Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 66 (2003) 425–430
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Inhibition of ATP-sensitive K^+ channels by substituted benzo[c]quinolizinium CFTR activators

Anne-Lise Prost[a], Renaud Dérand[a,b], Laurent Gros[c], Frédéric Becq[b], Michel Vivaudou[a,*]

[a]CEA, DRDC, Laboratoire de Biophysique Moléculaire et Cellulaire (UMR 5090), 17 rue des Martyrs, 38054 Grenoble, France
[b]LBSC, UMR 6558, CNRS, Université de Poitiers, 40 avenue du recteur Pineau, 86022 Poitiers, France
[c]Institut de Génétique Moléculaire, CNRS, 1919 route de Mende, 34090 Montpellier, France

Received 6 February 2003; accepted 4 April 2003

Abstract

The substituted benzo[c]quinolizinium compounds MPB-07 and MPB-91 are novel activators of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel. High homologies between CFTR and the sulfonylurea receptor (SUR), which associates with the potassium channel Kir6.2 to form the ATP-sensitive K^+ (K_{ATP}) channel, prompted us to examine possible effects of these compounds on K_{ATP} channels using electrophysiological recordings and binding assays. Activity of recombinant K_{ATP} channels expressed in *Xenopus* oocytes was recorded in the inside-out configuration of the patch–clamp technique. Channels were practically unaffected by MPB-07 but were fully blocked by MPB-91 with half-inhibition achieved at ~20 μM MPB-91. These effects were similar on channels formed by Kir6.2, and either the SUR1 or SUR2A isoforms were independent of the presence of nucleotides. They were not influenced by SUR mutations known to interfere with its nucleotide-binding capacity. MPB-91, but not MPB-07, was able to displace binding of glibenclamide to HEK cells expressing recombinant SUR1/Kir6.2 channels. Glibenclamide binding to native channels from pancreatic MIN6 cells was also displaced by MPB-91. A Kir6.2 mutant able to form channels without SUR was also blocked by MPB-91, but not by MPB-07. These observations demonstrate that neither MPB-07 nor MPB-91 interact with SUR, in spite of its high homology with CFTR, and that MPB-91 blocks K_{ATP} channels by binding to the Kir6.2 subunit. Thus, caution should be exercised when planning to use MPB compounds in cystic fibrosis therapy, specially MPB-91 which could nonetheless find interesting applications as the precursor of a new class of K channel blockers.

Keywords: CFTR; Sulfonylurea receptor; ATP-sensitive potassium channel; ABC transporter; Glibenclamide; Kir6.2

1. Introduction

CFTR is a chloride channel primarily found in epithelial cells. Mutations in the CFTR gene reducing the channel ability to transport anions are responsible for CF, the most common lethal genetic disease [1]. Many disease-causing mutations do not compromise the integrity of the channel protein but act in more subtle ways either by impairing normal trafficking and thereby reducing the number of channels able to reach the plasmalemma (e.g. ΔF508, the most common CF mutation responsible for ~70% of the cases) or by altering the gating mechanisms to yield an abnormally low open probability (e.g. G551D, the second most common CF mutation responsible for ~3% of the cases). In those situations, a possible treatment is conceivable using pharmacological agents designed to target CFTR and counteract the effects of the mutations [2]. This line of research led to the discovery of CFTR activators of the benzo[c]quinolizinium class [3]. In this family of molecules, designated MPB, some have been found not only to augment the open probability of wild-type and G551D CFTR [4] but also to partly correct the trafficking defect of the ΔF508 mutant [5]. Although the mechanism of action of MPB compounds is unclear, the evidence points to a direct interaction of MPB compounds with CFTR. It is therefore possible that these compounds could affect other proteins homologous to CFTR, such as other members of the ABC transporter family [6]. In terms of

* Corresponding author. Tel.: +33-4-38-78-48-67; fax: +33-4-38-78-54-87.
E-mail address: vivaudou@cea.fr (M. Vivaudou).

Abbreviations: ABC, ATP-binding cassette; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; DMSO, dimethyl sulfoxide; HEK, human embryonic kidney; K_{ATP}, ATP-sensitive potassium channel; Kir, inward-rectifier potassium channel; SUR, sulfonylurea receptor.

0006-2952/$ – see front matter
doi:10.1016/S0006-2952(03)00289-2

sequence, function and pharmacology, CFTR appears closest to the SUR, an ABC transporter which assembles with the inward-rectifying potassium channel Kir6.2 to form the K_{ATP} channels [7,8]: SUR displays ~37% primary sequence homology with CFTR, is part of an ion channel, and is sensitive to drugs, K channel blockers and openers [9], which also regulate CFTR [10,11].

In order to evaluate the specificity of action of two proven CFTR activators, MPB-07 and MPB-91, we have measured their effects on K_{ATP} channels and found that MPB-07 was ineffective while MPB-91 was a blocker of moderate affinity. Contrary to expectations, this effect of MPB-91 was found to be mediated by the Kir6.2 subunit and not the SUR subunit. Thus, in addition to their CFTR activating capacity, MPB compounds may have K channel blocking properties. These combined effects could interfere with therapeutic use against CF since in the case of MPB-91 the concentrations needed to activate CFTR are above those necessary to block K_{ATP} channels [4]. Nonetheless, this work demonstrates that MPB compounds have specificity for CFTR over its homologue SUR and that their K channel blocking activity may be curtailed by subtle changes in structure (e.g. MPB-07 vs. MPB-91) which do not compromise their CFTR activating potential.

2. Materials and methods

2.1. Electrophysiology

Methods and clones were as described previously [12]. The truncated construct Kir6.2Δ26 lacking the last 26 amino acids of Kir6.2 was created by engineering a premature stop codon at the correct position. Kir6.2 and SURs were coexpressed in *Xenopus laevis* oocytes and characterized by the patch–clamp technique in the excised inside-out configuration. Patch pipettes contained (total, in mM): 154 K^+, 146 Cl^-, 5 Mg^{2+}, and 10 PIPES (pH 7.1). The cytoplasmic face of the patch was bathed in solutions which all contained (total, in mM): 174 K^+, 40 Cl^-, 1 EGTA, 1 Mg^{2+}, 10 PIPES (pH 7.1), and methanesulfonate as the remaining anions. ATP (potassium salt; Sigma Chemical Co.) was added as specified.

The membrane potential was maintained at −50 mV. Experiments were conducted at room temperature (20–22°).

Applications of the various solutions to the intracellular face of the patch was performed using a RSC-100 rapid-solution-changer (Bio-Logic). Drops in current upon solution switching evident in some figures represent normal artefacts resulting from brief exposure to the ATP-containing oocyte bath solution.

2.2. Binding studies

Membrane extracts from HEK-293 cells expressing SUR1/Kir6.2 fused in tandem with an hexaglycine linker and from MIN6 cells (a mouse β cell line kindly provided by Dr. H. Ishihara, Tokyo, Japan) were prepared as previously described [13]. On the day of the experiment, membranes were resuspended in 50 mM Tris–HCl, pH 7.5. Membranes (200 μg protein/mL) were incubated for 1 hr at room temperature in a final volume of 500 μL with 3.5 nM [^{3}H]glibenclamide (50 Ci/mmol, DuPont-NEN), in the presence of various concentrations of MPB. Non-specific binding was determined in the presence of excess glibenclamide. Bound and free radiolabeled ligands were separated by filtration under vacuum on GF/B glass fiber filters (Whatman) before ^{3}H counting in a scintillation medium (ACS II, Amersham).

MPB-07 and MPB-91 were prepared from stock solution of 25 mM in water and 50 mM in DMSO, respectively. The vehicle DMSO by itself, at the concentrations used, had little or no effect on channel activity [14]. The highest concentration of DMSO used in binding assays, 6.3%, corresponding to 3.2 mM MPB-91, reduced only weakly glibenclamide binding to 94.3 ± 3.1% of its maximal value.

Model fitting to the data points obtained at various concentrations of MPB was using a standard Hill equation:

$$f(|\mathrm{MPB}|) = \frac{\mathrm{Max}}{1 + (|\mathrm{MPB}|/K_{1/2})^h}$$

where $K_{1/2}$ is the concentration for half-maximal inhibition and h is the Hill coefficient.

Results are displayed as mean ± SEM.

3. Results

We selected two MPB compounds for this study, MPB-07 (6-hydroxy-10-chlorobenzo[c]quinolizinium chloride) and MPB-91 (5-butyl-6-hydroxy-10-chlorobenzo[c]-quinolizinium chloride), which have been well documented for their activatory effects on normal and defective CFTR proteins [3–5]. The chemical structures of these molecules are shown in Fig. 1a. The functional effects of these drugs on recombinant K_{ATP} channels were assessed using the patch–clamp technique in the inside-out configuration. K_{ATP} channels were reconstituted in *Xenopus* oocytes by coinjecting cRNA coding for the two subunits of these channels, Kir6.2, and either the pancreatic/neuronal isoform SUR1 or the cardiac muscle isoform SUR2A. Both isoforms were tested since their responses to known blockers and openers greatly differ [12,14,15].

In the absence of internal ATP where K_{ATP} channels are maximally open, MPB-07 and MPB-91 at a concentration of 50 μM had very distinct effects. The former had little visible effect while the latter caused a channel blockade which took 20–40 s to reach its full extent and reversed within minutes (Fig. 1b and c). Results for seven patches show a <10% inhibition by MPB-07 and a ~75% inhibition by MPB-91 (Fig. 1d). If the responses of the cardiac SUR2A/Kir6.2 and pancreatic SUR1/Kir6.2 channels were

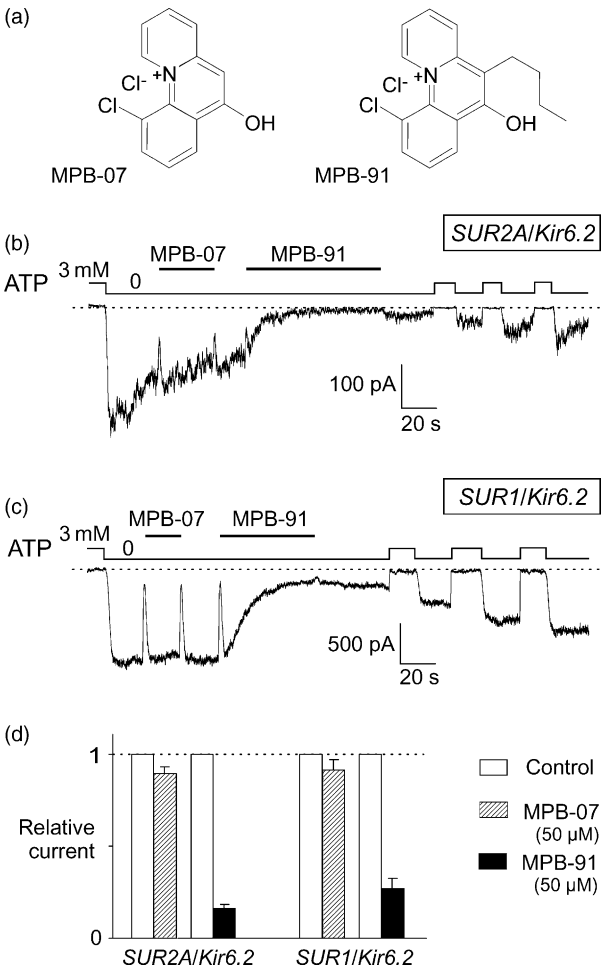

Fig. 1. Effects of benzo[c]quinolizinium compounds on recombinant K_{ATP} channels. (a) Chemical structure of the two compounds tested [3]. (b) Representative current trace recorded at −50 mV from an inside-out patch excised from a *Xenopus* oocyte coinjected with Kir6.2 and SUR2A and exposed to 50 µM MPB-07 and 50 µM MPB-91. (c) *Idem* for SUR1. (d) Average effects (mean ± SEM) of MPB-07 and MPB-91 observed in 14 distinct patches using the above experimental protocols. Currents are normalized to the control value recorded immediately prior to drug application.

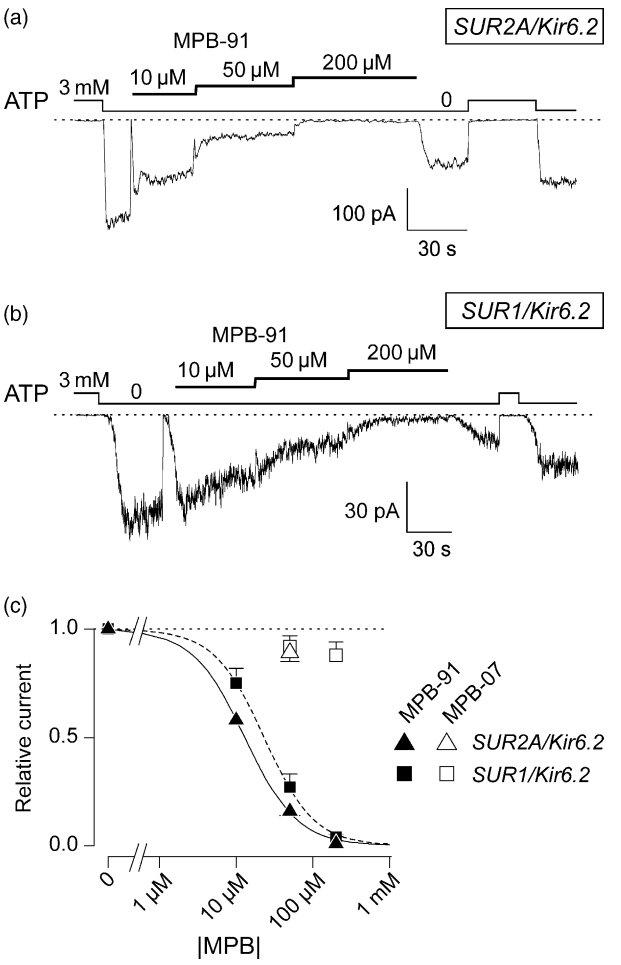

Fig. 2. Concentration-dependent block by MBP-91 of recombinant K_{ATP} channels. (a) Representative patch–clamp record illustrating the responses to increasing concentrations of MPB-91 of SUR2A/Kir6.2 channels expressed in *Xenopus* oocytes. (b) *Idem* for SUR1. (c) Average effects of various concentrations of MPB-91 (filled symbols) and MPB-07 (open symbols) on activity of SUR1/Kir6.2 channels (squares; N = 7) and SUR2A/Kir6.2 channels (triangles; N = 7). Fitting of a standard Hill equation to the MPB-91 data yielded $K_{1/2} = 13$ µM and $h = 1.3$ for SUR2A/Kir6.2 (continuous line) and $K_{1/2} = 23$ µM and $h = 1.3$ for SUR1/Kir6.2 (dashed line).

similar, the cardiac channels displayed a slightly greater sensitivity and a more rapid response to block by MPB-91. Kinetics were however not quantified because the rate constants for inhibition onset and offset were highly variable from patch to patch. As always with the *Xenopus* oocyte expression system, expression levels varied but we observed no correlation between current density and responses to MPB compounds.

The presence on intracellular nucleotides did not change these observations. When these experiments were repeated with SUR1/Kir6.2 channels in the presence of a partially-inhibiting concentration of ATP (100 µM), MPB-07 remained ineffective and MPB-91 at 50 µM blocked ∼60% of the current (57 ± 12%; three patches; data not shown). There was no obvious linkage between the degree of ATP inhibition and the magnitude of the MPB-91 block suggesting no preference for either open or closed state.

Figure 2 illustrates experiments with varying concentrations of MPB compounds up to 200 µM. The effects of MPB-07 was not significantly augmented at 200 µM vs. 50 µM (12% vs. 8% inhibition of SUR1/Kir6.2 channels). The concentration-dependent block of channel activity by MPB-91 corresponded well with the classical behaviour of a full antagonist. Fitting of the activity vs. concentration curves for either channel isoforms yielded a Hill coefficient close to 1 with half-inhibition concentrations of 13 and 23 µM for SUR2A and SUR1, respectively. Statistical tests on the relative current values measured at 50 µM MPB-91 (0.27 ± 0.06% for SUR1 and 0.16 ± 0.02% for SUR2A) show that the observed differences were not significant ($P > 0.1$; Student's unpaired *t*-test).

It was expected that any effect of MPB compounds on K_{ATP} channels would involve the SUR subunit because of its strong homology with CFTR. The similitude of the

responses of the cardiac and pancreatic channels led us to consider a possible interaction with the common Kir6.2 subunit. The intact Kir6.2 subunit cannot be tested alone since it possesses within its C-terminal extremity an endoplasmic reticulum retention signal which needs to be masked by SUR to enable it to leave the endoplasmic reticulum and reach the plasma membrane [16]. It is however possible to bypass this trafficking checkpoint by simply cutting off the C-terminal end of Kir6.2 thereby removing the retention signal. We have used such a truncated mutant, Kir6.2Δ26, which, when expressed in oocytes forms plasmalemmal ATP-inhibited K^+ channels [17] although at a lower density than SUR-carrying K_{ATP} channels. As shown in Fig. 3, this truncated channel was blocked by MPB-91 but not by MPB-07. The concentration-dependent inhibition by MPB-91 resembled that observed with the full-featured channels with a Hill coefficient of 1.1 and half-inhibitory concentration of 34 μM. This latter value is slightly higher than those recorded with SUR/Kir6.2 channels but the difference is small. At 50 μM MPB-91, the relative currents measured with Kir6.2Δ26 and SUR1/Kir6.2 were not statistically different ($P > 0.1$; Student's unpaired *t*-test).

These functional studies were completed by binding studies where we measured the displacement by MPB compounds of the binding of [^{3}H]glibenclamide to membrane

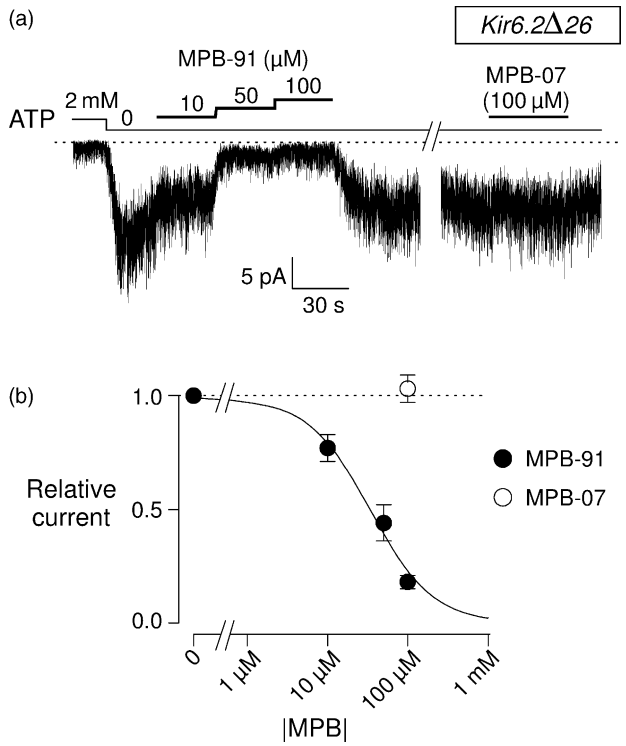

Fig. 3. MPB-91 block is mediated by the Kir6.2 subunit. (a) Representative patch–clamp record from an oocyte expressing a truncated Kir6.2 subunit able to form K_{ATP} channels without SUR subunits. (b) Average effects of MPB-91 (filled circles) and MPB-07 (open circles) measured in seven patches using above protocol. Fitting of a standard Hill equation to the MPB-91 data, shown as a continuous line, yielded $K_{1/2} = 34$ μM and $h = 1.1$.

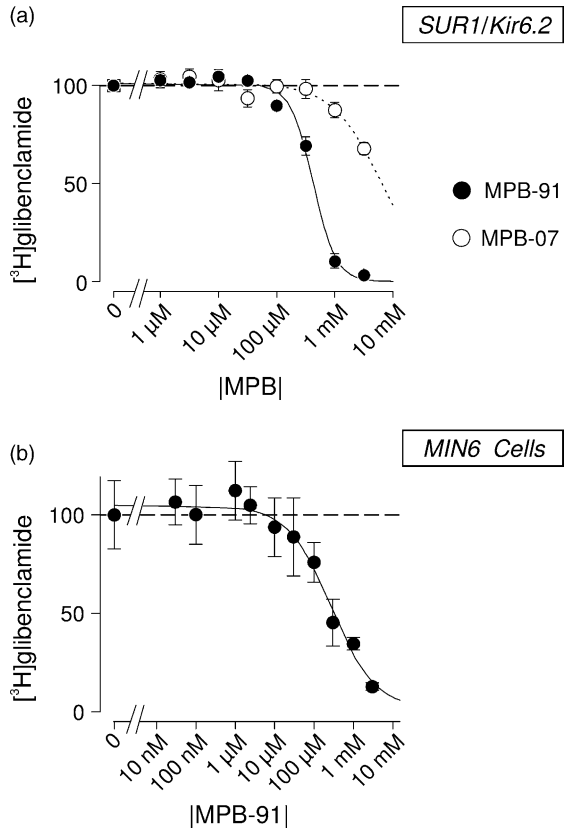

Fig. 4. MPB-91 displaces binding of glibenclamide to recombinant and native pancreatic K_{ATP} channels. (a) Membranes from HEK-293 cells transfected with a SUR1/Kir6.2 tandem construct were incubated with [^{3}H]glibenclamide in the presence of various concentrations of either MPB-91 (filled circles) or MPB-07 (open circles). Mean specific binding, expressed as a percent of its value in absence of MPB drugs, was measured in four (MPB-91) and five (MPB-07) separate experiments, each performed in triplicate. Fitting of a standard Hill equation yielded $K_{1/2} = 430$ μM and $h = 2.2$ for MPB-91 (continuous line) and $K_{1/2} = 6.4$ mM and $h = 1.0$ for MPB-07 (dashed line). (b) Results from similar experiments with MIN6 mouse insulinoma cells. Points are averaged from two experiments performed in duplicates. Best fit of the data points, shown as a continuous line, was obtained with $K_{1/2} = 270$ μM and $h = 0.8$.

preparations containing either native or recombinant K_{ATP} channels (Fig. 4). To study native channels, we used membrane preparations from the pancreatic cell line MIN6 which strongly express SUR1 and Kir6.2 [18]. These membranes were compared to membranes from HEK-293 cells overexpressing SUR1 and Kir6.2 fused together with an hexaglycine linker [13]. Non-specific binding measured with 100 mM glibenclamide accounted for 0.6 ± 0.05 and $0.8 \pm 0.1\%$ of total binding to membranes from HEK-293 transfected with the empty vector and the vector containing the SUR1/Kir6.2 cDNA, respectively. Glibenclamide at 10 mM was also used with equivalent results. Such high concentrations are unusual but were chosen to be at least 10-fold the highest concentration of MPB tested to ensure complete removal of all non-specific binding.

Qualitatively, the binding data matched the functional data. In HEK-293 cells transfected with the tandem SUR1/Kir6.2, MPB-91 was able to fully displace specific binding

with a half-effective concentration of 430 μM while partial displacement could only be achieved at 15-fold higher concentrations of MPB-07 (Fig. 4a). In MIN6 cells (where MPB-07 was not tested) MPB-91 displaced specific binding to native SURs in the same range of concentrations with a $K_{1/2}$ of 270 μM (Fig. 4b) with a lower Hill coefficient probably due to a greater variability in the measurements.

4. Discussion

CFTR and SUR have much in common in terms of primary structure and pharmacology. The objective of this study was to evaluate whether newly developed CFTR activators of the benzo[c]quinolizinium class [3] were able to also interact with SUR. Two promising compounds, MPB-07 and MPB-91, were tested in this work using electrophysiological and biochemical techniques.

Since SUR is physiologically associated with the K^+ channel protein Kir6.2 to form the K_{ATP} channel, we have examined with the patch–clamp technique the functional effects of MPB-07 and MPB-91 on K_{ATP} channels heterologously expressed in *Xenopus* oocytes. When applied to the cytoplasmic face of excised patches, MPB-07 had little or no effect on channel activity and MPB-91 was found to fully block the channels. These results applied to the two types of K_{ATP} channel tested, the β-cell channels formed by Kir6.2 and the isoform SUR1 [7] and the cardiac muscle channel formed by Kir6.2 and the isoform SUR2A [19]. Unexpectedly these observations were repeated with SUR-less channels constituted solely of Kir6.2Δ26, an altered version of Kir6.2 able to reach the plasmalemma on its own [17]. The IC_{50}s for MPB-91 block were 13, 23 and 34 μM for SUR2A/Kir6.2, SUR1/Kir6.2, and Kir6.2Δ26, respectively, and the Hill coefficient was near 1 in the three cases. These values are not identical but clearly close enough to support the hypothesis that MPB-91 closes channels by interacting only with Kir6.2 with a slight dependency on the quaternary structure of the channel complex.

Although the electrophysiological data demonstrate unambiguously the interaction of MPB compounds with K_{ATP} channels, this was further examined by measuring the displacement of a radiolabelled sulfonylurea, [^{3}H]glibenclamide, from both recombinant and native SUR1/Kir6.2 channels. Either subunit of the K_{ATP} channel possess inhibitory glibenclamide binding sites which are connected to the channel gate on Kir6.2 [15,20]. SUR binds glibenclamide with nM affinity and Kir6.2 with μM affinity. MPB-91 displaced all [^{3}H]glibenclamide binding but the concentrations required were at least an order of magnitude higher than those needed to trigger channel closure. MPB-07 up to 1 mM had feeble effects on [^{3}H]glibenclamide binding, in line with its failure to affect K_{ATP} channels.

Electrophysiological data demonstrate that MPB-91 binds to the Kir6.2 subunit. Since no radiolabelled Kir6.2-specific ligand was available, we used radiolabelled glibenclamide which binds to the SUR1 subunit with high affinity. Although MPB-91 and glibenclamide target different proteins, these proteins are tightly coupled within the K_{ATP} channel. Displacement of glibenclamide binding by MPB-91 is not therefore unexpected. Another example of such an interaction between a Kir6.2 ligand and a SUR ligand is that between ATP and K_{ATP} channel openers: ATP acting on the Kir6.2 inhibitory nucleotidic site shifts the apparent affinity of SUR for openers, and vice versa [21,22]. Also it has been reported that sulfonylurea binding to SUR is directly affected by the Kir6.2 subunit [23].

In agreement with the patch–clamp measurement, we do observe that MPB-91 interacts with native and reconstituted SUR1/Kir6.2 channels by displacing glibenclamide binding. However, the concentrations effective in the binding assay are significantly higher than in the functional assay.

Such discrepancies are not uncommon and could arise from the widely different environments of the channels in those experiments. They could also reflect a shift by glibenclamide of MPB-91 affinity resembling the shift by openers of ATP sensitivity [21,22]. Another possible explanation could be that binding to only one of the four Kir6.2 subunits of the channel is sufficient to cause closure. In that case, a roughly 5-fold shift between the functional and biochemical effects would be expected [24].

The observed differences between MPB-91 and MPB-07 contrast with the similitude of their structure. Both possess the benzo[c]quinolizinium core structure with a positively charged ammonium but MPB-91 has an added butyl group. This group should make the molecule more hydrophobic and more able to cross membranes to reach a possible external site when applied on the cytosolic face of an inside-out patch. This hypothesis does not hold, however, because MPB-07 is able to freely cross membranes since Becq *et al.* [3] described effect of MPB-07, applied extracellularly, on CFTR current recorded in cell-attached patches. This suggests that MPB-91 is a better inhibitor because it has a higher affinity for a specific binding site and not because it is more likely to reach a general MPB binding site.

MPB compounds were designed with CF in mind. Our findings demonstrate that these molecules can modulate other ion channels besides CFTR. The resulting side effects will have to be considered carefully since, in the case of MPB-91, there is a definite overlap between the CFTR-activating concentrations and the K^+-channel blocking concentrations: EC_{50} of MPB-91 have been reported to be 47 μM for activation of wild-type CFTR and 85 μM for activation of the mutant G551D-CFTR [4] while we measured here values of 13 and 23 μM for inhibition of SUR2A/Kir6.2 and SUR1/Kir6.2 channels. Controlling these side effects appears feasible since small structural changes around the MPB backbone (as between MPB-07 and MPB-91) are sufficient to abolish action on K^+ channels. On the other hand, MPB-91 could prove useful as a lead toward a novel class of K channel blockers.

In conclusion, two MPB compounds designed to activate CFTR were tested on K_{ATP} channels. MPB-07 was ineffective but MPB-91 acted on the Kir6.2 subunit to block these channels at μM concentrations. We found no evidence that these compounds interacted with SUR in spite of its close homology with CFTR.

Acknowledgments

We thank Y. Mettey and C. Marivingt-Mounir for the synthesis of MPB derivatives and E. Garcia for expert technical assistance. We are grateful to J. Bryan (Houston, TX) for SUR1, S. Seino (Chiba, Japan) for Kir6.2 and SUR2A and D. Logothetis (New York, NY) for the Kir6.2Δ26 construct.

This work was made possible by grants from AFLM (Association Française de Lutte contre la Mucoviscidose) with additional support provided by CEA (Commissariat à l'Energie Atomique), CNRS (Centre National de la Recherche Scientifique). A.L.P. and R.D. were supported by fellowships from CEA and AFLM, respectively.

References

[1] Greger R, Schreiber R, Mall M, Wissner A, Hopf A, Briel M, Bleich M, Warth R, Kunzelmann K. Cystic fibrosis and CFTR. Pflugers Arch-Eur J Physiol 2001;443:S3–7.

[2] Hwang TC, Sheppard DN. Molecular pharmacology of the CFTR Cl^- channel. Trends Pharmacol Sci 1999;20:448–53.

[3] Becq F, Mettey Y, Gray MA, Galietta LJV, Dormer RL, Merten M, Metaye T, Chappe V, Marvingt-Mounir C, Zegarra-Moran O, Tarran R, Bulteau L, Dérand R, Pereira MMC, McPherson MK, Rogier C, Joffre M, Argent BE, Sarrouilhe D, Kammouni W, Figarella C, Verrier B. Development of substituted benzo[c]quinolizinium compounds as novel activators of the cystic fibrosis chloride channel. J Biol Chem 1999;274:27415–25.

[4] Dérand R, BulteauPignoux L, Mettey Y, ZegarraMoran O, Howell LD, Randak C, Galietta LJV, Cohn JA, Norez C, Romio L, Vierfond JM, Joffre M, Becq F. Activation of G551D CFTR channel with MPB-91: regulation by ATPase activity and phosphorylation. Am J Physiol 2001;281:C1657–66.

[5] Dormer RL, Dérand R, Mcneilly CM, Mettey Y, BulteauPignoux L, Metaye T, Vierfond JM, Gray MA, Galietta LJV, Morris MR, Pereira MMC, Doull IJM, Becq F, Mcpherson MA. Correction of delF508-CFTR activity with benzo(c)quinolizinium compounds through facilitation of its processing in cystic fibrosis airway cells. J Cell Sci 2001;114:4073–81.

[6] Dean M, Rzhetsky A, Allikmets R. The human ATP-binding cassette (ABC) transporter superfamily. Genome Res 2001;11: 1156–66.

[7] Inagaki N, Gonoi T, Clement JP, Namba N, Inazawa J, Gonzalez G, Aguilar-Bryan L, Seino S, Bryan J. Reconstitution of I-KATP: an inward rectifier subunit plus the sulfonylurea receptor. Science 1995; 270:1166–70.

[8] Seino S. ATP-sensitive potassium channels: a model of heteromultimeric potassium channel/receptor assemblies. Annu Rev Physiol 1999; 61:337–62.

[9] Terzic A, Vivaudou M. Molecular pharmacology of ATP-sensitive K^+ channels: how and why? In: Archer SL, Rusch NJ, editors. Potassium channels in cardiovascular biology. New York: Kluwer Academic Publishers/Plenum Press; 2001. p. 257–77.

[10] Sheppard DN, Welsh MJ. Effect of ATP-sensitive K^+ channel regulators on cystic fibrosis transmembrane conductance regulator chloride currents. J Gen Physiol 1992;100:573–91.

[11] Cai Z, Lansdell KA, Sheppard DN. Inhibition of heterologously expressed cystic fibrosis transmembrane conductance regulator Cl^- channels by non-sulphonylurea hypoglycaemic agents. Br J Pharmacol 1999;128:108–18.

[12] Moreau C, Jacquet H, Prost AL, D'Hahan N, Vivaudou M. The molecular basis of the specificity of action of K-ATP channel openers. EMBO J 2000;19:6644–51.

[13] Gros L, Trapp S, Dabrowski M, Ashcroft FM, Bataille D, Blache P. Characterization of two novel forms of the rat sulphonylurea receptor SUR1A2 and SUR1BDelta31. Br J Pharmacol 2002;137:98–106.

[14] D'hahan N, Moreau C, Prost AL, Jacquet H, Alekseev AE, Terzic A, Vivaudou M. Pharmacological plasticity of cardiac ATP-sensitive potassium channels toward diazoxide revealed by ADP. Proc Natl Acad Sci USA 1999;96:12162–7.

[15] Gribble FM, Tucker SJ, Seino S, Ashcroft FM. Tissue specificity of sulfonylureas: studies on cloned cardiac and beta-cell K-ATP channels. Diabetes Care 1998;47:1412–8.

[16] Zerangue N, Schwappach B, Jan YN, Jan LY. A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K-ATP channels. Neuron 1999;22:537–48.

[17] Tucker SJ, Gribble FM, Zhao C, Trapp S, Ashcroft FM. Truncation of Kir6.2 produces ATP-sensitive K^+ channels in the absence of the sulphonylurea receptor. Nature 1997;387:179–83.

[18] Ashfield R, Ashcroft SJH. Cloning of the promoters for the beta-cell ATP-sensitive K-channel subunits Kir6.2 and SUR1. Diabetes Care 1998;47:1274–80.

[19] Inagaki N, Gonoi T, Clement JP, Wang CZ, Aguilar-Bryan L, Bryan J, Seino S. A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels. Neuron 1996;16: 1011–7.

[20] Gros L, Virsolvy A, Salazar G, Bataille D, Blache P. Characterization of low-affinity binding sites for glibenclamide on the Kir6.2 subunit of the beta-cell K-ATP channel. Biochem Biophys Res Commun 1999; 257:766–70.

[21] Thuringer D, Escande D. Apparent competition between ATP and the potassium channel opener RP 49356 on ATP-sensitive K^+ channels of cardiac myocytes. Mol Pharmacol 1989;36:897–902.

[22] Forestier C, Pierrard J, Vivaudou M. Mechanism of action of K channel openers on skeletal muscle K-ATP channels—interactions with nucleotides and protons. J Gen Physiol 1996;107:489–502.

[23] Hambrock A, LofflerWalz C, Russ U, Lange U, Quast U. Characterization of a mutant sulfonylurea receptor SUR2B with high affinity for sulfonylureas and openers: differences in the coupling to Kir6.x subtypes. Mol Pharmacol 2001;60:190–9.

[24] Dorschner H, Brekardin E, Uhde I, Schwanstecher C, Schwanstecher M. Stoichiometry of sulfonylurea-induced ATP-sensitive potassium channel closure. Mol Pharmacol 1999;55:1060–6.