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RECENT PROGRESS IN POTASSIUM CHANNEL OPENER PHARMACOLOGY

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Abstract—Potassium (K) channel openers comprise a diverse group of molecules capable of opening K channels in excitable cells. These agents exhibit their greatest potency in the smooth muscle system but K channels in cardiac muscle, neurones and in secretory cells are also affected. The development of tissue selectivity is currently one major focus of research and evidence is starting to emerge that this can be achieved. The profound effects of the K channel openers *in vivo* has led to the suggestion that an endogenous K channel opener might exist and exert an important role in blood pressure homeostasis. The discovery of such a substance—endothelium-derived hyperpolarising factor—has many implications and its role in cardiovascular regulation is currently under investigation. *In vivo*, initial studies with the K channel openers emphasized their antihypertensive properties. However, later studies have concentrated on the improvement to coronary blood flow produced by these substances together with their protective effect on the ischaemic myocardium, the basis of which is not fully understood. In spite of great efforts, the K channel which forms the target of these agents in smooth muscle is a matter of controversy. The ability of glibenclamide to antagonize the actions of the K channel openers initially led to the suggestion that an ATP-dependent K channel was their site of action in smooth muscle although the most recent data have implicated a smaller conductance K channel.

More variants of potassium (K) channels exist than of any other ion channel type [1] and this diversity confers on K channels as a group the properties of an excitation regulator of great complexity. It is thus hardly surprising that research into the modulation of K channels by drugs is one of the most rapidly growing areas of pharmacology and many investigators are focusing their attention on the group of drugs known as "potassium channel openers". Research in this area really began in the mid-1980s when it was discovered that a novel molecule, cromakalim, exerted its hypotensive and smooth muscle relaxant effects by opening K channels in vascular smooth muscle [2]. The subsequent realization that existing antihypertensive agents such as diazoxide, minoxidil and pinacidil were also K channel openers [3, 4, 5] stimulated the pharmaceutical industry to synthesize a variety of novel agents based largely on cromakalim and pinacidil [4, 6]. A further impetus to the development of new molecules has been the existence of so many different types of K channel [1]. The differences, which seem largely to be concerned with the biochemical and biophysical properties of the channel gating mechanisms, offer the possibility that K channel openers selective for specific K channel types can be developed. In parallel with these pharmacological developments has been the realization that endogenous molecules such as substance P and endothelium-derived hyperpolarizing factor (EDHF†) also exert some or all of their efforts via K channel opening [7, 8].

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† Abbreviations: EDHF, endothelium-derived hyperpolarizing factor; EDRF, endothelium-derived relaxing factor; K_{ATP}, ATP-sensitive K channel; BK_{Ca}, large conductance calcium-activated K channel; p5, picosiemens.

The objective of this brief review is to highlight selected aspects of research into the exciting field of K channel opener research. This promises to enhance our understanding of the K channel as the main "on–off" switch of excitable cells and to yield molecules suitable for the treatment of a wide variety of disease states.

Chemical progress: the search for potent, long-acting and tissue-selective synthetic molecules

The realization that the benzopyran derivative cromakalim exerts its effects via K channel opening [2] soon led to the appreciation that seven distinct chemical types of K channel opener exist [3]. To date, the greatest chemical research effort has been in the production of variations on the benzopyran nucleus. It thus seems appropriate to review the progress which has been made in varying the properties of this group of molecules. Comments will be made with reference to BRL 38227 (formerly lemakalim), the (−)-3*S*,4*R* enantiomeric component of the racemate cromakalim (Fig. 1). For further information on developments within the cyanoguanidine and thioformamide groups of K channel opener, the reader is referred to Robertson and Steinberg [4] and to Chapman [9].

Potency. Ro 31-6930 was an early development in the search for variants of BRL 38227 and is currently the best described, most potent variant of BRL 38227. Ro 31-6930 lacks chiral carbon atoms (Fig. 1). Its potency as an antihypertensive agent *in vivo* is approximately five times greater than that of BRL 38227 [10] and it is two to three times more potent as a bronchodilator [11]. Its effects on regional blood flow are different from those of cromakalim [12] although whether the basis for this is pharmacokinetic or pharmacodynamic is not known.

Duration of action. A relatively long half-life is an important property for a clinically useful K

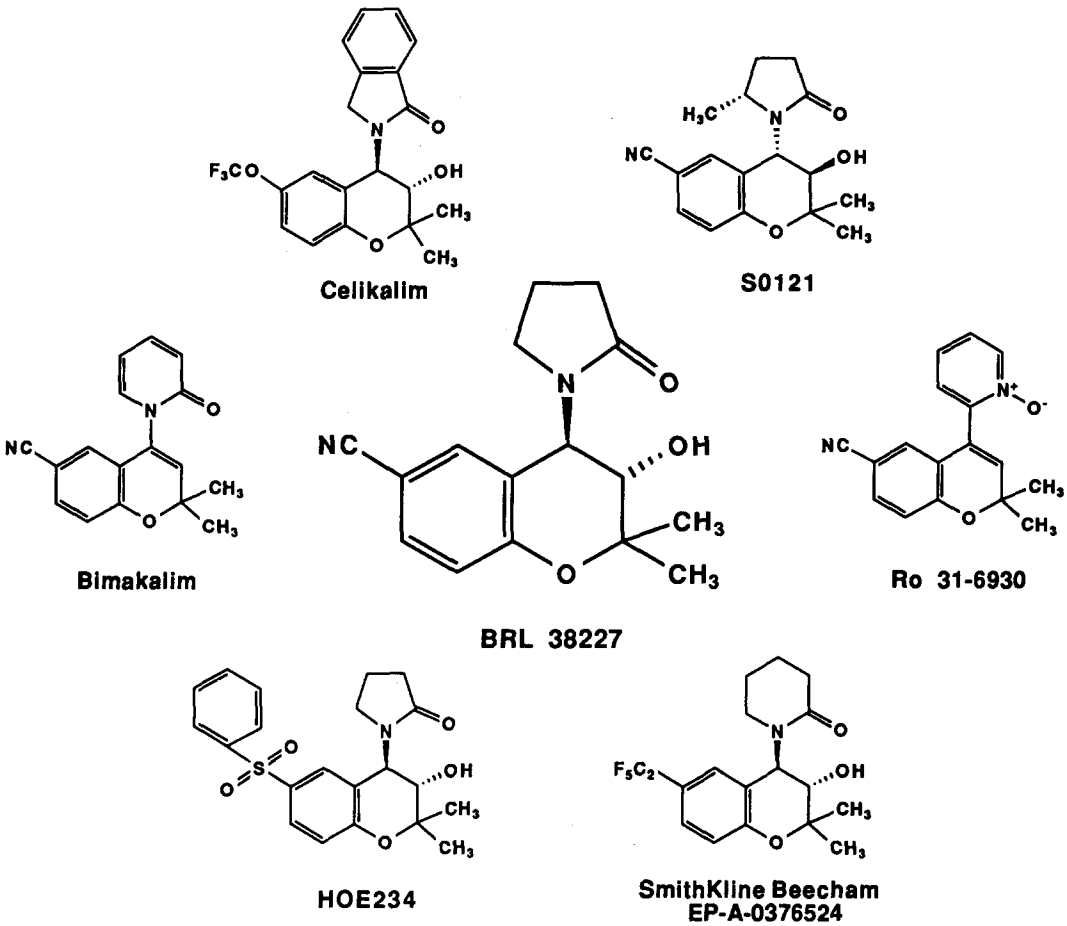

Fig. 1. K channel openers which are all variants on the benzopyran nucleus. BRL 38227 (SmithKline Beecham, Welwyn, U.K.) is the prototype molecule; celikalim (Wyeth-Ayerst, Princeton, U.S.A.), bimakalim (E Merck, Darmstadt, F.R.G.), Ro 31-6930 (Roche, Welwyn, U.K.) and S0121 (Hoechst, Frankfurt, F.R.G.) can all be regarded as first generation molecules which exhibit some differences with respect to duration of action, potency or tissue selectivity when compared with BRL 38227. HOE 234 (Hoechst) and the SmithKline Beecham molecule described in EP-A-0376524 are second generation compounds designed primarily to confer enhanced tissue selectivity.

channel opener as it will then necessitate only a once-a-day or at most a twice-a-day dosing regime. BRL 38227 itself has a very satisfactory half-life of approximately 18 hr in man [13]. Another first generation benzopyran K channel opener under development, celikalim (Fig. 1; formerly WAY 120,491) has a half-life even longer than that of BRL 38227 in dogs and SH rats [14].

Tissue selectivity. The ubiquitous nature of K channels in all excitable tissues means that the development of tissue-selective molecules is highly desirable. Within the smooth muscle system, most first generation K channel openers seem to exhibit a slightly greater potency in the vasculature. The benzopyran SO121 (Fig. 1) was reported to exert selective inhibitory effects on ureteric smooth muscle [15]. However, a more detailed study in which the effects of a variety of K channel openers on vascular and bladder tissues were compared [16] failed to confirm a low potency for SO121 in the vasculature. Further experiments with this agent are required and SO121 remains of interest because it does not conform to the usual 3*S*,4*R* stereochemical configuration normally associated with active molecules. The failure [16] to confirm the earlier report of tissue selectivity [15] does not exclude the possibility that SO121 could be tissue-selective *in vivo* because of its pharmacokinetic properties and data from such experiments are awaited with interest. Bimakalim (Fig. 1; formerly EMD 52692) is another first generation molecule with possible tissue-selective properties. This benzopyran produces significant changes in coronary collateral blood flow in dogs following acute coronary artery occlusion [17] with essentially no effect on systemic haemodynamics. Since this selective effect is derived from *in vivo* data, the degree of true tissue selectivity as opposed to the involvement of pharmacokinetic factors remains to be established.

Two second generation molecules, HOE 234 and a Smith Kline Beecham development (see Fig. 1), may represent important first steps in the

development of true tissue selectivity. Both exhibit interesting substituent groups (phenylsulphonyl, HOE234; pentafluoroethyl, SKB undesignated) at the 6-position of the benzopyran nucleus. Data describing their tissue selectivity for bronchial smooth muscle is essentially restricted to the patent literature [18] and further information is required before the existing brief claims can be substantiated.

Physiological progress: is there an endogenous K channel opener?

The possible role of endogenous vasodilator substances in the modulation of blood pressure in mammals is the subject of much current research effort. Interest in this area was stimulated several years ago by the recognition that the vascular endothelium was an important source of vasodilator substances [19]. The role of EDRF, its identity as nitric oxide (NO) or a carrier of NO-containing moieties, and the associated activation of smooth muscle soluble guanylate cyclase, have been widely reviewed [20–23].

With the realization that K channel opening in vascular smooth muscle cells is a powerful vasodilator stimulus [24], the possibility that synthetic K channel openers might be substituting for an endogenous K channel opener of unknown type became the subject of investigation. At the outset, EDRF did not seem to be a candidate since neither NO itself nor nitrovasodilator agents produce significant membrane hyperpolarization at less than supramaximal mechano-inhibitory concentrations [25–28]. Early microelectrode experiments had shown that carbachol could produce endothelium-dependent hyperpolarization in blood vessels [29] and indications that acetylcholine could release an endogenous K channel opening factor, different from EDRF, from the vascular endothelium were published soon afterwards [30, 31]. Confirmation that this factor was derived from the endothelium was obtained by Feletou and Vanhoutte [32] using the donor–acceptor tissue technique. Final confirmation that a novel, endogenous K channel opening substance could be released from the vascular endothelium and that this factor—EDHF—is pharmacologically distinct from EDRF was obtained by Chen *et al.* [33] (see Fig. 2). Further comprehensive data concerning the presence of EDHF in several blood vessels and the failure of NO synthase inhibitors to modify the resultant hyperpolarization were described recently [34–36].

In spite of the failure of many workers to detect NO-induced hyperpolarization, the possibility that NO could, in fact, contribute to the EDHF-induced hyperpolarization was described by Tare *et al.* [37, 38] following experiments in guinea-pig uterine artery. Further evidence that NO can produce hyperpolarization was also provided recently by Garland and MacPherson [39]. Further studies are required to determine the relative contributions which EDHF and NO make to endothelium-dependent hyperpolarization.

Several studies have been carried out to characterize the type of K channel involved in endothelium-dependent increases in membrane potential. In rabbit middle cerebral artery both Standen *et al.* [40] and Brayden [41] reported that acetylcholine-induced endothelium-dependent hyperpolarization was glibenclamide-sensitive. However, this K channel blocker failed to modify similar acetylcholine-induced changes in either guinea-pig coronary artery or in rat small mesenteric artery [34, 35]. In rat aorta, acetylcholine-induced hyperpolarization was reduced by glibenclamide [36] whereas in a separate study Bray and Quast [42] found that acetylcholine-stimulated ^{42}K efflux was reduced by tetraethylammonium but not by glibenclamide. To complicate matters further, the NO-induced hyperpolarization reported by Tare *et al.* [37, 38] was relatively unaffected by glibenclamide in contrast to the observations of Garland and McPherson [39] in which NO-induced changes were blocked by glibenclamide. Such conflicting data suggest that different hyperpolarizing factors may be liberated in different tissues or that the K channels modulated by NO and by EDHF may vary from tissue to tissue.

Similar uncertainty exists concerning the relative contributions which EDRF and EDHF make to the measured endothelium-dependent relaxation of blood vessels. The recent introduction of NO synthase inhibitors has allowed the contribution of EDRF to be assessed with the assumption that any remaining relaxation must be associated with the effects of hyperpolarizing factors. Such experiments involving agents such as L-NOARG, methylene blue and haemoglobin have shown that the contribution of EDHF is variable but may be as much as 30% of any given endothelium-dependent relaxation [33, 34, 41, 43]. Little is known about the stimuli which release EDHF (or indeed EDRF) *in vivo*. One factor which may be involved is haemodynamic sheer stress [44] although further experiments are required before a definitive statement can be made.

Clinical progress and prospects: the possible use of potassium channel openers as cardioprotective agents

The general decrease in the excitability of cells which follows K channel opening has resulted in many projections for the potential clinical use of agents with this property. Currently, K channel openers are in various stages of evaluation for use in the treatment of hypertension, bronchial asthma and the irritable bladder syndrome [45]. In addition, evidence is emerging that these agents are "cardioprotective" and capable of exerting beneficial effects on the heart, especially during periods of ischaemia. It is not yet clear whether such a protective action occurs via dilatation of coronary vessels or whether the cardiac myocyte is the site of this action, and it seems appropriate to review the current state of knowledge.

Effects on the coronary circulation. In vitro, the K channel openers produce relaxation of isolated coronary segments from a variety of species [24]. *In vivo*, the majority of experiments have been performed using nicorandil which increases coronary blood flow in anaesthetized and conscious dogs [46–48], and in guinea-pigs [49]. In general, these effects of nicorandil are seen more in the sub-epicardial layers of muscle than in the sub-endocardium. Although it could be argued that these effects of nicorandil are at least partly due to nitrovasodilation

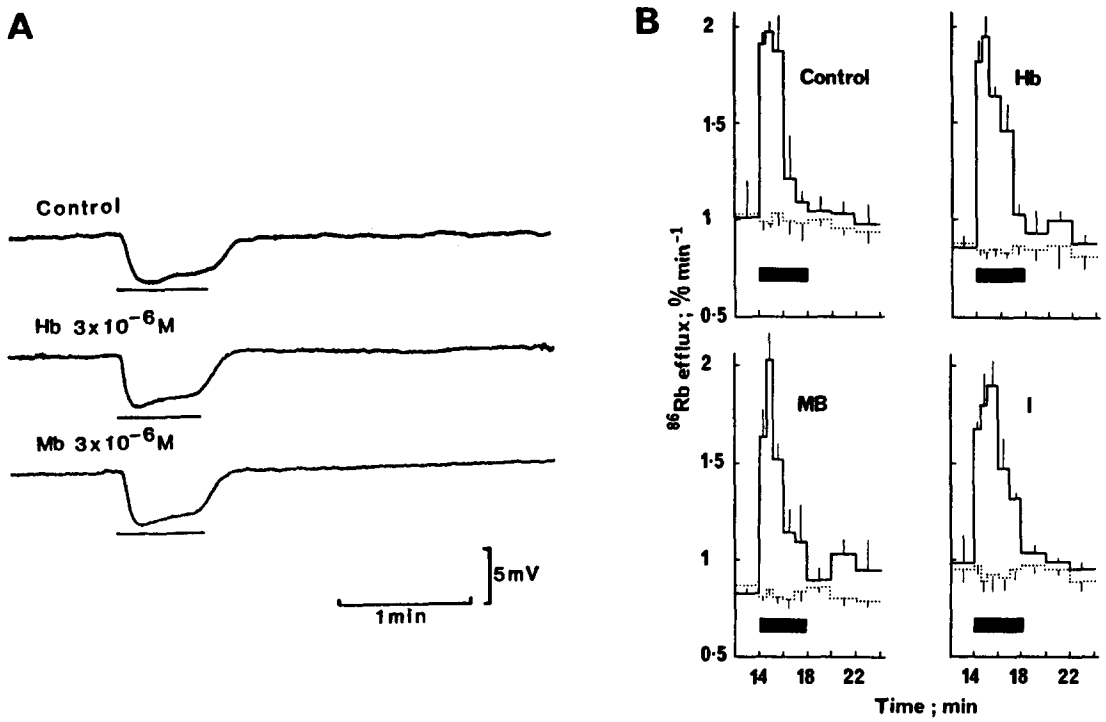

Fig. 2. Evidence for the existence of EDHF in rat blood vessels. (A) Acetylcholine-induced hyperpolarization is resistant to modifiers of the EDRF pathway, Methylene blue (Mb) and haemoglobin (Hb). (B) The increased ^{86}Rb efflux induced by acetylcholine indicates the opening of K channels, an effect resistant to Methylene blue (MB), haemoglobin (Hb) and indomethacin (I). For further details, see text. Adapted from Chen *et al.*, *Br J Pharmacol* **95**: 1165–1174, 1988 [33] with permission.

via guanylate cyclase activation rather than to its K channel opening properties, experiments in which pinacidil and other K channel openers have been used also indicate a significant increase in coronary blood flow *in vivo*. Thus, when pinacidil was given to either conscious or anaesthetized dogs, increases in sub-epicardial flow greater than those in the sub-endocardium were observed [50, 51]. More recent experiments with bimakalim (Fig. 1) have yielded similar results [17].

Although the above data demonstrate that the K channel openers are effective in normoxic or non-ischaemic conditions, clinical interest is centred around the effects of these agents following acute and/or chronic ischaemia. In the latter condition especially, blood flow is carried by newly developed collaterals. The first demonstration of a relatively selective increase in blood flow to collateral vessels was given by Angersbach and Nicholson [52] in rat skeletal muscle. In this tissue, cromakalim, pinacidil and nicorandil increased blood flow selectively to the previously ischaemic limb, an action not shared by calcium entry blocking agents. A similar result has been described in anaesthetized dogs for bimakalim at concentrations which were without significant effect on systemic blood pressure [17]. Nicorandil also increases coronary collateral flow but such effects can only be seen clearly if the associated systemic vasodilator actions of this agent are prevented [47, 53]. The basis of the selective vasodilation of newly developed collateral vessels is unknown.

Effects on cardiac muscle. The effects of several K channel openers have been evaluated in rat isolated hearts using a model in which 25 min of global ischaemia was followed by a 30-min reperfusion period under normoxic conditions at constant pressure [54]. Under these conditions, cromakalim and EMD 56431 produced significant improvement of cardiac function on reperfusion as measured by a reduced lactate dehydrogenase release and contracture generation. Similar effects were seen with the non-benzopyran K channel openers, pinacidil and aprikalim [54, 55]. The effective concentration range for these effects was between 1 and 30 μM and thus in general the K channel openers were not selectively cardioprotective. The effects of the K channel openers were antagonized by glibenclamide or sodium hydroxydecanoate indicating that the protective mechanism was associated with the opening of K channels. Although it could be argued that these effects were associated with a primary effect on coronary flow via dilation of the coronary vasculature, comparative experiments under constant flow conditions produced identical results to those under constant pressure [55]. Measurement of high energy phosphate levels following global ischaemia and during the reperfusion period showed that the ischaemia-induced reduction in ATP levels was significantly attenuated in the

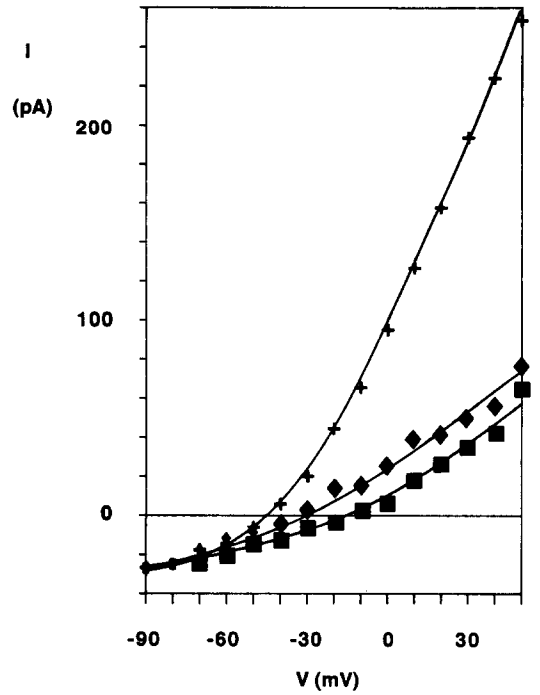

Fig. 3. Rat portal vein. Current–voltage relationships in a single isolated cell under control conditions (■) and in the presence of BRL 38227 10 μM (+) and BRL 38227 + glibenclamide, each 10 μM (◆). Noise analysis of the BRL 38227-induced current indicates that the K channel involved has a conductance of 21 pS at 0 mV. For further details see Ref. 74.

presence of cromakalim after at least a 10-min period of ischaemia [55].

The cardioprotective effects of the K channel openers are glibenclamide-sensitive and thus involve the opening of K channels sensitive to this agent, probably in both the coronary circulation and in the myocytes. Thus, delivery of oxygen to ischaemic areas would be enhanced and the cardiac muscle would tend to be in a "switched-off" state as a consequence of open glibenclamide-sensitive (presumably ATP-dependent) K channels. It therefore seems possible that the K channel *openers* effectively mimic a physiological mechanism associated with protection of the heart against hypoxia/ischaemia [56]. As would be predicted, exposure to a K channel *blocker* such as glibenclamide exacerbates the deleterious effects of myocardial stunning—the changes produced by a brief period of coronary occlusion followed by normoxic reperfusion [57].

If hearts are exposed to a brief period of coronary occlusion, the size of infarcts associated with subsequent, prolonged coronary occlusion (up to 4 hr) is greatly attenuated [58]. The beneficial effects of such preconditioning can be mimicked by substituting exposure to K channel openers provided that any decrease in mean arterial pressure is kept to a minimum [59]. Presumably in the preconditioning phase or on exposure to the K channel opener, the opened (possibly ATP-sensitive) K channels place the heart in a state which allows it to withstand a serious hypoxic insult.

Electrophysiological progress: which smooth muscle K channel is the target for the K channel openers?

The observations that the smooth muscle relaxant actions of the K channel openers could be antagonized by glibenclamide [60] resulted in the conclusion that an ATP-dependent K channel must be the most likely target for these agents. Such a view was reinforced by the ability of K channel openers (albeit at very high concentrations) to inhibit insulin release from isolated pancreatic islets [61] and to increase the opening probability of K_{ATP} in cardiac muscle [62]. The report by Standen *et al.* [40] of the existence of a K_{ATP} in rabbit mesenteric artery smooth muscle and of the ability of cromakalim to increase the open probability of this channel seemed further proof that K_{ATP} was indeed the target channel of the K channel openers.

However, reports from other laboratories have provided conflicting evidence and, at present, there is no clear consensus concerning the site of action of these agents. In one of the most comprehensive studies, Beech and Bolton [63] described the effects of a range of K channel blockers on whole-cell K currents elicited from isolated cells of rabbit portal vein. When the effects of these blockers on the current carried by cromakalim (I_{CK}) were compared with those on the other K currents, I_{CK} showed closest similarity to the delayed outward rectifier current, K_V. However, subsequent experiments with noxiustoxin, a blocker of K_V in neurones [64] showed that this agent failed to antagonize the relaxant effects of cromakalim in intact smooth muscle [65].

In a series of detailed studies, Gelband and coworkers [66–68] have obtained evidence that BK_{Ca} is involved in the action of the cromakalim. In experiments using either cultured aortic smooth muscle or following the incorporation of BK_{Ca} into planar lipid bilayers, the channel opened by cromakalim was indistinguishable from charybdotoxin-sensitive BK_{Ca} channels. However, charybdotoxin has no effect on cromakalim-induced relaxation in either blood vessels or airway muscle [69, 70] and Green *et al.* [70] failed to confirm an action of BRL 38227 on BK_{Ca}. The possible involvement of a small conductance K channel in the action of the K channel openers has also received support from several groups. Indeed, as pointed out by Beech and Bolton [63], the cromakalim-induced outward current in whole cells is associated with relatively low noise and as such is likely to be associated with channels of low unitary conductance. Evidence for the involvement of an ATP-sensitive, Ca-activated K channel following exposure to nicorandil and pinacidil was obtained by Kajioka and coworkers [71, 72]. A channel of similar size (7.5 pS) was observed following exposure to cromakalim in rabbit mesenteric artery but although this channel was Ca-dependent, no ATP-dependency could be detected [73]. Our own experiments [74] also favour the involvement of a small conductance K channel. In this study, carried out in freshly isolated whole portal vein cells, BRL 38227 induced a non-inactivating,

Ca-independent current with an underlying conductance of approximately 10 pS (Fig. 3). This current was activated by BRL 38227 and inhibited by glibenclamide in concentration ranges associated with mechano-inhibitory effects in whole tissues.

There is still much controversy about the identity of the K channel involved in the action of the K channel openers and no clear consensus has emerged. In the electrophysiological studies involving enzymatically dispersed cells it is possible that the separation techniques have introduced artifacts. It is thus important to ensure that the pharmacology of any potential target channel for the K channel openers is consistent with the pharmacology of these agents in whole tissues. Thus, studies with blocking agents in whole tissues and the selectivity of these substances assume great importance. It is quite clear that the target K channel must be glibenclamide-sensitive but the conclusion that sensitivity to the sulphonylureas indicates involvement of K_{ATP} has not yet been established for smooth muscle.

Conclusions

K channel openers are proving to be one of the most interesting of the recently discovered groups of novel pharmacological agent. The prospect that the K channels of excitable cells can be selectively modulated by such drugs raises many exciting therapeutic prospects. The results of on-going clinical trials are awaited with interest but it already seems that these agents are likely to become a therapeutic class of major importance in the years to come.

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