ELSEVIER

European Journal of Pharmacology 336 (1997) 243–249

ejp

The new anticonvulsant retigabine (D-23129) acts as an opener of K^+ channels in neuronal cells

Chris Rundfeldt *

Department of Pharmacology, Arzneimittelwerk Dresden GmbH, Corporate R&D, ASTA Medica Group, Meissner Strasse 35, D-01445 Radebeul, Germany

Received 31 July 1997; accepted 5 August 1997

Abstract

The patch-clamp technique was used to measure currents passing through K^+ channels in neuronal cell preparations. Retigabine (D-23129, *N*-(2-amino-4-(4-fluorobenzylamino)-phenyl) carbamic acid ethyl ester) activated a K^+ conductance in slightly depolarized NG108-15 neuronal cells in a dose-dependent manner (0.1–10 μM). At the K^+ reversal potential, no current could be elicited and in hyperpolarized cells the current was reversed. A similar current was elicited in primary cultures of mouse cortical neurones and in differentiated hNT cells, a cell line derived from human neuronal cells. At higher concentrations, retigabine also partially blocked voltage activated K^+ currents. None of the tested anticonvulsants, phenytoin, carbamazepine and valproate and none of the K^+ channel openers cromakalim, diazoxide and pinacidil exerted a similar effect. The current was not affected by the K^+ channel blocker glibenclamide (10 μM) but was fully blocked by application of Ba^{2+} (10.8 mM). Exchange of K^+ with cesium in the intracellular space also fully abolished the current. It can be expected that the K^+ channel opening effect contributes to the anticonvulsant activity of retigabine.

Keywords: Membrane potential; Antiepileptics; Cromakalim; Glibenclamide; Patch-clamp electrophysiology; NG 108-15 cells; hNT neurons; Cortical neurons, mouse

1. Introduction

Channels selective for K^+ ions play a vital role in the function of many cell types (Rudy, 1988; Hille, 1993). They are exceptionally diversified both in variety and function. Individual cells can, and normally do, express several kinds of channels.

Such channels are regulated through various mechanisms (Rudy, 1988) and can be grouped into different gene families. Differences between K^+ channels have also emerged clearly from molecular biology studies showing that they differ considerably in molecular structure (Takumi et al., 1988; Kubo et al., 1993; Rupersberg et al., 1993). K^+ channels are currently the target of diverse pharmacological manipulation. Voltage activated K^+ channels in the heart are blocked by class III antiarrhythmic drugs such as amiodarone and sotalol and this action delays the repolarization of the cardiac action potential and increases cardiac refractoriness (Colatsky et al., 1990). The antidiabetic sulfonylureas glibenclamide and tolbutamide are blockers of the ATP-sensitive K^+ channel, K_{ATP}, and these drugs affect insulin producing β-cells (Bernardi and Lazdunski, 1993). K^+ channel openers such as levcromakalim, aprikalim and pinacidil, currently being evaluated for the treatment of hypertension, peripheral ischemia and obstructive airway diseases, also influence the K_{ATP} channel (Edwards and Weston, 1993). Modulators of a subtype of Ca^{2+} activated K^+ channels, namely the large conductance Ca^{2+} activated K^+ channels (BK_{max}), are being evaluated for neuroprotective activity. Such Ca^{2+} activated K^+ channels are expressed in most neurones, smooth and striated muscle cells and secretory epithelial cells (McKay et al., 1994). Inward rectifying K^+ channels, which are open in resting cells, are involved in the generation of the resting membrane potential which is mainly due to the concentration gradient of K^+ ions. While the whole cell conductance of such channels is fairly small, the contribution to cell membrane potential is large since the input resistance of neuronal cells is high and open channels do not desensitize. A direct pharmacological modulation of inward rectifying K^+ channels in neuronal cells is cur-

* Tel.: (49-351) 834-3329; Fax: (49-351) 834-3320.

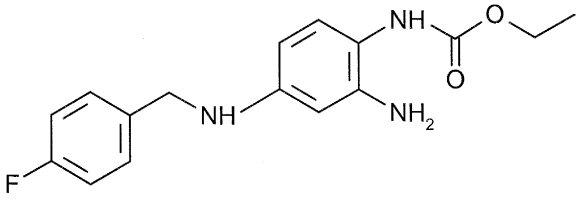

Fig. 1. Chemical structure of retigabine (*N*-(2-amino-4-(4-fluorobenzylamino)-phenyl) carbamic acid ethyl ester). Molecular weight: 303.3 g/mol; molecular formula: $C_{16}H_{18}FN_3O_2$.

rently not possible due to a lack of specific drugs. A selective opening of such channels is discussed as a therapeutic target for several diseases including epilepsy and neurodegeneration. (Doupnik et al., 1995).

Retigabine (D-23129, *N*-(2-amino-4-(4-fluorobenzylamino)-phenyl) carbamic acid ethyl ester) is a new anticonvulsant currently undergoing phase II clinical trials. The drug is structurally unrelated to currently marketed anticonvulsant drugs (see Fig. 1) and exerts potent anticonvulsant activity in a broad range of seizure models at nontoxic doses (Tober et al., 1995, 1996; Rostock et al., 1996). In a hippocampal slice preparation, D-20443, the hydrochloride of retigabine, was unique in its ability to suppress 4-aminopyridine induced interictal like epileptiform spikes and to abolish stimulus induced bursts (Yonekawa et al., 1995). Since 4-aminopyridine is a blocker of voltage sensitive K^+ channels, we were interested to see, if the anti-4-AP effect of the drug is mediated through a direct effect on K^+ channels.

Some of the data have been published in abstract form (Rundfeldt et al., 1995a,b; Rundfeldt and Bartsch, 1996).

2. Methods

2.1. Cell culture

Most electrophysiological recordings were performed using the neuronal cell line NG 108-15, which was originally derived from the fusion of a neuroblastoma cell of the mouse with a glioma cell of the rat (European Collection of Cell Cultures, ECACC, Porton Down, UK). Cells were cultivated and differentiated using a method described previously (Bodewei et al., 1985). Briefly, the cell clone was maintained in Dulbecco's modified Eagle's medium (DMEM with 4.5% glucose) supplemented with 10% fetal calf serum, 4 mM glutamine and 2% hypoxantin, aminopterin, thymidin (HAT)-supplement (all obtained from Life Technologies, Eggenstein, Germany). For differentiation, the cells were seeded on collagen-coated cover slips and treated with differentiation medium containing 1% fetal calf serum and 1 mM dibutyryl cycloadenosinemonophosphate (Sigma, Deisenhofen, Germany) for 3–7 days (Bodewei et al., 1985).

To verify the data obtained in NG 108-15 neuronal cells, some experiments were repeated in human tissue derived neuronal cells (hNT) and in mouse cortical neurones. The neuronal precursor cell NT2 was obtained from American Type Culture Collection (ATCC, Rockville, MD, USA) and can be differentiated into postmitotic central nervous system neurones (hNT cells) (Pleasure et al., 1992; Lee et al., 1994). NT2 cells were maintained in DMEM (4.5% glucose) supplemented with 10% fetal calf serum, 4 mM glutamine, penicillin (100 IE/ml) and streptomycin (100 μg/ml). For differentiation, the cells were treated with 10 mM retinoic acid (Sigma, Deisenhofen, Germany) for 4 weeks (2×10^6 cells in a 75 cm^2 flask), replated 1:6 and harvested differentially on the following 2 days. These cells were seeded on poly-L-lysine (Sigma, Deisenhofen, Germany) coated cover slips and cultivated for 1 week with DMEM containing 10% fetal calve serum and supplemented with 1 μM cytosiene-1β-D-arabinofuranoside, 10 μM fluorodeoxyuridine and 10 μM uridine, 100 I.E./ml penicilline and 100 μg/ml streptomycine (Sigma, Deisenhofen, Germany). From the second week on, cytosine-1β-D-arabinofuranoside was omitted. The cells were used for tests within the following two weeks.

Mouse cortical neurons were obtained from cortical tissue of 18 day old fetal mice (NMRI, Winkelmann, Borchen, Germany) and cultivated together with the astrocytes on poly-L-lysine coated glass cover slips at a density of 2×10^5 cells/cm^2. The cells were cultivated in DMEM supplemented with 10% fetal calf serum, 10% horse serum and glutamine (2 mM). Cytosine-1β-D-arabinofuranoside (5 μM) was added from day 3 to 5 to prevent overgrowth of the neurons by astrocytes. For the experiments neurones between day 8 and 16 were used.

2.2. Patch-clamp recording

The whole-cell configuration of the patch-clamp technique was used for the voltage clamp experiments (Hamill et al., 1981). The micropipettes were pulled from borosilicate glass capillaries (Science Products, Günzburg, Germany) and heat polished at the tip. They had resistances between 2 and 4 MΩ after filling with pipette solution containing (in mM) KCl, 135; $MgCl_2$, 2; *N*-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 10; glucose, 5; 1,2,-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA), 10 and Na_2-ATP, 2; pH 7.3–7.4. The intracellular (pipette) solution was prepared in advance and deep-frozen in aliquots of 2 ml. To fill the recording pipettes, the solution was thawed every morning and fresh ATP was added. For cytocompatibility, only solutions with an osmolarity between 300 and 330 mosmol were used. The pH was adapted to 7.3–7.4 with 1 M KOH or CsOH. The intracellular Ca^{2+} concentration was kept at a low value by buffering with BAPTA. Current signals were amplified by an EPC-7 amplifier (HEKA Elektronik, Lam-

brecht Germany) digitised, stored and analysed using the TIDA system (HEKA Elektronik, Lambrecht, Germany). The data were sampled, digitally filtered (Bessel 50 Hz) and stored on computer disk at a frequency of 200 Hz for drug induced and 2000 Hz for voltage activated K^+ currents. For recording, a cover slip with differentiated cells was transferred into the recording chamber and permanently superfused with modified extracellular solution containing in mM: NaCl, 136; KCl, 5.3; $CaCl_2$, 1.8; $MgSO_4$, 0.8; HEPES, 10; glucose, 21.5 and NaH_2PO_4, 1; pH 7.3–7.4. For the evaluation of drug effects, drugs or control solutions were locally applied onto the cell using a 2 channel gravity fed locale application system allowing the drug to reach the cell within less than 100 ms. Effects on membrane conductance were observed before, during and after drug application while continuously clamping the cell at a fixed membrane potential. For evaluation of voltage activated currents, cells were clamped at a fixed membrane potential and superfused for at least 1 min with control or drug solution before initiating the channel opening by a series of depolarising voltage steps.

2.3. Drugs and solutions

Retigabine, phenytoin and carbamazepine were obtained from Arzneimittelwerk Dresden (Radebeul, Germany). Sodium-valproate was obtained from Synopharm (Hamburg, Germany). The K^+ channel openers pinacidil and diazoxide were obtained from Biotrend (Köln, Germany). Cromakalim, tetrodotoxin, 4-aminopyridine, glibenclamide, dimethylsulfoxide (DMSO), buffers and ions for intra- and extracellular solutions were obtained from Sigma (Deisenhofen, Germany). For drug application, fresh stock solutions were prepared daily and kept refrigerated (4°C) for the day. To prepare stock solutions, an appropriate amount of retigabine was dissolved in 0.1 ml of 1 M HCl, diluted to 10 ml with deionized water and ultrasonicated for 3 min; phenytoin was dissolved in 0.2 ml 1 M NaOH, diluted to 10 ml with demineralized water and ultrasonicated for 3 min. All other stock solutions were prepared as follows: an appropriate amount of the respective drug was dissolved in 2 ml DMSO, diluted with 18 ml extracellular solution and ultrasonicated for 3 min. For final concentration, an aliquot of the stock was added to extracellular solution and ultrasonicated. The pH of all buffers and solutions was adjusted to 7.3–7.4 using 1 M HCl and 1 M NaOH, respectively. The solutions in the drug application system were changed every hour to avoid degradation artifacts. For cytocompatibility, only solutions with an osmolarity between 300 and 330 mosmol were used.

2.3.1. Evaluation of K^+ conductance initiated by application of retigabine

To investigate currents passing through K^+ channels which are open in resting cells, cells were clamped at −40 mV and superfused with extracellular solution containing no drug. The 'leakage current' flowing across the cell membrane in this state was evaluated. After 1 min, the superfusion was switched to an extracellular solution containing the desired concentration of retigabine for 30–40 s while observing the change in 'leakage current'. The drug effect was expressed as change in current flow (pA) before and during drug application and plotted against drug concentration. The reversal of the drug effect was evaluated by switching back to control superfusion. No voltage steps were applied to the cells to avoid activation of voltage sensitive channels. To verify the nature of the ions passing through the open channels, the experiment was repeated at different membrane potentials and using an extracellular solution containing 40 instead of 5.3 mM K^+ to shift the reversal potential of K^+ to more positive values (Na^+ concentration was adjusted accordingly). For further validation of the nature of the ions passing through the channels, different blockers were applied. Intracellular solution containing cesium instead of K^+ was used in one set of experiments to block all K^+ channels. Using normal extracellular solution, Na^+ channel blocker, tetrodotoxin (300 nM), the Ca^{2+} channel blocking ion, cobalt (2 mM), non-specific blocker of voltage activated K^+ channels, 4-aminopyridine (500 μM), blocker of ATP sensitive K^+ channels, glibenclamide (10 μM) and Ba^{2+} (10.8 mM) as a non-selective blocker of inwardly rectifying K^+ channels were added to the superfusion medium, respectively, to further evaluate the nature of the current induced by retigabine.

For comparison, the effects of the K^+ channel openers active in smooth muscle cells, cromakalim (10 and 500 μM), pinacidil (10 and 500 μM) and diazoxide (10 and 500 μM) and the anticonvulsants phenytoin (100 μM), carbamazepine (30 μM) and valproate (300 μM) were tested using the same paradigm as described above. To evaluate if the effect measured in NG108-15 neuroblastoma cells is restricted to this cell line, some experiments were repeated in two other cell preparations, i.e., in hNT cells, which are derived from human neuronal tissue, and in primary cultures of mouse cortical neurones.

2.3.2. Effects on voltage activated K^+ currents

To elicit currents passing through voltage activated K^+ channels, cells were clamped at a membrane potential of −40 mV. A series of depolarizing voltage steps (200 ms duration, each s 1 depolarization) was then applied starting at −40 mV in steps of 10 mV. To evaluate drug effects on these currents, artificial extracellular solution containing the desired concentration retigabine was then applied onto the cell via the two channel application system. After 1 min of superfusion, the voltage protocol was again activated. Comparing individual control currents and currents elicited while drug was applied, the effect on maximal as well as on the sustained currents at the different depolarized potentials was evaluated. The amount of current re-

maining while drug is applied was expressed as percent control current. To exclude effects of the fluid application system, all cells were superfused with vehicle for all control currents.

3. Results

3.1. Effects on K^+ channels which are open in resting cells

Retigabine significantly increased the membrane permeability for K^+ in differentiated NG108-15 neuronal cells (see Fig. 2, inset for sample trace). The effect was dose dependent and could be evaluated in slightly depolarized cells. The effect could be observed within less than 1 s after the start of superfusion and quickly reached a plateau; within 30–40 s, no desensitization was observed. A significant effect was visible already after application of 0.1 μM; the maximum effect was present at 10 μM. At 100 μM, the effect was slightly less pronounced (see Fig. 2). To verify that the current was carried by K^+ ions, K^+ was exchanged by cesium in the pipette solution to block K^+ channels. Under this condition, no drug induced current could be elicited by application of retigabine 10 μM (see Fig. 2, open square).

To prove, that the current elicited by retigabine is carried by K^+ ions and to test for voltage dependence of the K^+ channel opening effect, the current was induced by 10 μM retigabine clamping the cells at different membrane potentials. In cells clamped at −40 mV, the current

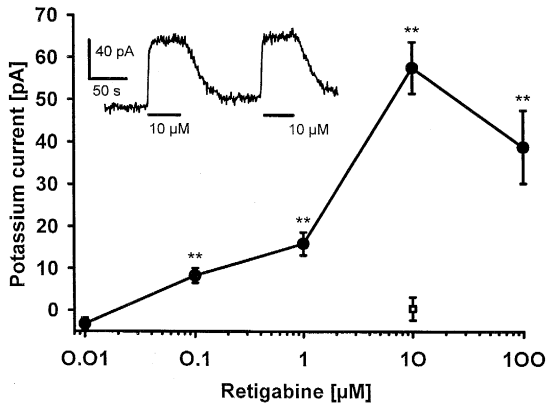

Fig. 2. Dose-dependent effect of D-213129 on K^+ conductance in differentiated NG 108-15 cells. The cells were clamped at −40 mV and the current flowing across the membrane ('leakage current') was evaluated before, during and after application of retigabine, 0.01–10 μM. The difference in current before and during drug application was expressed in pA. For each concentration, 8–10 cells were evaluated and displayed as mean ± S.E.M. The duration of application was 30–40 s; the effect occurred within less than 1 s. (●) Current induced using solutions containing potassium; (□) intracellular solution containing cesium instead of potassium was used. A significant change in leakage current was indicated by ** ($P < 0.01$, paired *t*-test) Inset: Original trace of a sample cell, retigabine was applied twice for 40 s onto the same cell.

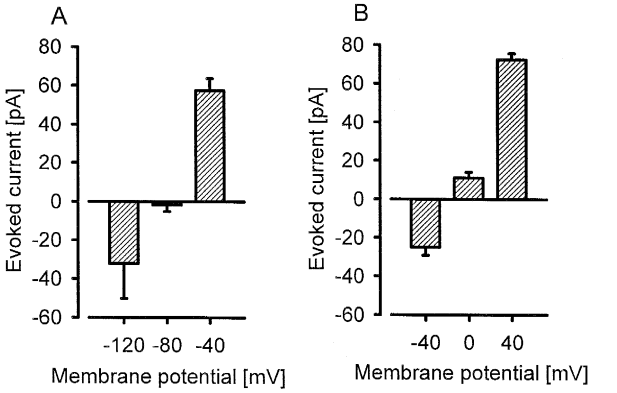

Fig. 3. Effect of retigabine on potassium conductance at different resting membrane potentials in NG 108-15 cells. Mean ± S.E.M. of 5–10 cells is plotted against the membrane potential. Retigabine was used at a concentration of 10 μM. (A) Currents evoked using extracellular solution containing 5.3 mmol potassium yields to a potassium reversal potential of approx. −84 mV; (B) Currents were evoked using an external solution containing 40 mmol potassium (the concentration of sodium was reduced to match the osmolarity) yielding to a potassium reversal potential of approx. −30 mV.

could be induced. Clamping the cell at −80 mV, which is close to the K^+ reversal potential (approximately −84 mV), no significant current flow could be initiated; at hyperpolarized membrane potential (−120 mV), the ion flow was reversed (see Fig. 3A). The reversal potential calculated from these data amounted to −87 mV. These results strongly support the hypothesis, that the current is carried by K^+ ions. As further proof, the concentration of K^+ in the external solution was changed to 40 mM to reduce the concentration gradient. To restore the osmolarity, the concentration of Na^+ was reduced accordingly. In this instance, the reversal potential for K^+ shifts from −84 mV to approximately −32 mV. Using this modified extracellular solution (Fig. 3B), at −40 mV the current induced was already reversed compared to the standard solution (Fig. 3A). The reversal potential calculated from these data amounted to −16 mV giving additional proof for K^+ as main charge carrier.

3.2. Influence of ion channel blocker on the current initiated by retigabine

In further experiments, we tried to block the current. While the exchange of K^+ by the K^+ channel blocking ion cesium in the intracellular solution yielded a total block of the current elicited by retigabine (10 μM) (Fig. 2, open square), the addition of tetrodotoxin (0.3 μM) or $CoCl_2$ (2 mM) normally used to block Na^+ and Ca^{2+} channels, respectively, had no significant effect. Even with the blocker present, K^+ conductance could be elicited by application of retigabine (not illustrated). The K^+ channel blocker 4-aminopyridine is a convulsant with limited specificity for different K^+ channels. In our experiments,

application of 4-aminopyridine had no effect on the current induced by retigabine even at a concentration of 500 μM. The blocker of ATP sensitive K^+ channels, glibenclamide (10 μM) applied with retigabine to the cells, did not abolish the induced current (without glibenclamide: 60.7 ± 11.5 pA, with glibenclamide: 54.3 ± 13.8 pA, $n = 5$ cells). Application of extracellular solution containing $BaCl_2$ (10.8 mM) instead of $CaCl_2$ to the cells reduced the leakage current observed prior to application of retigabine indicating a block of K^+ channels. Application of retigabine to these cells did not longer elicit an increase in K^+ conductance (see Fig. 4A).

3.3. Evaluation of effects of anticonvulsants and K^+ channel openers

All tested openers of ATP sensitive K^+ channels (cromakalim, pinacidil, diazoxide, 10 and 500 μM) did not elicit a current similar to retigabine at both high and low concentrations. Cells which were not responsive to these drugs though were capable of generating the current in response to application of retigabine (see Fig. 4B for a sample trace). Like the K^+ channel openers, none of the tested anticonvulsants (carbamazepine 30 μM, valproate 300 μM, phenytoin 100 μM) induced a K^+ current in NG 108-15 cells.

3.4. Effect of retigabine on K^+ channels in other cell preparations

To verify, that the effect of retigabine is not restricted to NG108-15 neuronal cell, we also tried to induce the current using a human tissue derived neuronal cell line (hNT) and primary cultures of mouse cortical neurones. In all 6 hNT cells clamped at −40 mV, an increase in K^+ conductance could be elicited by application of 10 μM retigabine. This increase was similar to that seen in NG 108-15 cells. The magnitude of the current was somewhat smaller reaching 16.8 ± 2.4 pA ($n = 6$) compared to 57.6 ± 6.1 pA ($n = 10$) in NG108-15 cells. A current could be also elicited by application of retigabine (10 μM) to mouse cortical neurones. The average current elicited in 6 cells reached 41 ± 2.6 pA.

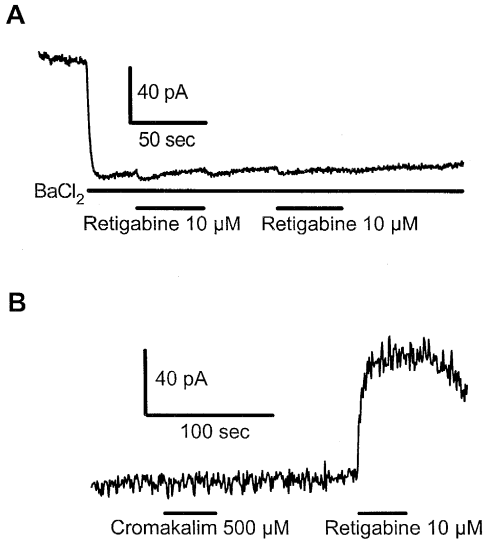

Fig. 4. Sample traces obtained with retigabine in different preparations. (A) Using Ba^{2+} (10.8 mM) as a blocker of inward rectifying potassium channels, the membrane conductance of the cell clamped at −40 mV was considerably reduced; while $BaCl_2$ was present, no current could be elicited by retigabine. (B) While local application of cromakalim (500 μM) did not elicited any potassium current using the conditions described elsewhere, retigabine was able to induce a potassium conductance in the same cell.

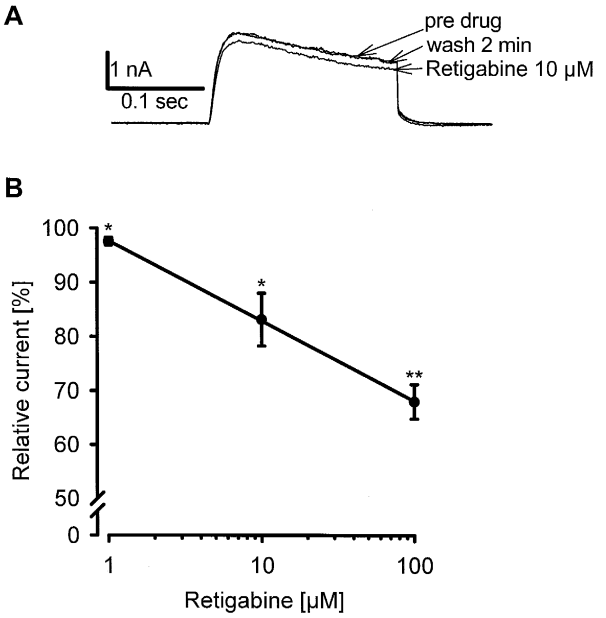

Fig. 5. Inhibition of voltage activated K^+ currents by retigabine. (A) Sample trace of a current elicited by a 200 ms voltage step from −40 mV to +20 mV before, during and after application of retigabine, 10 μM. (B) Dose dependence of the blocking activity. The current elicited under the presence of the respective concentration of retigabine was normalized on the respective control current elicited before drug application and is plotted against the concentration of retigabine (mean ± S.E.M., 6–9 cells were evaluated for each concentration). From the different voltage steps used to depolarize the cell, the step from −40 mV to +20 mV was selected; other voltage steps yielded similar results. A significant block was indicated by * ($P < 0.05$) and ** ($P < 0.01$), paired t-test.

3.5. Effects of retigabine on voltage gated K^+ channels

Voltage sensitive K^+ currents were activated by applying depolarising steps of 200 ms duration to NG 108-15 cells held at a membrane potential of −40 mV. Evaluating the peak current elicited by a depolarizing step from −40 to +20 mV, retigabine did reduced the peak and sustained part to a similar extend. Application of 10 μM resulted in a block of the peak current by 16.5 ± 4.6% (see Fig. 5A for a sample trace). Similar results were obtained, if other voltage steps were evaluated. At 1 μM, the block reached only 2.4 ± 0.7% and only at the very high concentration of 100 μM a block of 32.0 ± 3.2% was reached (see Fig. 5B).

4. Discussion

We were able to demonstrate, that retigabine initiates a membrane conductance which is selective for K^+ ions. The characterization reveals, that the effect can be initiated in depolarized as well as in hyperpolarized neuronal cells and that the current can be blocked by $BaCl_2$, while the non-selective blocker of voltage activated channels, 4-aminopyridine, had no effect. These data indicate, that the potent effect of retigabine against 4-aminopyridine induced discharges in the hippocampal slice preparation is not due to a direct counteraction of the effect of 4-aminopyridine. retigabine had a weak blocking effect on voltage activated K^+ channels. The question arises, which K^+ channel subtype is involved in the opening effect of retigabine and if this mechanism can be related to the potent block of discharges in the 4-aminopyridine model. Some hints can be taken from the current voltage relationships of different channels. Outward rectifying channels are closed at hyperpolarized membrane potentials; inward rectifying channels are open at hyperpolarized potentials, but closed upon depolarization; Ca^{2+} gated channels are operated by intracellular Ca^{2+} concentration and some are also operated by voltage. The current–voltage relationship of the action of retigabine implies that the drug either removes the voltage sensitivity of such channels or interacts with channels which are not or only slightly voltage gated such as leakage channels like TWIK1 or ORK1 (Leasge et al., 1996; Goldstein et al., 1996) or ATP sensitive channels. K^+ channel openers of the cromakalim type, which are active on ATP sensitive channels, are currently under discussion for anticonvulsant therapy; an intracerebroventricular (i.c.v.) application of cromakalim has shown to exert some anticonvulsant activity (Del Pozo et al., 1990; Popoli et al., 1991). In our cell preparation, the three openers of ATP sensitive K^+ channels, cromakalim, pinacidil and diazoxide, did not act as K^+ channel openers and the current induced by retigabine was not blocked by the selective blocker of ATP sensitive channels, glibenclamide, indicating that retigabine interacts with a different K^+ channel subtype. A separation between retigabine and cromakalim is of great advantage. While derivatives of cromakalim might be active in neuronal cells as well as smooth muscle and cardial cells on K^+ channels and other binding sites, their therapeutic use for central nervous system based indications is hampered by their strong and non-selective effects on blood pressure, circulation and respiration. Indeed, data exist for retigabine supporting the idea of separation of effects on smooth muscles, cardiac cells and neurones. In phase I clinical studies, no effect of retigabine on blood pressure and heart function could be observed (unpublished data).

To address the question, whether the effect of retibabine on K^+ channels is restricted to NG 108-15 cells, experiments were repeated in two different cell cultures, i.e., the mouse cortical neurones and in a cell line derived from human neuronal tissue, the hNT cell line. In both preparations, the effect of retigabine on K^+ channels was present. Summarizing these data, the effect of retigabine can be seen in three neuronal cell preparations, but is different from that of K^+ channel openers of ATP sensitive channels. The target channel for retigabine can not be defined from these experiments.

Although no direct interaction between 4-aminopyridine and retigabine could be shown, the K^+ channel opening effect can be of importance for the anti-burst activity in the 4-aminopyridine brain slice model. Administration of 4-aminopyridine to the slices induces a release of both excitatory and inhibitory neurotransmitters which results in the initiation of spontaneous actions potential bursts (Yonekawa et al., 1995). Prior to the induction of bursts, a slight depolarization of the neuronal cells can be observed in this model (Heinemann, personal communication). Thus, the K^+ channel opening induced by retigabine should lead to a repolarization of the neuronal cells. This should reduce the amount of neurotransmitters released by 4-aminopyridine. Indeed, in a previous study, Kapetanovic et al. (1995) showed, that the increased release of newly synthesized neurotransmitters was reduced by retigabine.

K^+ channel opening is not a mechanism of antiepileptic drugs but has been discussed as an interesting approach (Alzheimer and Ten Bruggencate, 1988; Rogawski and Porter, 1990; Del Pozo et al., 1990; Popoli et al., 1991). Application of retigabine induced a K^+ current in slightly depolarized cells while no current was induced in cells clamped close to the K^+ reversal potential of -84 mV. Transferring this into the epileptic brain, cells which are partially depolarized and therefore prone to generating action potentials in response to excitatory postsynaptic potentials are repolarized; cells with a membrane potential close to the reversal potential of K^+ are clamped to this membrane potential preventing the surpassing of the spike threshold.

While retigabine acted as an opener of K^+ channels, some blocking activity on voltage activated outward rectifying K^+ channels was observed. This effect, though only at concentrations above 10 μM, reached a substantial level. Since the plasma concentration found to be effective in pharmacological experiments was in the range of 0.1 and 3 μM (Jainta et al., 1995; Rostock et al., 1996) and even below 0.1 μM (Tober et al., 1996) the effect on outward rectifying K^+ channels can be considered to be not relevant for anticonvulsant activity. In previous studies, we and others have reported further mechanisms of action of retigabine including potentiation of γ-aminobutyric acid (GABA) induced currents, weak blocking effects on Na^+ and Ca^{2+} channels (Rundfeldt et al., 1995b) and glutamate antagonistic effects (Skeen et al., 1995). Comparing the concentrations needed to induce the effects, both the GABA potentiation and the K^+ channel opening are the most sensitive mechanisms (Kapetanovic and Rundfeldt, 1996).

In conclusion, on the cellular level retigabine exerts multiple effects on several targets all contributing to the anticonvulsant activity. The activation of K^+ channels is different from cromakalim, opening up the perspective of clearly separating effects on neurones and smooth muscle cells. Activation of K^+ channels can be considered a new approach to anticonvulsant therapy. Such an effect might also be useful in other disease states related to depolarized cells like brain ischemia and neurodegeneration. Further work utilizing cloned receptors is under way to define the K^+ channel subtype at which retigabine is active.

Acknowledgements

The work was supported in part by 'Sächsisches Staatsministerium für Wirtschaft und Arbeit', PT-Nr. 1791.

References

Alzheimer, C., Ten Bruggencate, G., 1988. Actions of BRL 34915 (Cromakalim) upon convulsive discharges in guinea pig hippocampal slices. Arch. Pharmacol. 337, 429–434.

Bernardi, H., Lazdunski, M., 1993. Characterization of the sulfonylurea-sensitive ATP-modulated potassium channel. In: Glossmann, H., Striessnig, J. (Eds.), Molecular and Cellular Biology of Pharmacological Targets. Plenum Press, New York, NY.

Bodewei, R., Hering, S., Schubert, B., Wollenberger, A., 1985. Sodium and calcium currents in neuroblastome×glioma hybrid cells before and after morphological differentiation by dibutyryl cyclic AMP. Gen. Physiol. Biophys. 4, 113–127.

Colatsky, T.J., Follmer, C.H., Starmer, C.F., 1990. Channel specificity in antiarrhythmic drug action. Mechanism of potassium channel block and its role in suppressing and aggravating cardiac arrhythmiasis. Circulation 82, 2235–2242.

Del Pozo, E., Barrios, M., Baeyens, J.M., 1990. Effects of potassium channel openers on pentylenetetrazole-induced seizures in mice. Pharmacol. Toxicol. 67, 182–184.

Doupnik, C.A., Davidson, N., Lester, H.A., 1995. The inward rectifier potassium channel family. Curr. Opin. Neurobiol. 5, 268–277.

Edwards, G., Weston, A.H., 1993. The pharmacology of ATP-sensitive potassium channels. Annu. Rev. Pharmacol. Toxicol. 33, 597–637.

Goldstein, S.A.N., Price, L.A., Rosenthal, D.N., Pausch, M.H., 1996. ORK 1, a potassium-selective leak channel with two pore domains cloned from *Drosophila melanogaster* by expression in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 93, 13256–13261.

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J., 1981. Improved patch clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflüg. Arch. 391, 85–100.

Hille, B., 1993. Ionic Channels of Excitable Membranes, 2nd ed. Sinauer, Sunderland, MA.

Jainta, H., Tober, C., Kronbach, T., 1995. Exploratory pharmacokinetics of the new anticonvulsant D-23129 in rats and dogs investigated by column-switching HPLC. Naunyn-Schmiedenbergs Arch. Pharmacol. 351 (Suppl.), R51.

Kapetanovic, I.M., Rundfeldt, C., 1996. D-23129: A new anticonvulsant compound. CNS Drug Rev. 2, 308–321.

Kapetanovic, I.M., Yonekawa, W.D., Kupferberg, H.J., 1995. The effects of D-23129, a new experimental anticonvulsant drug, on neurotransmitter amino acids in the rat hippocampus in vitro. Epilepsy Res. 22, 167–173.

Kubo, Y., Baldwin, T.J., Jan, Y.N., Jan, L.Y., 1993. Primary structure and functional expression of a mouse inward rectifier potassium channel. Nature 362, 127–133.

Lee, V., McGrogan, M., Lernhardt, W., Huvar, A., 1994. hNT Neurons: Human postmitotic CNS neurons. Strategies 7, 28–31.

Leasge, F., Guillemare, E., Fink, M., Duprat, F., Lazdunski, M., Romey, G., Barhanin, J., 1996. TWIK-1, a ubiqitous human weakly inward rectifying K^+ channel with a novel structure. EMBO J. 15, 1004–1011.

McKay, M.C., Dworetzky, S.I., Meanwell, N.A., Olesen, S.-P., Reinhart, R.H., Levitan, I.B., Adelman, J.P., Gribkoff, V.K., 1994. Opening of large-conductance calcium-activated potassium channels by the substituted benzimidazolone NS004. J. Neurophysiol. 71, 1873–1882.

Pleasure, S.J., Page, C., Lee, V.M., 1992. Pure, postmitotic, polarized human neurons derived from NTera 2 cells provide a system for expressing exogenous proteins in terminally differentiated neurons. J. Neurosci. 12, 1802–1815.

Popoli, P., Pezzola, A., Sagratella, S., Zeng, Y.C., Scotti de Carolis, A., 1991. Cromakalim (BRL 34915) counteracts the epileptiform activity elicited by diltiazem and verapamil in rats. Br. J. Pharmacol. 104, 907–913.

Rostock, A., Tober, C., Rundfeldt, C., Bartsch, R., Engel, J., Polymeropoulos, E.E., Kutscher, B., Löscher, W., Hönack, D., White, H.S., Wolf, H.H., 1996. D-23129: A new anticonvulsant with a broad spectrum activity in animal models of epileptic seizures. Epilepsy Res. 23, 211–223.

Rogawski, M.A., Porter, R.J., 1990. Antiepileptic drugs: Pharmacological mechanisms and clinical efficacy with consideration of promising developmental stage compounds. Pharmacol. Rev. 42, 223–286.

Rudy, B., 1988. Diversity and ubiquity of potassium channels. Neuroscience 25, 729–749.

Rundfeldt, C., Bartsch, R., 1996. The new anticonvulsant D-23129 acts as a potent potassium channel opener in neuronal cells. Naunyn-Schmiedenbergs Arch. Pharmacol. 353 (Suppl.), R97.

Rundfeldt, C., Tober, C., Ganquly, A., Wolf, H.H., White, H.S., 1995a. Potassium channel opening properties of D-23129, an anticonvulsant with potent effects in rodent models for generalized and focal seizures. Soc. Neurosci. Abstr. 21, 203.

Rundfeldt, C., Rohlfs, A., Netzer, R., 1995b. Multiple actions of the new anticonvulsant D-23129 on voltage-gated inward currents and GABA-induced currents in cultured neuronal cells. Naunyn-Schmiedebergs Arch. Pharmacol. 351 (Suppl.), R160.

Rupersberg, J.P., Ermler, M., Knopf, M., Kues, W., Jonas, P., Koenen, M., 1993. Properties of shaker-homologues potassium channels expressed in the mammalian brain. Cell Physiol. Biochem. 3, 250–258.

Skeen, G.A., Wolf, H.H., White, H.S., 1995. D-23129 attenuates glutamate-evoked whole-cell currents of mouse cortical neurons through an interaction with kainate-sensitive ion channels. Soc. Neurosci. Abstr. 21, 353.

Takumi, T., Ohkubo, H., Nakanishi, S., 1988. Cloning of a membrane protein that induces a slow voltage-gated potassium current. Science 242, 1042–1045.

Tober, C., Rundfeldt, C., Rostock, A., Bartsch, R., Nickel, B., Szeleny, I., Herbst, M., Dieter, R., Olbrich, A., Kutscher, B., Sauer, W., Jainta, H., Jahn, W., Engel, J., 1995. D-23129. Drugs Future 20, 1112–1115.

Tober, C., Rostock, A., Rundfeldt, C., Bartsch, R., 1996. D-23129: A potent anticonvulsant in the amygdala kindling model of complex partial seizures. Eur. J. Pharmacol. 303, 163–169.

Yonekawa, W.D., Kapetanovic, I.M., Kupferberg, H.J., 1995. The effects of anticonvulsant agents on 4-aminopyridine induced epileptiform activity in rat hippocampus in vitro. Epilepsy Res. 20, 137–150.