New drugs review

Potassium channels and airway function: new therapeutic prospects

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Potassium (K^+) channels have long been associated with recovery of excitable cells after depolarisation and drugs that block these channels, such as tetraethylammonium and 4-aminopyridine, cause an increase in excitability. In airway smooth muscle these K^+ channel blocking drugs result in spontaneous action potentials and a reduced threshold of excitation,^{1,2} which may be similar to the electrophysiological changes described in asthmatic airways.³

The recent development of drugs that open K^+ channels in smooth muscle has reawakened interest in potassium channels because such drugs relax airway smooth muscle and may reduce the hyperreactivity of asthma. It is now clear that there is great diversity of K^+ channels in different cells, raising the possibility that selective drugs may be developed for specific cell types, with therapeutic benefit. We review here some of the recent developments in understanding K^+ channels in airways and the therapeutic prospects for new K^+ channel activators.

Potassium channels and their types

Recent electrophysiological studies and the use of new pharmacological agents have shown that K^+ channels are present on cells of several types, including nerves, secretory cells, and skeletal and smooth muscle cells.^{4,5} K^+ channels are now seen to be diverse and may be modulated by changes in membrane potential, ion concentration, or receptor activation. All cells in the body are likely to have K^+ channels and they may have more than one type.

VOLTAGE SENSITIVE K^+ CHANNELS

Depolarisation of excitable cells leads to opening of a K^+ channel, which brings about repolarisation or hyperpolarisation of the cell. These channels, therefore, function to inhibit excitatory processes (fig 1). Voltage sensitive K^+ channels are well described in nerves and skeletal muscle and several types have been described, with different conductances and timing of opening.⁴

CALCIUM ACTIVATED K^+ CHANNELS

Calcium activated K^+ channels open when the concentration of intracellular calcium ions rises

(either from entry of calcium through voltage dependent or receptor operated calcium channels or from release from intracellular stores). Again, several types of channel, with high, intermediate, or low conductances, have been recognised. These may be blocked selectively by different pharmacological agents. Calcium activated K^+ channels have been described in airway smooth muscle.⁶

RECEPTOR OPERATED K^+ CHANNELS

Activation of certain surface receptors, such as muscarinic and adenosine receptors, may open these channels. Some receptors may be linked directly with the K^+ channel, whereas others may depend on intermediary G protein.⁴

ATP CONTROLLED K^+ CHANNELS

These K^+ channels may respond to reduced intracellular concentrations of ATP.⁷ The best described example is in pancreatic islet β cells, in which ATP controlled K^+ channels are activated by drugs such as diazoxide to inhibit insulin secretion, and blocked by sulphonylurea drugs, such as tolbutamide and glibenclamide, which therefore stimulate insulin secretions.⁸ These channels may also be important in human airway smooth muscle because glibenclamide is active in blocking K^+ channels in these cells.⁹

Figure 1 Diversity of K^+ channels.

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Agonists and antagonists

One of the factors responsible for the recent interest in K^+ channels is the discovery and development of pharmacological agents that discriminate between different K^+ channels, and drugs that are even more selective are likely to become available in the future. K^+ channel modulators include antagonists or blocking drugs, which inhibit the movement of K^+ ions through the channels, and agonists, openers, or activators, which open the channels (fig 1).

K^+ CHANNEL BLOCKERS (ANTAGONISTS)

Several K^+ channel blockers are now recognised that have differing degrees of selectivity. Tetraethylammonium, the best known agent, has effects on various channels and is rather non-selective.⁵ It has additional pharmacological properties at higher concentrations, which make it an unsuitable drug for some experimental uses.¹⁰ 4-Aminopyridine¹¹ is also non-selective but its range of effects is different from that of tetraethylammonium. Apamin is an 18 amino acid basic peptide derived from bee venom, which selectively blocks the small conductance Ca^{2+} activated K^+ channel.¹² Another toxin with similar selectivity is leiurotoxin I, derived from the venom of the Israeli scorpion. Charybdotoxin is also derived from the venom of this scorpion, but it appears to block selectively the large conductance of Ca^{2+} activated K^+ channel found in skeletal and smooth muscle.¹³⁻¹⁵ Sulphonylureas, such as tolbutamide and glibenclamide, are known to function as hypoglycaemic agents by blocking the ATP sensitive K^+ channels in pancreatic β cells, thus stimulating insulin secretion.¹⁶ These drugs are also effective in other cells, such as cardiac myocytes and the cells of smooth muscle (including airway smooth muscle). Quinine and quinidine have the effect of blocking Ca^{2+} activated K^+ channels,¹⁷ but they have actions on other types of K^+ channel in addition to other pharmacological effects, such as Ca^{2+} channel antagonism,¹⁸ so are unsuitable as probes.

K^+ CHANNEL ACTIVATORS

Several drugs have recently been developed which open K^+ channels in various types of cell. Nicorandil, developed as a coronary vasodilator, was the first drug shown to hyperpolarise smooth muscle by opening K^+ channels, though part of its vasodilator action is due to stimulation of guanylate cyclase.¹⁹ The vasodilators minoxidil and pinacidil have also been shown to open K^+ channels in vascular smooth muscle. Cromakalim (BRL 34915) is the most selective and potent K^+ channel activator so far developed. Cromakalim itself is a racemic mixture and the active L-enantiomer (BRL 38227) is now being developed for clinical use. Several other drugs, such as RP 49356, which is less active, are also being developed for clinical use. Cromakalim has been shown to hyperpolarise various smooth muscles, including rat portal vein,²⁰ rat aorta,²¹ guinea pig trachea,²² guinea pig bladder,²³ and rat uterus.²⁴

The evidence that the effects of cromakalim

are due to an action on K^+ channels is based on the fact that it stimulates the efflux of labelled rubidium ions ($^{86}Rb^+$), which are transported by K^+ channels, from these smooth muscle preparations. Stimulation of $^{42}K^+$ efflux²⁵ by cromakalim provides more direct evidence. Electrophysiological studies in vascular smooth muscle cells show that cromakalim and pinacidil open both large conductance Ca^{2+} activated K^+ channels and ATP dependent channels.²⁶⁻²⁷ The effect of these drugs is, however, selectively blocked by glibenclamide, which suggests that the ATP dependent channel is the channel that is most likely to be affected by these drugs in smooth muscle.^{5, 28-29} The K^+ channel in smooth muscle is clearly different from the classical ATP dependent K^+ channel of pancreatic β cells because the latter is unaffected by cromakalim (which therefore has no effect on plasma glucose concentrations). By contrast, diazoxide opens ATP dependent K^+ channels in both smooth muscle and pancreatic β cells (and therefore vasodilates and also increases plasma glucose³⁰). As glibenclamide alone has no effect on smooth muscle tone, this indicates that the K^+ channel in question is not open under resting conditions.²⁸⁻²⁹

Potassium channels in airway smooth muscle

IN VITRO STUDIES

Three different K^+ channel activators—namely cromakalim, nicorandil, and pinacidil—have been studied in vitro in airway smooth muscle of various species. Evidence of K^+ channel opening is supported by a reduction of smooth muscle tone (either spontaneous or induced by contractile agonists), hyperpolarisation, an increase in ^{86}Rb efflux, and of course reversal or inhibition of these effects by non-selective K^+ channel blockers such as 4-aminopyridine and tetraethylammonium.

Airway smooth muscle appears to differ in certain respects from smooth muscle in other organs. It usually has a low level of electrical excitability and does not have spontaneous action potentials. Instead, it displays slow wave activity, which may represent action potentials that are attenuated by the opening of K^+ channels and the consequent increase in K^+ conductance. This appears to be the case in human airway smooth muscle cells because action potentials are only generated when K^+ channel activity is reduced.³¹ This indicates that the airway smooth muscle is strongly rectified (that is, more resistant to the effects of a depolarising than a hyperpolarising current) and that this rectification is mediated through K^+ channel activation. Should the airway smooth muscle cell lose the ability to rectify, a state of hyperresponsiveness could occur. It is not difficult to see therefore that K^+ channel blockade could cause hyperresponsiveness. Possibly the continual presence of inflammatory mediators with an excitatory action in vivo could induce such a condition. There is some evidence to suggest that airway smooth muscle cells in asthma are more

Figure 2 Schematic representation of an airway smooth muscle cell and the mode of action of K^+ channel activators. The concentration of intracellular calcium ions rises as a result of calcium influx from the extracellular space through receptor operated or voltage dependent channels, or by release from intracellular stores. A raised intracellular calcium ion concentration, reduced ATP, or depolarisation stimulate efflux of K^+ through K^+ channels, leading to hyperpolarisation and relaxation of smooth muscle. K^+ channel activators such as cromakalim activate the K^+ channel and may have additional actions on intracellular calcium stores, receptor operated channels, and ion pumps, which remove Ca^{2+} from cells (dotted lines).

excitable,³ and the contractile properties of airway smooth muscle of animals^{32,33} and man³⁴ are altered after sensitisation. This could possibly be related to changes in ion flux through channels in the smooth muscle membrane.

Modulation of K^+ channel opening can result from several cellular signals (such as GTP binding proteins or protein kinase C) but in many cases the channel is activated by any process that raises intracellular calcium ion concentration (fig 2). As K^+ channel opening produces hyperpolarisation and opposes the depolarisation induced opening of voltage dependent Ca^{2+} channels, the possibility arises that K^+ channel activators are acting merely as Ca^{2+} channel blockers. K^+ channel activators, however, have different *in vitro* properties from those of Ca^{2+} channel antagonists. They induce greater relaxation of spontaneous tone, which may equal the relaxation in response to isoprenaline.³⁵ Cromakalim also inhibits cholinergically induced contractions in human bronchus,^{9,36} a property not shared by verapamil.³⁷ The mechanism of these additional actions of the K^+ channel activators is not yet clear, though they could be related to modification of the electrogenic ion pumps that remove Ca^{2+} from cells,³⁸ or to effects on intracellular Ca^{2+} stores or perhaps on receptor operated Ca^{2+} channels.³⁵ Thus K^+ channel activators are far more effective at relaxing airway smooth muscle than are Ca^{2+} channel antagonists and they therefore have greater therapeutic potential in asthma.

The actions of K^+ channel activators in airway tissue seem to vary with the experimental conditions and in this respect they differ from other relaxants, such as β adrenoceptor agonists and methyl xanthines. They

produce relaxation of spontaneous tone in guinea pig^{22,35,36,39,40} and human³⁶ airways, with an efficacy approaching that of isoprenaline. Contractions induced by histamine, prostaglandin D_2 , and leukotriene C_4 are also inhibited.^{35,36,39} Although K^+ channel activators show little or reduced efficacy against cholinergically induced tone in nearly all animal airway preparations,^{22,36,41} cromakalim reverses cholinergic tone in human airways, which, however,^{9,36} highlights the differences in excitation contraction coupling mechanisms between animal and human isolated airways.

Rubidium-86 is used as a marker for K^+ efflux in many excitable cells, and an increase in ^{86}Rb efflux indicates an increase in K^+ conductance in the airway smooth muscle cell membrane. Both nicorandil⁴⁰ and cromakalim²² increase the efflux of ^{86}Rb from guinea pig tracheal smooth muscle but this has yet to be studied in human airways. This property of increasing ^{86}Rb efflux is not shared by other smooth muscle relaxants such as isoprenaline.⁴⁰

ELECTROPHYSIOLOGICAL STUDIES

Intracellular electrophysiological recording and sucrose gap experiments can detect changes in membrane potential and activity of ion channels. Nicorandil and cromakalim both produce hyperpolarisation of tracheal smooth muscle cells which can be reduced by K^+ channel blockers.^{22,40}

The K^+ channel present in airway smooth muscle has not yet been fully characterised. The fact that glibenclamide inhibits the effect of cromakalim on airway smooth muscle suggests that it is some type of ATP sensitive channel. Electrophysiological studies, how-

ever, indicate that a large conductance Ca^{2+} dependent channel is also present,⁶ and this may be responsible for the rapid repolarisation of airway smooth muscle.

IN VIVO STUDIES

In anaesthetised guinea pigs both cromakalim and pinacidil give dose dependent protection against 5-hydroxytryptamine induced bronchoconstriction, the dose giving half maximal protection being 80 $\mu\text{g}/\text{kg}$ for cromakalim and 560 $\mu\text{g}/\text{kg}$ for pinacidil. By contrast, the calcium antagonist nifedipine provides no appreciable protection.³⁵ Cromakalim is only weakly effective against acetylcholine induced bronchoconstriction in vivo,⁴² which reflects the poor inhibitory effect against cholinergic contraction of guinea pig trachealis in vitro.³⁶⁻⁴¹ Cromakalim provided no protection against bronchoconstriction induced by intravenous substance P.⁴²

Few clinical studies of K^+ channel activators have been reported as yet but orally administered cromakalim protects against nocturnal bronchoconstriction in asthmatic patients,^{43,44} which may reflect an inhibitory action on the vagal cholinergic bronchoconstriction that occurs at night.⁴⁵ Orally administered cromakalim (2 mg) also protects against histamine induced bronchoconstriction in healthy subjects.⁴⁶

Potassium channels on other cells

Although most attention has focused on the role of K^+ channels in airway smooth muscle, these channels are likely to be important also in other cells. This may be relevant in airway disease and these effects may contribute to any antiasthma effect of K^+ channel activators.

AIRWAY NERVES

K^+ channels have an important role in hyperpolarisation of nerves after a depolarising stimulus and in the release of neurotransmitters. In an innervated guinea pig tracheal tube preparation cromakalim had a greater inhibitory action on preganglionic (vagus nerve) stimulation than on postganglionic stimulation (electrical field stimulation), indicating that K^+ channels have a modulatory effect on neurotransmission through airway parasympathetic ganglia.^{41,47} In guinea pigs treated with capsaicin (to destroy sensory nerves containing neuropeptides) cromakalim caused a greater inhibition of bronchoconstriction induced by vagus nerve stimulation than of that induced by intravenous acetylcholine, suggesting an inhibitory effect on cholinergic neurotransmission in vivo.⁴²

Cromakalim has an even greater effect on non-adrenergic, non-cholinergic (NANC) bronchoconstriction in guinea pigs (induced by vagus nerve stimulation in the presence of atropine) than on cholinergic bronchoconstriction. The bronchoconstrictor response is due to release of neuropeptides such as neurokinin A and substance P from sensory nerves in airways. Cromakalim caused more than 70% inhibition of NANC induced bronchoconstriction while having no effect on bronchoconstriction induced by substance P, suggesting that it

was inhibiting the release of sensory neuropeptides.⁴² This effect of cromakalim is inhibited by glibenclamide, indicating that the K^+ channel on sensory nerves may be similar to that on airway smooth muscle. K^+ channels are important in modulating the effects of certain neuromodulators, such as γ aminobutyric acid (GABA), opiates, and α_2 agonists in central neurones.^{48,49} This raises the possibility that other modulators of NANC nerves in airways, such as opiates,^{50,51} GABA,⁵² H_3 agonists,⁵³ and α_2 agonists,⁵⁴ may work by opening K^+ channels in sensory nerve endings. This may be relevant in airway disease, because release of sensory neuropeptides via an axon reflex mechanism may contribute to the inflammatory reaction in asthmatic airways.⁵⁵ K^+ channel activators may therefore have anti-inflammatory activity in addition to being bronchodilators.

EPITHELIUM

Airway epithelium modulates the contractile response of airway smooth muscle to several spasmogens,⁵⁶ possibly by releasing a relaxant substance.⁵⁷ Possibly K^+ channels on airway epithelial cells are concerned in the release of this relaxant factor, and also in the ion transporting function of epithelium.

MICROVASCULAR LEAKAGE

Most inflammatory mediators cause microvascular leakage in airways by effects on post-capillary venules.^{58,59} Leakage is probably due to retraction of endothelial cells, thus allowing extravasation of plasma. Some antiasthma drugs may reduce airway microvascular leakage by preventing this contraction,^{60,61} and this raises the possibility that K^+ channel activators also reduce plasma extravasation by preventing the contraction of endothelial cells. Cromakalim, however, did not inhibit airway microvascular leakage induced by platelet activating factor in guinea-pig airways (D Rogers *et al*, unpublished observations).

INFLAMMATORY CELLS

Several inflammatory cell types may participate in the inflammatory response in asthmatic airways.⁶² Possibly K^+ channels play a part in the chemoattraction of inflammatory cells and release of inflammatory mediators, but almost no information is currently available. Preliminary studies, however, suggest that cromakalim is ineffective in preventing activation and degranulation of eosinophils (C Kroegel and P Barnes, unpublished observations). K^+ channels have been identified on human alveolar macrophages by electrophysiological techniques,⁶³ and may therefore modulate the activation of these cells.

MUCUS SECRETION

K^+ channels have an important role in secretion but whether K^+ channel activators or blockers influence airway mucus secretion is not yet known.

Clinical applications

BRONCHODILATORS

K^+ channel activators represent a new class of

drugs that may have a useful clinical application in the treatment of asthma.⁶⁴ It is clear that the K⁺ channel activator cromakalim relaxes guinea pig and human airway smooth muscle in vitro and is likely to be a bronchodilator. It has also been shown to protect against histamine induced bronchoconstriction in normal individuals.⁴³ The long plasma half life of the drug (22.5 hours) after oral administration⁶⁵ makes it particularly suitable for treating nocturnal asthma, where a long duration of protection is required, and two preliminary studies have confirmed that oral administration (0.5–2.0 mg) gives useful protection against early morning bronchoconstriction in asthmatic patients.^{43,44} The effect of K⁺ channel activators against other bronchial challenges in asthmatic patients must now be studied systematically. The recent finding that cromakalim is effective against NANC bronchoconstriction in guinea pigs⁵³ may indicate an effect against neurogenic inflammation in airways; further information about anti-inflammatory effects in human airways is now required.

COMPARISON WITH CALCIUM ANTAGONISTS

Calcium antagonists have proved to be very disappointing in the treatment of asthma.^{66,67} They have no bronchodilator action and provide only weak and clinically irrelevant protection against various bronchoconstrictor challenges. The reason for their ineffectiveness is likely to be that they block calcium entry only from voltage dependent calcium channels and are ineffective against release of Ca²⁺ from intracellular stores. The latter appears to be the dominant mechanism by which spasmogens cause contraction of human airway smooth muscle.

As K⁺ channel activators reverse the depolarisation of airway smooth muscle cells, which might be accompanied by calcium entry via voltage dependent calcium channels, it might be predicted that K⁺ channel activators would have a similar functional effect to calcium antagonists. K⁺ channel activators, however, as discussed above, appear to be effective bronchodilators both in vitro and in vivo. This strongly suggests that this class of drug is having effects in addition to hyperpolarisation of the smooth muscle cell after depolarisation and presumably may act to extrude Ca²⁺ from the cell (via the Na⁺-Ca²⁺ exchange pump) or sequester Ca²⁺ into intracellular stores.

SIDE EFFECTS

Side effects may be the limiting factor in the clinical application of any new drug, particularly as the most useful drugs for treating asthma, inhaled β_2 agonists, inhaled corticosteroids, and inhaled cromoglycate have very few unwanted effects. Cromakalim was introduced for the treatment of hypertension as it is a vasodilator and hypotension might therefore be a troublesome side effect in asthmatic patients. This has not been a problem in single dose studies so far.⁴³ If systemic side effects do prove to be a problem, an inhaled preparation

may be useful. Cromakalim appears to be safe and, in contrast to diazoxide, has no effect on plasma glucose concentration or on other biochemical measurements.

Cromakalim relaxes rat and human pulmonary vessels in vitro (D Crawley *et al*, unpublished observations) and therefore may increase shunting by reversing the hypoxic vasoconstriction that occurs in acute asthma, causing a fall in arterial oxygen tension. In practice, this may be overcome by increasing the inspired oxygen concentration. Cromakalim also relaxes urinary bladder smooth muscle²³ and might potentially cause problems with urinary retention. This has not been observed, however, and indeed K⁺ channel activation of this type may be useful treatment for bladder syndromes such as detrusor overactivity.⁶⁸

Future directions

K⁺ channel activators represent a novel class of drug that appear to be effective as bronchodilators and may have a role in the management of asthma. If they also have anti-inflammatory effects they may prove useful in asthma prophylaxis. The long duration of action of oral cromakalim, the prototype of such drugs, would be an obvious advantage in the management of chronic asthma as once daily administration would be feasible.

It is now evident that K⁺ channels are very diverse and this raises the possibility that highly selective activators may be developed in the future. Advances in molecular biology have now made it possible to clone these channels and rapid progress in this direction is being made. The diversity of K⁺ channels is in sharp contrast to the uniformity of voltage dependent Ca²⁺ channels in different tissues, which has meant that it has not proved possible to develop selective calcium antagonists for specific tissues.⁶⁹ The development of more selective K⁺ channel activators (and blockers) in the future with more action on one type of smooth muscle than another (for example, airway as opposed to vascular smooth muscle) might allow the development of drugs without appreciable side effects.

We thank Ann McGregor for her careful preparation of the manuscript.

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