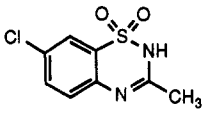
RP-49356 (**24**)



Minoxidil sulfate (**25**)



Niguldipine (**26**)



Diazoxide (**27**)

C-4 of the benzopyran ring,[138,139] and analogue **19** (SR-44994) was reported to be over 10-fold more potent that cromakalim as an inhibitor of the spontaneous contractions of isolated rat portal veins.[138] As previously described, ring-opened analogues of cromakalim such as **21** retain their ability to open K channels,[127] and the furanoyl derivative **22**, bearing a trifluoromethoxy group in place of cromakalim's nitrile, is a potent K channel opener.[140] Finally, a benzene ring may be fused onto the lactam of cromakalim (**23**).[140]

A variety of other structural classes of K channel openers have appeared, and one of the more potent examples is RP-49356 (Chart VI). This drug is essentially equipotent with chromakalim and is effective as a hypotensive agent in rats, dogs, and cats.[97,102] The vasoactive sulfate metabolite (**25**) of minoxidil is reported to be a potent K channel opener,[141] as is the vasodilator diazoxide.[142] Diazoxide opens ATP-dependent K channels in β-cells, leading to insulin secretion, and this effect may be mediated by protein phosphorylation.[143] With patch-clamp techniques in rat insulinoma cells, diazoxide, but not cromakalim, was found to open K channels,[144] and these differences have been confirmed in whole animal studies.[92] Diazoxide is also much more potent than pinacidil in opening pancreatic ATP-dependent K channels.[145] (+)-Niguldipine, developed originally as a long-acting nicardipine-like Ca channel blocker, also increases the open probability of Ca-activated K channels and may be an interesting example of a compound acting in opposite fashions on two distinct ion channels. Both the Ca channel blocking and the K channel opening actions of the drug manifest stereoselectivity. For example, in electrophysiological studies the (+)-enantiomer of niguldipine was a K channel opener, whereas the (−)-enantiomer behaved as a K channel blocker.[87] These purported K channel openers have been characterized much less completely than nicorandil, cromakalim, and pinacidil, and further study is warranted.

Possible Therapeutic Applications of K Channel Openers. Because of the prominent physiological significance of K channels, K channel openers have important pharmacological effects on a variety of tissues, including smooth and cardiac muscle. In this section we will provide an overview of some of the more logical therapeutic targets. However, well-controlled clinical studies have been reported *only* for hypertension, and considerable experimental work remains before the validity of other indications can be determined.

Hypertension. Extensive clinical studies demonstrated pinacidil is an effective antihypertensive drug. In a series of double-blind comparator trials, pinacidil was more effective than placebo and somewhat more effective than prazosin or hydralazine. Mild-to-moderate hypertension can be treated with 12.5 mg of pinacidil alone or 25 mg of pinacidil plus 12.5 mg of hydrochlorothiazide, given twice a day. The most prominent side-effects are those typical of direct-acting vasodilators, including reflex tachycardia, headache, flushing, and edema.[146] Coadministration with either β-blockers or diuretics provided additive antihypertensive efficacy and attenuated some of the side effects produced by pinacidil monotherapy.[147] The pharmacokinetics of pinacidil in hypertensive patients has been extensively studied.[148]

In man, cromakalim dose-dependently decreases blood pressure and appears to be a well-tolerated antihypertensive.[1] Administration of 0.75 mg/day of cromakalim to hypertensive patients led to a reduction of diastolic and systolic blood pressure, and the only side effects appeared to be reflex tachycardia and headache.[149] In *normal* subjects, the drug may activate the renin–angiotensin–aldosterone and noradrenergic systems, and these neuroendocrine changes could be due to a direct effect on kidney juxtaglomerular cells.[150] However, hypertensive subjects treated with 1.5 mg/day of cromakalim experienced a significant reduction in systolic and diastolic blood pressure, with no untoward neuroendocrine effects.[151] Cromakalim was initially developed as the racemate, but development of the racemate for chronic treatment of hypertension has been suspended in favor of the more active levorotatory enantiomer BRL-38227.[152]

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From reports to date, K channel openers appear to be at least as effective as other vasodilators in treating hypertension, including Ca channel antagonists. In contrast to some antihypertensive medications, pinacidil does not adversely affect the serum lipid profile and may increase the HDL/LDL ratio, although coadministration of a diuretic could attenuate this beneficial effect on serum cholesterol. Importantly, the drug does not appear to activate untoward neuroendocrine changes[146,153,154] and may decrease serum aldosterone concentrations by direct inhibition of aldosterone secretion at the level of the glomerulosa cells.[155] Compared to nifedipine, pinacidil appeared to be more effective in reversing left ventricular hypertrophy and improving left ventricular diastolic function.[156] However, whether the mechanism of K channel opening confers advantages or disadvantages relative to other vasodilators must await careful, double-blind comparisons among these agents. Because of the previously reviewed mechanistic and pharmacologic differences between K channel openers and Ca channel blockers, there is reason to believe K channel openers will find a useful niche in treatment of hypertension.

Asthma. Potassium channels are important in mediating airway smooth muscle tone. In addition to the well-known effects of β-agonists to elevate cAMP and decrease intracellular Ca, these drugs also act via protein kinase A to open Ca-dependent K channels, thereby producing membrane hyperpolarization.[24] Similar effects would be expected with K channel openers. Cromakalim and pinacidil are potent relaxers of bronchial smooth muscle constricted with a variety of spasmogens including PGE_2, LTD_4, 5HT, and histamine. However, both pinacidil and cromakalim appear to be less effective than many bronchodilators at blocking contractions of airway smooth muscle elicited by carbachol.[157] Cromakalim is effective in relaxing isolated human bronchioles,[158] and in healthy humans, 2-mg oral doses of cromakalim (which do not lower blood pressure) attenuated histamine-induced bronchoconstriction.[159] Because of its long half-life, cromakalim may be useful in patients with nocturnal asthma, and in a small, randomized, double-blind study, doses of 0.5 and 1.5 mg of cromakalim produced an improvement in early morning lung function in patients with nocturnal asthma.[160] Similar results have been obtained in a second study with 0.5 mg of cromakalim in 22 patients.[161]

Urogenital Malfunction. Urinary incontinence resulting from bladder hyperactivity secondary to bladder hypertrophy or partial outflow obstruction is common, and the existing therapeutic regimens are often ineffective or poorly tolerated. Animal studies in a variety of species have shown that pinacidil decreases the spontaneous contractile activity of bladder smooth muscle, especially in hypertrophic bladders.[162,163] In guinea pigs, the pharmacological effects of cromakalim resulted from a concentration-dependent increase in membrane K conductance.[76] Intravenous administration of cromakalim to pigs with urethral obstruction attenuated untoward, pathological contractions of the bladder. Importantly, isolated bladders treated with cromakalim could still respond to appropriate stimuli such as carbachol or intrinsic nerve stimulation, suggesting that K channel openers should not interfere with normal, physiologically mediated voiding.[76,164] In isolated human bladder smooth muscle, pinacidil blocked contractions induced by electrical-field stimulation, carbachol, or low concentrations of K and stimulated the efflux of ^{86}Rb. This latter effect could be antagonized with TEA and procaine, but not by apamin,[165] a pattern of blockade like that seen in cardiac muscle or vascular smooth muscle. Patients with hyperactive detrusor muscles, when treated with 0.5 or 1 mg/day of cromakalim, had an improvement in their symptoms of incontinence,[166] but this small study needs to be confirmed in large, double-blind, placebo-controlled studies.

Cardiac Effects. Cardiac tissue has a variety of K channels, and the three most-studied voltage-dependent channels have been referred to previously. Moreover, a high-conductance, ATP-inhibited K channel has been well-characterized in cardiac muscle.[58]

An increase in K conductance should selectively shorten APD, an effect opposite to that of the class 3 antiarrhythmics, and indeed, nicorandil, cromakalim, and pinacidil all shorten APD without an effect on the rate of rise of the action potential upstroke.[61,167,168] APD shortening is correlated with reductions in contractility in isolated tissues[116] and in perfused whole hearts.[169] These reductions in APD appear to be highly selective, and as previously discussed, dramatic stereoselectivities are seen with the enantiomers of pinacidil, **12**, and cromakalim.[94,170] The cardiac K channel affected by these openers appears to be the ATP-inhibited K channel upon the basis of patch clamp electrophysiologic evidence and the ability of sulfonylureas to block their effects.[171,172] These data, and the excellent correlation between the abilities of a series of pinacidil analogues to reduce APD and to produce antihypertensive effects,[168] provide additional indirect pharmacological evidence for the existence of ATP-dependent K channels in vascular smooth muscle with at least qualitative similarities to those in the heart.

Because of the pivotal nature of K flux in the regulation of normal cardiac conductance, the most obvious cardiac

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indication for K channel openers is suppression of certain forms of arrhythmias, and all three of the prototypic K channel openers have shown some antiarrhythmic activity than cannot be explained on the basis of their hypotensive effects.[65,116,173] In situations where prolonged action potentials produce early afterdepolarizations and arrhythmias, pinacidil has antiarrhythmic effects both in vitro and in vivo.[174] However, under certain conditions which predispose cardiac tissue to reentrant arrhythmias, K channel openers could also have a proarrhythmic effect.[62] Another possible application of K channel openers is amelioration of ischemia-induced cardiac damage. Effects of pinacidil and cromakalim have been studied recently in isolated globally ischemic rat hearts. Perhaps by repolarization of ischemic tissue, these drugs produced significant improvements in cardiac compliance and reperfusion function, and these beneficial effects could be reversed with glyburide.[175] Intracoronary administration of pinacidil and cromakalim to dogs with occluded coronary arteries, followed by reperfusion, resulted in a decrease in infarct size and ischemia-induced fibrillation.[176]

CNS Effects. The control of cellular membrane potential is integrally involved in the physiology of neuronal tissue, and neurotransmitters are often released by depolarization. Moreover, activation of a variety of neurotransmitter receptors, including dopamine D_2, $5HT_3$, α_2, opioid, and somatostatin receptors, alters the flux of K ions.[177,178] Therefore, K channel modulators might be expected to exert pharmacological effects in the CNS (central nervous system).

Brain tissue contains a high density of K channels that can be revealed with tritium-labeled glyburide, and the SAR for displacement of this radioligand from brain binding sites correlated with similar studies using heart or pancreatic tissues.[179] Increasing extracellular K concentrations led to ^{86}Rb efflux in rat brain synaptosomal or slice preparations. This effect could be blocked by TEA or 3,4-diaminopyridine, but K efflux was not affected by pinacidil or cromakalim.[180] Potassium channel openers ameliorated seizures induced by administration of a mast cell degranulating peptide isolated from bee venom, but not seizures induced by other K channel blockers such as dendrotoxin or 4-aminopyridine.[181] Finally, K channel openers reduce seizures in genetically epileptic rats.[182]

Miscellaneous Effects. In gastrointestinal smooth muscle, kinetically slow K channels, carrying outward current, may be responsible for slow-wave activity,[183] suggesting that K channel openers may have antimotility effects. Cromakalim blocked contractions in guinea pig small intestines evoked by electrical stimulation or $5HT_3$ and nicotinic receptor agonists, stimuli which act via release of endogenous acetylcholine. Spontaneous phasic contractions were abolished by cromakalim, but release of acetylcholine from the myenteric plexus was unaltered.[184]

Topically administered minoxidil (presumably acting via minoxidil sulfate) enhances hair growth in certain forms of male pattern baldness.[185] This, coupled with the knowledge that structurally diverse K channel openers such as pinacidil and diazoxide induce hair growth, provides circumstantial evidence that this effect is mediated via K channels. Whereas hypertrichosis occurs in 80–100% of minoxidil-treated patients, the incidence is 2–13% in pinacidil-treated patients,[153] but this may relate to different doses of the drugs. Minoxidil sulfate, the metabolite implicated in the antihypertensive effects of minoxidil, was found to be 14 times more potent than the parent drug in stimulating hair growth in cultured follicles from neonatal mice,[186] and this metabolite may be produced in hair follicles.[187] Compound **20** (Chart V) was reported to stimulate hair growth in a mouse model.[139] There is no evidence that cromakalim, nicorandil, or RP-49356 stimulate hair growth, but this possibility warrants further investigation.

Cromakalim enhanced K flux in skeletal muscle fibers derived from human patients, and this effect may be mediated via ATP-dependent channels since it was blocked with tolbutamide. The authors suggested K channel openers may have some role in the treatment of pathological muscle fatigue or paralysis resulting from excessive membrane depolarization.[188]

Conclusions and Research Opportunities

In this perspective we reviewed the tremendous advances that have occurred recently in the biology, pharmacology, and medicinal chemistry of K channels. The advent of potent and specific antagonists and openers of some K channel subtypes has increased our understanding of these channels, and has provided some promising drug candidates. The increasing sophistication of our experimental techniques, devices, and chemical probes, coupled with our enhanced understanding of K channel biochemistry and physiology, augurs well for the rational design of additional medicines that modulate K channels. However, true advances will probably arise not by discovery of pinacidil or cromakalim mimics, but by the discovery of structurally and mechanistically distinct K channel modulators. It is interesting to recall that a plethora of nifedipine analogues have been prepared, and scores of them have been taken into clinical trials, but these efforts, for the most part, have been neither scientifically nor commercially rewarding.

The molecular pharmacology and electrophysiology of K channel modulators needs to be carefully explored. For example, what is the exact nature of the antagonism of K channel openers by sulfonylureas? Do the drugs occupy different binding regions on the same K channel or do they behave as functional antagonists by acting independently

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on different K channels? Do the K channel openers "activate" K channels or do they inhibit channel inactivation? Is phosphorylation/dephosphorylation a common means for regulation of K channel function? All of these questions represent important issues that will require exhaustive exploration.

The differences among existing K channel openers needs to be carefully explored in both animals and man. Just as the different structural classes of Ca channel blockers, exemplified by nifedipine, verapamil, and diltiazem, have certain pharmacological and therapeutic differences, the different structural classes of K channel openers may find distinct therapeutic niches. Diazoxide is much more potent than cromakalim or pinacidil in opening pancreatic ATP-dependent K channels, whereas cromakalim and pinacidil are more potent vasodilators, suggesting that not all K channel openers are alike.

Selectivity will likely be the key to future advances. There is a large diversity of K channel subtypes, and furthermore, there is a wide range of regulatory mechanisms for these subtypes. True tissue selectivity—if this goal is achievable—would represent a bona fide advance. In fact, the key to exploiting K channel openers for treatment of diseases other than hypertension will ultimately be determined by the issue of tissue selectivity. Selectivity can be electrophysiologic in that tissues may respond differently to a K channel opener on the basis of membrane potential differences. For example, in vitro studies reveal that cells with resting membrane potentials close to E_k (e.g., cardiac cells) are affected minimally by drugs such as pinacidil and cromakalim, whereas those with resting membrane potentials positive to the E_k are hyperpolarized (e.g., smooth muscle). Differences between normal and diseased tissues could also be exploited. In cardiac cells, chronic ischemia may result in partial membrane depolarization and action potential prolongation, rendering diseased tissues preferentially susceptible to K channel openers. Potential selectivity would also be obtained if K channel openers could be developed with selectivity for known subtypes of K channels that may be important in modulating physiological effects in different tissues. The discovery of suitable radioligands to label selectively K channel subtypes would also be an important advance. It is possible to label the high-conductance, Ca-activated K channel with radioiodinated charybdotoxin and the ATP-dependent K channel with [^{3}H]glyburide, but beyond this there is a dearth of radiolabeled K channel blockers. Moreover, no radioligands exist for the site of action of the K channel opening vasodilator drugs.

With the few exceptions noted in this Perspective, there are relatively few K channel openers or blockers available for widespread clinical application, and it will be some time before we know if the K channel modulators will become as pervasive in medicine as the Ca channel blockers. However, the field is poised for tremendous scientific advances through the diligent application of new experimental techniques and chemical probes and, at the very least, new vistas in ion channel science will be revealed.

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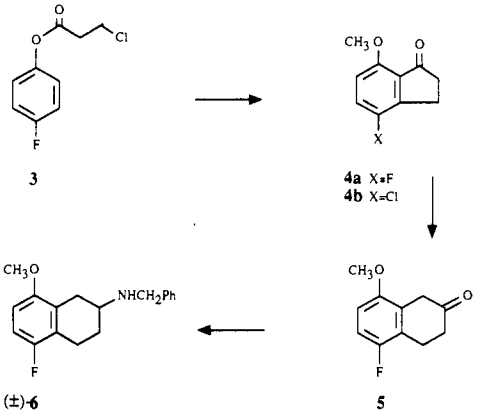
Communications to the Editor

(*S*)-5-Fluoro-8-hydroxy-2-(dipropylamino)tetralin: A Putative 5-HT$_{1A}$-Receptor Antagonist

Sir:

The 5-HT$_{1A}$ receptor[1,2] appears to be involved in a number of important brain functions such as regulation of mood, sleep, and sexual behavior. However, its functional role is not fully understood. This may be due to the lack of adequate pharmacological tools, that is, selective 5-HT$_{1A}$-receptor antagonists.[3,4] In this communication, we report that introduction of a C5-fluoro substituent into the potent 5-HT$_{1A}$-receptor agonist (*S*)-8-hydroxy-2-(di-

Scheme I



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