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Activation of KCNQ5 channels stably expressed in HEK293 cells by BMS-204352

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Abstract

The novel anti-ischemic compound, BMS-204352 ((3*S*)-(+)-(5-chloro-2-methoxyphenyl)-1,3-dihydro-3-fluoro-6-(trifluoromethyl)-2*H*-indol-2-one)), strongly activates the voltage-gated K^+ channel KCNQ5 in a concentration-dependent manner with an EC_{50} of 2.4 μM. At 10 μM, BMS-204352 increased the steady state current at −30 mV by 12-fold, in contrast to the 2-fold increase observed for the other KCNQ channels [Schrøder et al., 2001]. Retigabine ((D-23129; *N*-(2-amino-4-(4-fluorobenzylamino)-phenyl) carbamic acid ethyl ester) induced a smaller, yet qualitatively similar effect on KCNQ5. Furthermore, BMS-204352 (10 μM) did not significantly shift the KCNQ5 activation curves (threshold and potential for half-activation, $V_{1/2}$), as observed for the other KCNQ channels. In the presence of BMS-204352, the activation and deactivation kinetics of the KCNQ5 currents were slowed as the slow activation time constant increased up to 10-fold. The M-current blockers, linopirdine (DuP 996; 3,3-bis(4-pyridinylmethyl)-1-phenylindolin-2-one) and XE991 (10,10-bis(4-pyridinylmethyl)-9(10*H*)-anthracenone), inhibited the activation of the KCNQ5 channel induced by the BMS-204352. Thus, BMS-204352 appears to be an efficacious KCNQ channels activator, and the pharmacological properties of the compound on the KCNQ5 channel seems to be different from what has been obtained on the other KCNQ channels.

Keywords: M channel; Linopirdine; XE 991; M current

1. Introduction

M currents are a subset of native K^+ currents recorded from various neurones such as sympathic, hippocampal and cortical neurones (Brown, 1988; Marrion, 1997). These currents are voltage-dependent currents activated by depolarisation, they are non-inactivating, blocked by muscarinic M1 receptor stimulation and serve to stabilize the membrane potential, and thus, reduce neuronal excitability. KCNQ channels have been suggested to be the molecular counterpart of the M channel and M-like channels (Wang et al., 1998; Schroeder et al., 2000; Selyanko et al., 2000; Shapiro et al., 2000; Søgaard et al., 2001). KCNQ channels are voltage-gated K^+ channels expressed in both excitable and epithelial tissues (Jentsch, 2000). They possess a membrane topology similar to the K_v channels, comprising six transmembrane-spanning segments (S1–S6) with a typical S4 domain, which is the voltage sensor, a pore loop linking S5 and S6, and intracellular N and C termini. KCNQ1 is expressed in the heart where it co-assembles with KCNE1, a small β-subunit protein with a single transmembrane domain, to generate the slowly activating delayed rectifier Ik_s current (Barhanin et al., 1996; Sanguinetti et al., 1996; Yang et al., 1997). Mutations in either subunit result in prolongation of the cardiac action potential and an increased risk of ventricular arrhythmia in patients with long QT-syndrome (Keating and Sanguinetti, 1996; Chouabe et al., 1997; Sanguinetti, 1999; Suessbrich and Busch, 1999). KCNQ2 and KCNQ3 are expressed exclusively in the nervous system (Tinel et al., 1998; Yang et al., 1998). Mutations in either KCNQ2 or KCNQ3 have been linked to the Benign Familial Neonatal Epilepsy (Bievert et al., 1998; Charlier et al., 1998; Singh et al., 1998), a specialized form of epilepsy caused by a reduction of just 25% in KCNQ channel conductance (Schroeder et al., 2000).

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KCNQ2, KCNQ3 and KCNQ2/KCNQ3 heteromeric channels can be modulated by muscarinic stimulation when expressed with the M1 muscarinic receptor in mammalian cells (Selyanko et al., 2000; Shapiro et al., 2000). M currents are sustained currents in the range of the action potential initiation and their slow activation and deactivation are important for their function as a brake for repetitive action potential firing. The inhibition of M currents therefore leads to enhance neuronal excitability. Linopirdine and XE991, specifics inhibitors of M current (Lamas et al., 1997), block KCNQ2/KCNQ3 with similar affinity. The notion that neuronal KCNQ channels mediate M current is interesting in the context that mutations in at least KCNQ2/KCNQ3 may cause epilepsy. Positive M-current modulators may thus prove useful in the treatment of epilepsy. KCNQ4 is another member of this ion channel subfamily and is expressed, in sensory outer hair cells of the cochlea, as well as certain nuclei and tracts of the brain stem (Kubisch et al., 1999; Kharkovets et al., 2000). The KCNQ4 gene is mutated in one form of nonsyndromic autosomal dominant deafness (Coucke et al., 1999; Kubisch et al., 1999). KCNQ4 yields currents that kinetically resemble to M-currents (Selyanko et al., 2000; Søgaard et al., 2001) and can also be inhibited by M1 muscarinic receptor agonist (Selyanko et al., 2000). Recently, one additional member of the KCNQ family, the KCNQ5 channel, has been cloned (Lerche et al., 2000; Schroeder et al., 2000). KCNQ5 is expressed in skeletal muscle and shows widespread expression in the central nervous system. The expression of KCNQ5 is in many brain regions overlapping with the expression of KCNQ2 and KCNQ3 (Lerche et al., 2000; Schroeder et al., 2000). As with KCNQ2 and KCNQ3, in situ hybridisation has revealed broad expression of KCNQ5 in the brain, including the cortex and the hippocampus. It is also expressed in rat superior cervical ganglion and in NG108-15 cell (Schroeder et al., 2000), a cell line on which many studies on M current have been conducted. Like the other KCNQ subunits, KCNQ5 yields currents that activate slowly upon depolarisation. It may form heteromeric channels with KCNQ3, but not with KCNQ1, KCNQ2 or KCNQ4 (Lerche et al., 2000; Schroeder et al., 2000). KCNQ5 is inhibited by the M1 muscarinic receptor activation and shows pharmacological properties suggesting that it plays a role in generating M currents or M-like currents (Lerche et al., 2000; Schroeder et al., 2000). These findings suggest that KCNQ2–5 channel subunits might combine to produce different variants of M current in different parts of the nervous system.

Retigabine (D-23129) is a novel anti-epileptic compound with a broad spectrum and potent anticonvulsant properties, both in vitro and in vivo (Rundfeldt, 1997). The compound was shown to activate a K^+ current, which has a pharmacology displaying concordance with that of the M channel (Rostock et al., 1996; Main et al., 2000; Rundfeldt and Netzer, 2000; Wickenden et al., 2000, 2001). Retigabine acts as an opener of KCNQ2/KCNQ3 channels (Main et al., 2000; Rundfeldt and Netzer, 2000; Schrøder et al., 2001; Wickenden et al., 2001) by dramatically shifting the voltage activation curve to more negative potentials, and by accelerating the rate of activation and slowing the deactivation (Main et al., 2000; Wickenden et al., 2000). Subsequent studies have shown that this compound also activates the homomeric KCNQ2, KCNQ3, KCNQ4, and the heteromeric KCNQ4/KCNQ3 and KCNQ5/KCNQ3 channels (Schrøder et al., 2001; Tatulian et al., 2001; Wickenden et al., 2001).

Recently, another compound, BMS-204352 has been demonstrated to activate KCNQ channels (Schrøder et al., 2001). BMS-204352 is a fluoro-oxindole BK channel opener effective in the treatment of experimental acute stroke (Cheney et al., 2001; Gribkoff et al., 2001). However, the molecular mechanism of this compound might also include opening of KCNQ2, KCNQ2/KCNQ3, KCNQ4 and KCNQ3/KCNQ4 channels (Schrøder et al., 2001). BMS-204352 activates KCNQ2-KCNQ4 channels to a similar degree, as does retigabine.

In the present study, we have used the patch clamp technique to examine the effect of the BMS-204352 on the KCNQ5 channel stably expressed in Human Embryonic Kidney cells (HEK293). In contrast to what was previously observed with the KCNQ2, KCNQ2/KCNQ3, KCNQ4, KCNQ3/KCNQ4 (Schrøder et al., 2001), we show here that the KCNQ5 channel is activated by the BMS-204352 compound with a higher efficacy than by retigabine.

2. Materials and methods

2.1. Plasmids and cell lines

The KCNQ5 expressing plasmid, pKCNQ5-pac, was constructed by substituting the KCNQ2 in the pKCNQ2-pac (Schrøder et al., 2001) plasmid with the KCNQ5 coding sequence (polymerase chain reaction (PCR) generated) from pTNPN-KCNQ5 (kindly provided by T. Jentsch). The KCNQ5 sequence is initiated by a Kozak consensus sequence. After PCR and subcloning, the integrity of the KCNQ5 sequence was confirmed by sequencing.

HEK293 cells were grown in Dulbecco's Modified Eagle Medium (D-MEM, Life Technologies) supplemented with 10% fetal calf serum (Life Technologies) at 37 °C in 5% CO_2. One day prior to transfection, 2×10^6 cells were plated in a cell culture T75 flask (Nunc). Cells were transfected with 2 μg of plasmid using Lipofectamine and PLUS reagent (Life Technologies) according to the manufacturer's instructions. Two days post transfection cells were selected in media supplemented with 1 μg/ml puromycin (Sigma). Transfected cells were grown in selection media for more than 14 days, where after the appearing colonies were pooled and single cells from this cell population was used for monoclonal cell lines. The expression level of the monoclonal cell lines was measured by electrophysiology.

2.2. Electrophysiology

Transfected cells were cultured on glass coverslips (3 mm ∅), which were transferred to a small recording chamber mounted on an inverted microscope (IX-70 Olympus). The recording chamber was continuously perfused with Na–Ringer at a rate of 1 ml/min to give a complete exchange of the bath solution (15 μl chamber) each second. Borosilicate glass pipettes were pulled to have resistances in the range of 1.5–3.0 MΩ. A Ag/AgCl pellet electrode was permanently embedded in the chamber. The electrode off-set potential was zeroed just before the giga-sealing. C-fast and C-slow cancellations were done just after establishment of the cell attached and the whole cell configurations, respectively. Series resistance values (Rs) were updated before each current sweep, and experiments where the Rs value increased to 10 MΩ or more were discharged. A constant Rs compensation of 70% was performed throughout the experiments. The electrophysiological recordings were performed with an EPC-9 amplifier (HEKA electronics, Germany). A steady current level was always obtained prior to electrophysiological analysis and drug application. The current was not leak-substracted. All experiments were performed at room temperature. The experimental protocols were designed by Pulse software. The IGOR program (WaveMetrics, Lake Oswego, USA) and the GraphPad Prism software (GraphPad Software, San Diego, CA) were used for analysis and presentation of the data.

2.3. Solutions and chemicals

2.3.1. Extracellular ringer

The Na-Ringer consisted of (in mM): 140 NaCl, 4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES. The extracellular solution was adjusted to pH 7.4 by NaOH.

2.3.2. Intracellular ringer

The intracellular pipette solution used was (in mM): 120 KCl, 5.37 $CaCl_2$, 1.75 $MgCl_2$, 30/10 KOH/EGTA, 10 HEPES, 1.6 ATP. The free intracellular Ca^{2+}, Mg^{2+} and ATP concentrations were calculated to be 100 nM, 200 μM and 1 mM, respectively (using Eqcal software, Biosoft, Cambridge, UK). The intracellular pipette solutions were adjusted to pH 7.2 by KOH.

2.3.3. Chemicals

Linopirdine (DuP 996; 3,3-bis(4-pyridinylmethyl)-1-phenylindolin-2-one) was purchased from RBI (Natick, MA, USA). BMS-204352 ((3*S*)-(+)-(5-chloro-2-methoxyphenyl)-1,3-dihydro-3-fluoro-6-(trifluoromethyl)-2*H*-indol-2-one)) and XE991 (10,10-bis(4-pyridinylmethyl)-9(10*H*)-anthracenone) were synthesized at NeuroSearch, Retigabine ((D-23129; *N*-(2-amino-4-(4-fluorobenzylamino)-phenyl) carbamic acid ethyl ester) was a gift from Dr. Thomas Kronbach, Asta Medica. Drugs were dissolved in 100% dimethyl sulfoxide (DMSO) and diluted 1000 times in the extracellular ringer. The vehicle did not affect the recorded currents (not shown).

2.4. Calculations

Dose–response curves were obtained by plotting the relative increase in steady-state current at −30 mV as a function of the drug concentration. The peak tail currents (I_{tail}) measured after stepping back to −120 mV have been plotted as a function of the preceding step potential. The equations used for curve fitting have been described previously (Schrøder et al., 2001). The kinetics of activation and deactivation of the KCNQ5 channel were investigated by eliciting currents from a holding potential of −90 mV to test potentials in the range from −40 to +40 mV (10 mV increments, 600 or 2000 ms in duration). The currents were

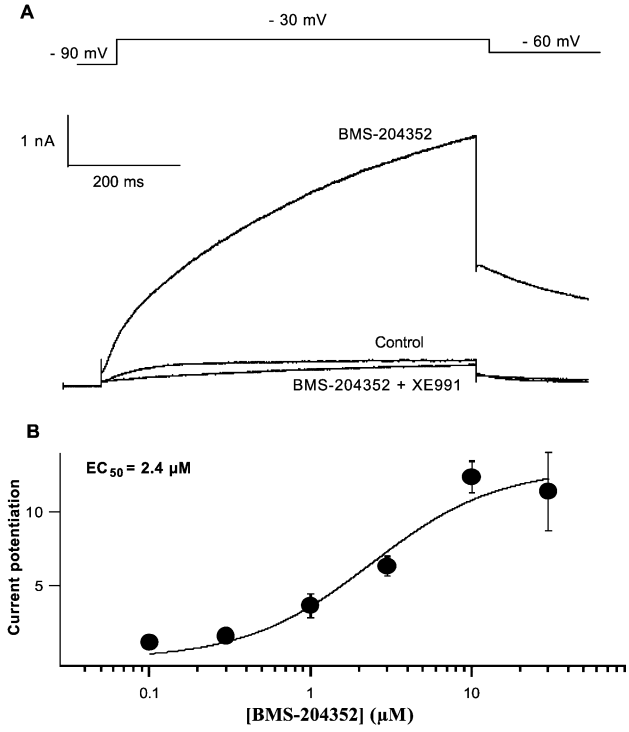

Fig. 1. Activation of KCNQ5 channels by BMS-204352. (A) Effect of BMS-204352 on KCNQ5 currents in the absence or presence of XE991. Representative whole-cell current traces of one HEK293 cell stably transfected with KCNQ5 channel. Recordings were obtained before and after application of either 10 μM BMS-204352 or co-administration of 10 μM BMS-204352 with 10 μM of XE991. The cells were held at −90 mV and every 5 s, the KCNQ5 currents were activated by depolarising steps to −30 mV (600 ms), followed by a step to −60 mV (200 ms). The traces were sampled every 1 ms and filtered at 333 Hz. The experiments were performed in Na^+ Ringers solution with the pipette having a free Ca^{2+} concentration of 100 nM and 1.6 mM of ATP. (B) KCNQ5 currents elicited by increasing concentrations of BMS-204352 (0.1, 0.3, 1, 3, 10 and 30 μM). Concentration–response relationship of BMS-204352 was constructed using the mean value±S.E.M. of current potentiation at each concentration of BMS-204352 (*n* is between 5 and 21 for each concentration). The solid line shows the Hill fit to average values. The EC_{50} value was 2.4 μM and the Hill coefficient of 1.1.

best fitted to a double-exponential function, according to the following equation

$$I(t) = A_{\text{fast}} \times (1 - \exp(-t/\tau_{\text{fast}}) + A_{\text{slow}} \times (1 - \exp(-t/\tau_{\text{slow}}),$$

where $I(t)$ is the current at time t; A_{fast}, A_{slow} are the current amplitudes at maximal times; and τ_{fast}, τ_{slow} are the time constants of the fast and slow components, respectively. In general, data are presented as mean±S.E.M. (standard of error of mean), with n indicating the number of experiments. Statistical significance was determined by Student's t-test. $P<0.05$ was considered to be significant.

3. Results

KCNQ5 channel were stably expressed in HEK293 cells (monoclonal) and the effect of the BMS-204352 was evaluated using whole-cell voltage-clamp techniques performed in solutions with a physiological K^+ gradient. Fig. 1A shows the activation of the KCNQ5 channel in the absence or presence of 10 μM BMS-204352. At the step to −30 mV, a slowly activating current was obtained after 600 ms. The current ranged from 0.2 to 1.5 nA in the transfected cells. The threshold for activation was measured at −62 ± 2.5 mV ($n=15$) with halfmaximal activation obtained at 46.5 ± 2.2 ($n=21$) mV. Using the same voltage-protocol, no current was observed on native HEK293 cells, as previously described (Søgaard et al., 2001). Application of BMS-204352 (10 μM) induced a mean increase of 12.4 ± 1.0 ($n=24$) fold at −30 mV. At −60 mV, the effect of BMS-204352 was more pronounced with a mean increase of 18.3±2.2 ($n=24$) fold. The compound induced a prominent slowing of the activation kinetics. After 2000 ms at −30 mV, the voltage dependant activated current reached a stable

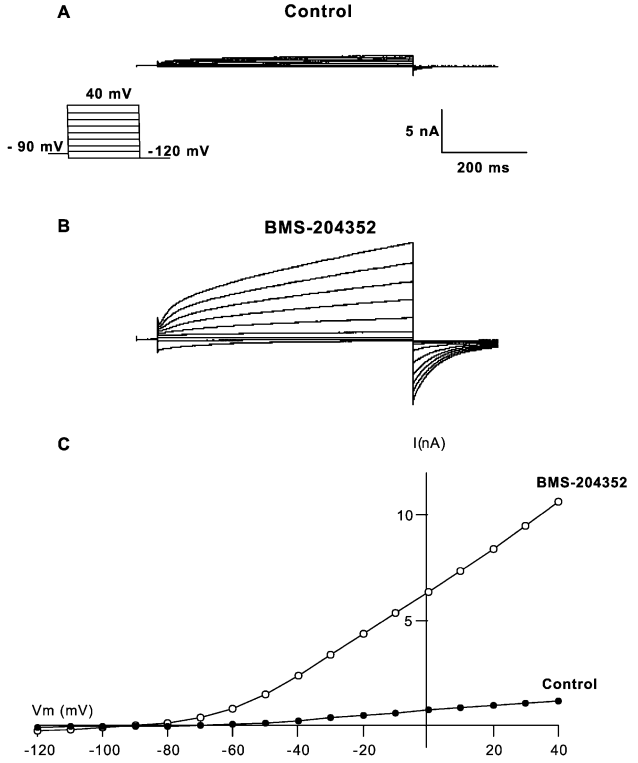

Fig. 2. Effect of BMS-204352 on KCNQ5 current–voltage relationship. (A, B) The whole-cell currents were activated by voltage steps from a holding potential at −90 mV to voltages ranging from −120 to +40 mV (10 mV increments, 600 ms duration). Tail currents were obtained at −120 mV (200 ms). The sweep interval was 2 s, data were sampled at every 400 μs, and filtered at 1.67 kHz. (C) Steady-state currents (measured at 89–91% of the second segment of the voltage protocol) have been plotted as a function of the membrane potential. The current–voltage relationship in the control condition is shown open circles and in the presence of BMS-204352 is depicted with filled circles.

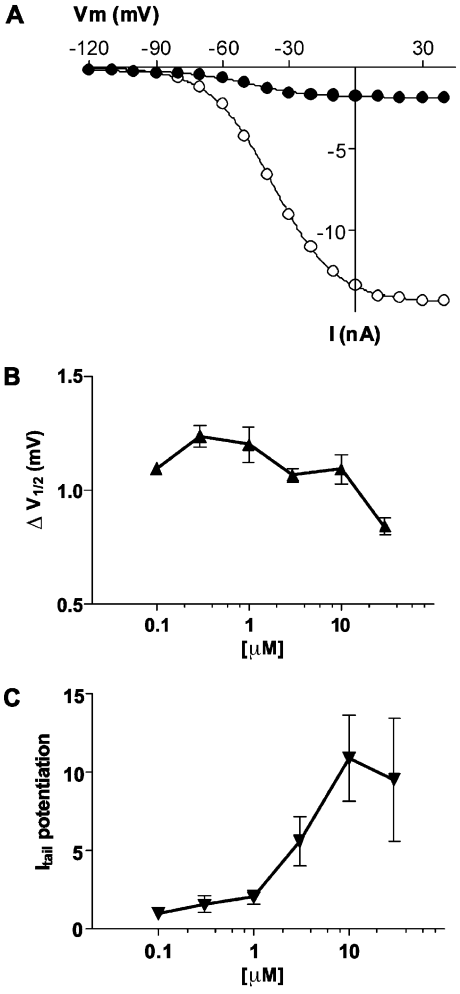

Fig. 3. Effects of BMS-204352 on tail currents. (A) Tail current measured at −120 mV as function of the step potential (from −120 to +40 mV, 2000 ms duration) in the absence (●) or in the presence of 10 μM of BMS-204352 (○). The solid line shows the current values fitted to the Boltzmann equation. (B) The mean values of the shift in the voltage of half-activation ($\Delta V_{1/2}$) are shown as a function of increasing concentration of BMS-204352 (0.1, 0.3, 1, 3, 10 and 30 μM). (C) The mean values of the relative increase in maximal tail currents I_{tail} (∞) is plotted as function of the concentration of BMS-204352. The bars represent mean data±S.E.M. with n between 3 and 11 for each concentration.

value and no decrease of the KCNQ5 current was observed in the absence or presence of the compound. However, as previously described for the KCNQ4 current (Schrøder et al., 2001), a brief transient increase in the amplitude of the tail current measured at −60 mV was observed before deactivation in the absence or presence of the compound, which could be explained as recovery from a very weak inactivation.

XE991 is a blocker of M-type currents, and recently Schroeder et al. (2000) have shown that the KCNQ5 current is also blocked by the XE991 in *Xenopus* oocytes. In order to determine whether the XE991 could block the BMS-204352-induced KCNQ5 current, XE991 (10 μM) was administered to the cells together with 10 μM BMS-204352. As shown in the Fig. 1A, XE991 reversed the current potentiation obtained in the presence of BMS-204352 to a lower level than the control. Note that the activation-kinetics is slowed, indicating an interaction of BMS-204352 with the channels. Similar results were obtained with 10 μM of linopirdine, another M-current blocker (data not shown). In Fig. 1B, the mean current potentiation of the outward plateau current measured at −30 mV as a function of the BMS-204352 concentration is shown. The maximal current potentiation was obtained at 10 μM. The washout of the compound was difficult at 10 and 30 μM, and application of those concentrations of BMS-204352 produced an unspecific leak current, which may have induced some variability in the current measurements. The EC_{50} of BMS-204352 was calculated at 2.4 μM with a Hill coefficient of 1.1. This value is similar to what was obtained on the KCNQ4 channel with BMS-204352 (Schrøder et al., 2001).

Current–voltage (I–V) relationships of the KCNQ5 currents were generated in the voltage range from −120 to +40 mV in the absence or in the presence of 10 μM BMS-204352 (Fig. 2). The steady-state outward current has been plotted as function of the membrane potential, and the threshold for activation of the KCNQ5 channel in the absence of drug was -62.0 ± 2.5 mV ($n = 15$). Superfusion of the same cell with 10 μM of BMS-204352 changed the threshold for channel activation to -68.6 ± 2.8 ($n = 7$). BMS-204352 potentiated the voltage activated KCNQ5 current at all potentials tested. The peak tail current was analysed after a step to −120 mV. Fig. 3A shows the tail current as a function of the preceding step potential in the absence or presence of 10 μM BMS-204352. Curves were fitted to a Boltzmann equation in order to determine the potential of half-maximal activation ($V_{1/2}$), and the maximal

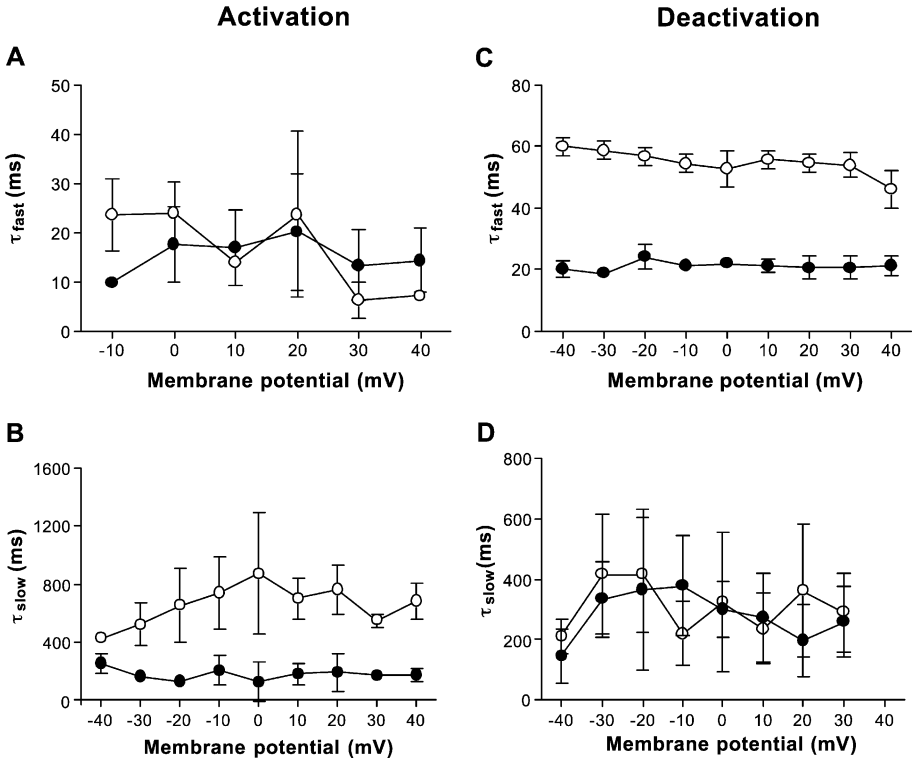

Fig. 4. Effects of BMS-204352 on KCNQ5 channel kinetics. (A, B) Activation kinetics was recorded from current traces by stepping to potentials ranging from −40 to +40 mV from a holding potential of −90 mV. Activation kinetics in the absence (●) or in the presence of 10 μM BMS-204352 (○) was thereafter fitted to a double-exponential function. τ_{fast} (A) and τ_{slow} (B) are shown as a function of the step potential. (C, D) The deactivation kinetics was measured from tail currents elicited at −120 mV following steps to potentials ranging from −40 to +40 mV. The deactivation kinetics was fitted to a double exponential function. The τ_{fast} (C) and the τ_{slow} (D) are shown as a function of the step potential and the symbol used are the same as described in (A) and (B). The bars represent the S.E.M. values and n is comprised between 3 and 6 for each potential.

obtainable tail current (I_{tail} (∞)). In the experiment shown, the $V_{1/2}$ was calculated at −47.5 mV (mean −46.5 ± 1.8 mV, n=21) and the maximal tail current to be −1.8 nA (mean −1.2±0.2 nA, n=21) in the control situation. In the presence of BMS-204352 (10 μM), $V_{1/2}$ was calculated at −37.3 mV (mean −46.4 ± 2.9, n=11) and the maximal tail current to be −14 nA (mean −9.4 ± 1.3 nA, n=11), giving an I_{tail} potentiation of 7.7-fold (mean 10.9 ± 2.8, n=11). BMS-204352 did not significantly (P>0.05) modify the potential for half-maximal activation, but it increased the maximal KCNQ5 tail current. Furthermore, the tail current was analysed in the presence of different concentrations of the compound. Fig. 3B shows the change in $V_{1/2}$ ($\Delta V_{1/2}$) as a function of the BMS-204352 (0.1–30 μM). BMS-204352 had no significant effect on the $V_{1/2}$ of activation of the channel at any concentration ($\Delta V_{1/2}$=1.0–1.2 mV; Fig. 3B). Fig. 3C shows the potentiation of I_{tail} obtained in the presence of increasing concentrations of BMS-204352 (0.1–30 μM). The tail current potentiation increased with the concentration of BMS-204352. The maximal tail current potentiation induced by 10 μM was calculated at 10.9 ± 2.8 fold (n=11).

To further examine the mechanism by which the KCNQ5 current was increased, we studied the effect of BMS-204352 on the time- and voltage-dependent activation and deactivation of the KCNQ5 current. The effects of the compound on the activation and deactivation kinetics were addressed by calculating the time-constant for activation at potentials between −40 and +40 mV, as well as the time constant for deactivation at −120 mV after step in potentials from −40 to +40 mV. Activation and deactivation kinetics were fitted to a double exponential function.

The effect of BMS-204352 in the contribution of the fast activation time constant (τ_{fast}) was evaluated at ±10 mV. In the absence of compound, the τ_{fast} accounted for 25 ± 5% (n=4) of the maximal current at −10 mV and for 31 ± 4% (n=5) at +10 mV. In the presence of BMS-204352, the τ_{fast} contribution of activation was decreased to 18±3% (n=5) both at −10 mV and significantly decreased to 17±2% (n=5, P<0.05) at +10 mV. Similarly, the contribution of the τ_{fast} deactivation time constant was evaluated in the absence or presence of 10 μM BMS-204352 at ±10 mV. In the control condition, the contribution of the τ_{fast} was 85 ± 2% (n=6) at −10 mV and 75±7% (n=6) at +10 mV. Application of 10 μM of BMS-204352 did not significantly modify the value of the contribution of the τ_{fast} of activation (77 ± 9% n=6 at −10 mV and 68±10% n=6 at +10 mV). Fig. 4 shows the activation and deactivation time constants (τ_{fast} and τ_{slow}, respectively) as a function of the membrane potential in the absence or presence of BMS-204352 (10 μM). In the absence of BMS-204352, τ_{fast} for activation was voltage-insensitive and the mean value was calculated at 15.5 ± 7.5 ms (Fig. 4A). Addition of BMS-204352 had no significant effect on τ_{fast} for activation. In contrast, application of 10 μM BMS-204352 modified the slow kinetic of activation of the KCNQ5 current. In the control condition, the τ_{slow} was voltage-insensitive and was calculated at 177 ± 39.3 ms (n=4). When the BMS-204352 was added to the same cell, τ_{slow} was significantly enhanced at all the potentials from −40 to +40 mV to a mean value of 616.4 ± 126 ms (n=4). The fast time constant of KCNQ5 tail current deactivation was changed by addition of BMS-204352. In the control condition, the τ_{fast} of deactivation was independent on the voltage and the mean value was calculated at 21.1 ± 2.0 ms (n=6). Application of the BMS-204352 significantly enhanced τ_{fast} from approximately 21 to 54.5±3.8 ms (n=6, Fig. 4C). The τ_{slow} of deactivation was voltage insensitive and not influenced by the application of BMS-204352 (Fig. 4D).

BMS-204352 acted as a strong activator of KCNQ5 current and enhanced KCNQ5 currents to a higher degree than seen for the other KCNQ channels. To investigate whether this was a compound-specific action or a feature of KCNQ5 homomeric channels, the effect of BMS-204352 was compared to the effect obtained with retigabine. The effect of retigabine was studied at −30 and −60 mV as described in the Fig. 1. Current–voltage (I–V) relationships as described in the legend to Fig. 2 were performed in the presence of retigabine (traces are not shown). A summary of the data obtained for potentiation of plateau current, tail current (at −120 mV) as well as effects on $V_{1/2}$ are shown in Table 1. In the presence of retigabine (10 μM), the current increased by 3±0.4 fold at −30 mV (n=10) and by 7±4 fold at −60 mV (n=10). Thus, the effect of retigabine on the activation of the KCNQ5 channel was significantly

Table 1
Effects of BMS-204352 and retigabine on KCNQ5 curents and tail currents

	Control	BMS-204352, 10 μM (±S.E.M.)	Retigabine, 10 μM (±S.E.M.)
Potentiation of plateau current (fold increase)			
−30 mV	–	12.4±1.1	3.0±0.4
−60 mV	–	18.3±2.2	7.0±3.6
Potentiation of I_{tail} (fold)		10.9±2.8	1.9±0.3
Threshold for activation (mV)	−62.0±2.5	−68.6±2.8	−71.2±1.3
V half of activation (mV)	−46.5±2.2	−46.4±2.9	−49.5±5.2
$\Delta V_{1/2}$ (mV)		1.1±0.1	1.0±0.1

Whole-cell KCNQ5 currents were measured under voltage-clamp in external Na^+-Ringer as described in Figs. 1 and 3. The effects of BMS-204352 (10 μM) and retigabine (10 μM) were measured at potentials of −30 and −60 mV. Current–voltage relationship measurements have been performed as described in Fig. 2. Threshold of activation has been determined in the absence (control condition) or in the presence of either 10 μM BMS-204352 or retigabine, respectively. Tail currents were measured at −120 mV as function of the step potential (from −120 to +40 mV) in the absence or presence of the compound and fitted to the Boltzmann equation. The maximal potentiation of plateau current, I_{tail}, $V_{1/2}$, and $\Delta V_{1/2}$ were determined. All data represent means values±S.E.M. from 7 to 24 experiments.

smaller than the channel activation obtained with BMS-204352 (10 μM). The difference in channel activation was more pronounced at −30 mV than at −60 mV (Table 1). Current–voltage relationship in the presence of retigabine (10 μM) showed a threshold of activation of −71.2±1.3 mV (n=8), not significantly different from that obtained in the control condition (−62.0±2.5 mV, n=15) or in presence of 10 μM BMS-204352 (−68.6±2.8 mV, n=7). Regarding the tail current, retigabine (10 μM) potentiated the maximal tail current by 1.9 fold (±0.9, n=7), a much smaller effect than that BMS-204352 (10 μM) (10.9 fold ±2.8, n=11). However, evaluating the voltage for half activation ($V_{1/2}$), no difference was observed between the two compounds. In the presence of retigabine, the voltage for half activation was −49.5±5.2 mV (n=7), and, thus, no shift in $V_{1/2}$ was obtained in the presence of either 10 μM retigabine or 10 μM BMS-204352 as compared to the control.

4. Discussion

The KCNQ5 channel has been stably expressed in HEK293 cells and the effects of BMS-204352 on the channel have been characterised. The molecular target of BMS-204352 has been reported to be BK channels (Gribkoff et al., 2001). However, it has recently been shown that BMS-204352 is also an activator of the KCNQ2 and KCNQ4 channels as well as of the heteromeric KCNQ2/KCNQ3 and KCNQ3/KCNQ4 channels (Schrøder et al., 2001). In the present study, we have shown that the BMS-204352 is an efficient opener of the KCNQ5 channel increasing the activity by more than 12-fold. The effect of BMS-204352 on the maximal current was concentration-dependent with an EC_{50} of 2.4 μM. The compound did not induce any shift in the $I–V$ relationship, but significantly slowed the activation kinetics. Retigabine showed qualitatively similar albeit much smaller effects.

A slowly activating and deactivating M-like current was obtained when the membrane of stably KCNQ5 transfected HEK293 cells was depolarised to −30 mV. Application of the BMS-204352 produced an increase in this plateau current at −30 mV with a potency identical to that obtained at the KCNQ4 channels (EC_{50} 2.4 μM; Schrøder et al., 2001). The specific M-current blockers XE991 and linopirdine inhibited the BMS-204352 induced KCNQ5 currents. Recently, Schrøder et al. (2001) have shown that the activating effect of BMS-204352 on KCNQ4 channels stably expressed in HEK293 cells could be described as being composed of two actions: a shift of the activation curve towards more negative potentials and an increase in maximal current. In the case of the KCNQ5 channel, BMS-204352 only caused an increase in the maximal current at all potentials without any shift of the $I–V$ relation curve. Further, the compound strongly slowed the activation of KCNQ5 channels, whereas it accelerated the KCNQ4 channel activation kinetics. Finally, BMS-204352 exerted a much larger effect on the activity of the KCNQ5 channels than on any other KCNQ channel. Taken together these findings suggest that the compound elicit a different effect on KCNQ5 than on KCNQ4 channels.

The effect of retigabine has also been analysed on the KCNQ5 channel. Retigabine activates KCNQ2/KCNQ3 expressed in Chinese Hamster Ovary (CHO) cells with an EC_{50} value of 1.7 (Rundfeldt and Netzer, 2000) and 0.34 μM when KCNQ2/KCNQ3 were expressed in *Xenopus* oocytes (Wickenden et al., 2000). The property of retigabine to enhance K^+ current through KCNQ2/KCNQ3 channels was extended to currents through homomeric KCNQ2, KCNQ3 and KCNQ4 channels expressed in HEK293 and CHO cells (Tatulian et al., 2001; Schrøder et al., 2001). Recently, Wickenden et al. (2001) have also shown that retigabine can activate the KCNQ5/KCNQ3 heteromer expressed in CHO cells. In the present study, retigabine enhanced the KCNQ5 current at −30 and −60 mV, by 3 and 7 fold, respectively. These data are in the same range as what has been obtained on the KCNQ4 channel (Schrøder et al., 2001). These findings confirm that the retigabine compound is a non-selective activator of the KCNQ family of K^+ channels. Retigabine activated the KCNQ5 channel by increasing the current in a concentration-dependent way. In comparison to BMS-204352, retigabine was less efficacious in potentiating the KCNQ5 current, which is in contrast to the fairly similar effects obtained with the two compounds on the other KCNQ subunits (Schrøder et al., 2001). BMS-204352 thus seems to be an opener of KCNQ channels exerting a particularly large effect on homomeric KCNQ5 channels.

We found that the kinetics of the KCNQ5 channel was slowed in the presence of BMS-204352. Two exponential functions have been used to fit the activation and deactivation kinetics. The τ_{slow} kinetic constant for activation was significantly prolonged in the presence of the compound and the contribution of τ_{slow} versus τ_{fast} was increased. Together, a pronounced slowing of the activation kinetics was obtained being reminiscent of the effects of the KCNE1 subunit (minK) on KCNQ1. Application of BMS-204352 also slowed the voltage-independent KCNQ5 channel deactivation. The slowing of KCNQ5 channel deactivation may have pronounced functional effects by progressively increasing the K^+ current during action potential bursts, and will thereby tend to terminate these. This result confirms that BMS-204352 could be a potential drug candidate to modulate the neuronal excitability. Cheney et al. (2001) have shown that BMS-204352 attenuates regional cerebral oedema and neurological motor brain impairment after experimental brain injury. As the BMS-204352 acts also on KCNQ5 channel, it could suggest that opening KCNQ5 channel may be implicated in the action of BMS-204352 in the treatment of experimental traumatic brain injury.

In conclusion, BMS-204352 a neuroprotectant K^+ channel opener known to open BK channels, is also an activator of KCNQ channels showing a much larger effect on

KCNQ5 channels than on any other KCNQ channel subtype. KCNQ channels lend themselves to physiological modulation via coupling to several G-protein coupled receptors such as muscarinic M1 receptors, via interaction with β-subunits (KCNEs) and also to pharmacological modulation. It is most likely that the activation of KCNQ2-5 channels by BMS-204352 is important for the functional effect of the compound, and that the channels constitute a very interesting molecular therapeutic target.

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