ELSEVIER

European Journal of Pharmacology 386 (1999) 165–171

ejp

www.elsevier.nl/locate/ejphar

Retigabine strongly reduces repetitive firing in rat entorhinal cortex

Robert Hetka [a,1], Chris Rundfeldt [b,*], Uwe Heinemann [a], Dietmar Schmitz [a]

[a] *Department of Neurophysiology, Institute of Physiology, Charité,Humboldt University Berlin, Tucholskystraße 2, 10117 Berlin, Germany*
[b] *Department of Pharmacology, Corporate R&D, ASTA Medica Group, Arzneimittelwerk Dresden, Meissner Strasse 35, D-01445 Radebeul, Germany*

Received 19 October 1999; accepted 2 November 1999

Abstract

Retigabine (D-23129) [*N*-(2-amino-4-(4-fluorobenzylamino)phenyl) carbamic acid ethyl ester] is a novel antiepileptic drug. The compound was shown to possess anticonvulsant properties both in vivo and in vitro. We investigated the effects of retigabine on neurones in the rat medial entorhinal cortex using conventional intracellular recordings in combined hippocampal–entorhinal cortex slices. Retigabine strongly reduced the number of action potentials elicited by 1 s long depolarising current injections. Both the amplitudes of monosynaptic inhibitory postsynaptic potentials/currents (IPSP/Cs) and the amplitudes of excitatory postsynaptic potentials (EPSPs) remained unaffected. The drug increased outward rectification and induced a membrane-potential hyperpolarisation in most of the tested neurones. The findings suggest that retigabine exerts its anticonvulsant effects by activation of a K^+ conductance, however it cannot be excluded from our experiments that other mechanisms may be involved in the effect of retigabine on membrane properties.

Keywords: Retigabine; Anticonvulsant; Entorhinal cortex; Spike frequency habituation; EPSP (excitatory postsynaptic potential); IPSP (inhibitory postsynaptic potential); K^+ channel opener

1. Introduction

Retigabine (D-23129) [*N*-(2-amino-4-(4-fluorobenzylamino)phenyl) carbamic acid ethyl ester] is a novel antiepileptic drug currently undergoing phase II clinical trials. In animal models of epilepsy, retigabine presented a broad spectrum of anticonvulsant activity. It was effective in threshold and supramaximal electroshock and pentylenetetrazol seizure models and against seizures induced by picrotoxin and *N*-methyl-D-aspartate (NMDA) (Rostock et al., 1996). Retigabine also was effective against focal and secondary generalised seizures in amygdala-kindled rats after both intraperitoneal and oral administration (Tober et al., 1996). In addition, retigabine has activity against audiogenically-induced convulsions in the genetically epilepsy-prone rat (Dailey et al., 1995) and DBA/2 mouse (Rostock et al., 1996).

Retigabine has been proposed to possess multiple mechanisms of action (Kapetanovic and Rundfeldt, 1996). It has been shown to activate voltage-sensitive K^+ channels (Rundfeldt, 1997, 1999), to potentiate the action of γ-aminobutyric acid (GABA) at the $GABA_A$ receptor (Rundfeldt et al., 1995), and, at higher concentrations, to increase GABA synthesis (Kapetanovic et al., 1995) and block Na^+ and Ca^{2+} channels (Rundfeldt et al., 1995; Kapetanovic and Rundfeldt, 1996). The contribution of any, or all, of these mechanisms to the anti-epileptic actions of the drug remains to be determined.

The aim of the present study was to test to what extend the different proposed mechanisms contribute to the excitability of neuronal cell in vitro. We investigated the effect of retigabine on repetitive firing induced by depolarising current injection and on stimulus-induced synaptic potentials of neurones in layer II and III in the medial entorhinal cortex of rats.

The entorhinal cortex is involved in a number of diseases including temporal lobe epilepsy (Du et al., 1993). In vitro studies showed that the entorhinal cortex is one of the most seizure prone structures in the central nervous system (Walther et al., 1986; Stanton et al., 1987; Jones and Heinemann, 1988). The entorhinal cortex is intimately

* Corresponding author. Tel.: +49-351-8343329; fax: +49-351-8343320.
E-mail address: dr_chris.rundfeldt@astamedica.de (C. Rundfeldt)
[1] Current address: Institute of Physiology, First Faculty of Medicine, Charles University, Albertov 5, 12800 Prague, Czech Republic.

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00786-4

linked to the hippocampus which sends its main output to the deep layers of entorhinal cortex. Because of its synaptic connectivity, the entorhinal cortex may play a role in the propagation and generalisation of temporal lobe–limbic system seizures, but it could also potentially develop a primary focus (Jones, 1993). Neurones of the superficial layers of the entorhinal cortex form the two branches of the perforant path (Steward and Scoville, 1976). Layer 2 cells project to the dentate gyrus and the CA3 region and layer 3 neurones project directly to the CA1 and the subiculum. The morphological and electrophysiological properties of different types of neurones in the entorhinal cortex have been described in detail (Alonso and Klink, 1993; Jones, 1994; Gloveli et al., 1997).

2. Materials and methods

2.1. Slice preparation

Adult Wistar rats of both sexes (140–250 g body weight) were used. The animals were decapitated under deep ether anaesthesia and the brains quickly removed. Horizontal slices (400 μm thick) containing the hippocampus, entorhinal, perirhinal and temporal cortices, as described previously (Dreier and Heinemann, 1991), were cut using a vibroslicer (Campden Instruments, Loughborough, UK). The slices were immediately transferred to a standard interface chamber and perfused at a rate 1.4–1.8 ml/min with prewarmed (35°C) artificial cerebrospinal fluid (ACSF), containing in mM: NaCl, 129; NaH_2PO_4, 1.25; $MgSO_4$, 1.8; $CaCl_2$, 1.6; KCl, 3.0; glucose 10; $NaHCO_3$, 21; saturated with 95% O_2 and 5% CO_2 (pH 7.4).

2.2. Drugs

Retigabine was obtained from Arzneimittelwerk Dresden (Radebeul, Germany). For application, the solution was freshly prepared before every single measurement. 3.03 mg retigabine was dissolved in 100 μl of 1 M HCl and then diluted to 0.5 ml with demineralised water. When the cell had become stabilised, the whole stock solution was applied into 100 ml of prewarmed and carbogenated ACSF. The pH was equilibrated back to 7.4 using NaOH. The final retigabine concentration was 100 μM. For induction of monosynaptic inhibitory postsynaptic potentials, excitatory synaptic transmission was blocked by adding 10 μM 6-nitro-7-sulphamoylbenzo(*f*)quinoxaline-2,3-dione (NBQX; a gift from Novo Nordisk) and 30 μM DL-2-amino-5-phosphonovaleric acid (±-APV; Research Biochemicals, Natick, MA, USA) to the perfusate.

2.3. Electrophysiological recordings

Intracellular recordings were made with electrodes pulled with a horizontal puller (P-87, Sutter Instrument, Novato, CA, USA) from borosilicate glass (o.d.: 1.2 mm; Science Products, Hofheim, Germany) and filled with 2.5 M K^+acetate. Electrode resistances varied between 40 and 120 MΩ. Recordings were made using a Neurodata IR 183 (Neurodata Instruments, New York, USA) or a SEC10L (NPI Electronic, Tamm, Germany) amplifier. Potential changes were filtered at 3 kHz, then sampled at a rate of 6.4 to 8 kHz by a CED 1401 (Cambridge Electronic Design, Cambridge, UK) or ITC-16 (Instrutech, Great Neck, NY, USA) analogue to digital converter and stored on the disk of an IBM PC compatible computer using SIAVG (Cambridge Electronic Design, Cambridge, UK) or WINTIDA (HEKA, Lambrecht, Germany) capturing software. Stimulation protocols were generated with a Master-8 (AMPI, Jerusalem, Israel) programmable pulse generator or directly via the ITC-16 board. Measurements were performed using the conventional bridge mode circuit. The bridge balance was monitored throughout the experiment. The transient suppression was used to remove the capacitance artefacts. Only cells with membrane potentials more negative than −60 mV, input resistance of more than 30 MΩ and with overshooting action potentials were accepted. Resting membrane potentials of the cells were estimated by subtraction of the tip potential following withdrawal from the cell. In some experiments we monitored the resting membrane potential on a chart recorder (Dash IVxl, Astro-Med, WestWarwick, RI USA). ''Switched'' single-electrode voltage clamp recordings were performed with conventional sharp microelectrodes using a SEVC amplifier (SEC10L). After clamping the cell close to the resting membrane potential, we optimised the gain, capacitance compensation and switching frequency (15–25 kHz). The headstage voltage was continuously monitored and decayed sufficiently before the next current injection (1/4 duty cycle).

3. Results

3.1. Spike frequency habituation

The neurones ($n = 46$) used in this study had an average membrane potential of -69.9 ± 0.7 mV and an average input resistance of 61.1 ± 2.7 MΩ. To elicit a train of action potentials we applied depolarising current pulses of 1 s with varying current intensities. In a first set of orienting experiments ($n = 10$ neurones) we used retigabine concentrations of 10, 50, and 100 μM for application periods of 2 to 20 min. The number of action potentials evoked by a 1 s pulse was always reduced. With lower concentrations it took longer time to see the effect. When 100 μM retigabine had been applied for more than 10 min, we did not see any recovery even after 1.5 h washout time. For a more quantitative study we therefore always applied 100 μM retigabine for only 2 min ($n = 36$ neurones). This treatment schedule assured a fast onset of the effects and

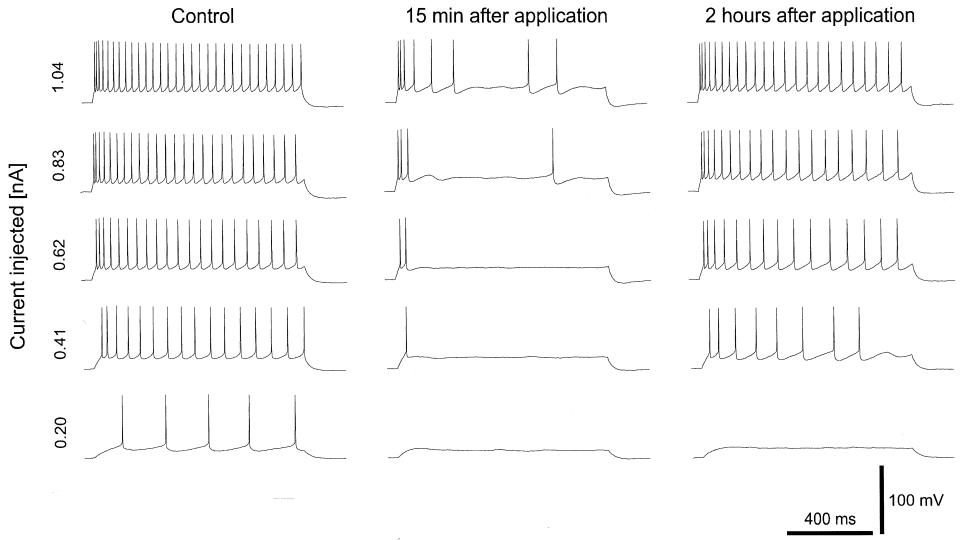

Fig. 1. Effect of retigabine on repetitive firing in a neurone of layer III of the medial entorhinal cortex. The drug reversibly reduced the number of action potentials elicited by 1 s long depolarising current injections. The membrane potential was constant prior to the depolarising current step (-69.9 ± 0.7 mV on average). Calibration on the right corner applies to all records in this figure.

an at least partial recover upon washout. However, due to the short application period, the actual concentration of retigabine at the site of action can be assumed to have been considerably lower than 100 μM. Fig. 1 illustrates effects of retigabine on neuronal firing elicited by different current intensities. The figure illustrates the strong effect of retigabine in a sample cell on repetitive firing which was reversible 2 h after application of retigabine. The effect was quantified in 6 cells (see Fig. 2 for results of a sample cell). Under control conditions (filled diamonds) current injections of >100 pA resulted in first spikes; with higher currents injected, the number of induced spikes increased nearly linear. With injection of 300 to 600 pA an average of 22.2 ± 2 (mean $\pm$ S.E.M., $n = 6$) action potentials were elicited. Upon application of retigabine (filled squared), higher currents had to be injected to induce first spikes (300 pA). In addition, even at currents as high as 1 nA, the number of spikes induced was considerably lower. With injection of 300 to 600 pA, an average reduction of 94% could be seen. At this current, only 1–2 remaining action potentials could be elicited. In the sample cell displayed in Fig. 2, the effect of retigabine was partially reversible upon washout (filled triangles). The effect was reproducible in all of the 6 cells (3 recovered) in which it was tested, regardless of whether we recorded from different types of projection cells in layer II or layer III. To monitor the time course of the effect, in an other set of experiments depolarising current injections were adjusted to elicit between 15 and 30 action potentials. These current pulses were repeated every 30 s before, during and after application of retigabine. A typical experiment is illustrated in Fig. 3. Full depression was reached within 10 to 20 min ($n = 9$) after application of 100 μM retigabine for 2 min. Recovery begun usually after 40 to 70 min.

3.2. Effects on membrane-potential properties

The resting membrane potential hyperpolarized in 6 out of 8 cells upon application of retigabine. The maximum change was 4 mV. The average hyperpolarisation was 1.4 ± 0.5 mV. The hyperpolarisation commenced within 9.2 ± 1.6 min after application of retigabine and lasted until 20.2 ± 2.2 min after application of retigabine. The hyperpolarisation was also noted as an outward current in voltage clamp recordings of about 100–200 pA (see Fig. 5b for a sample plot).

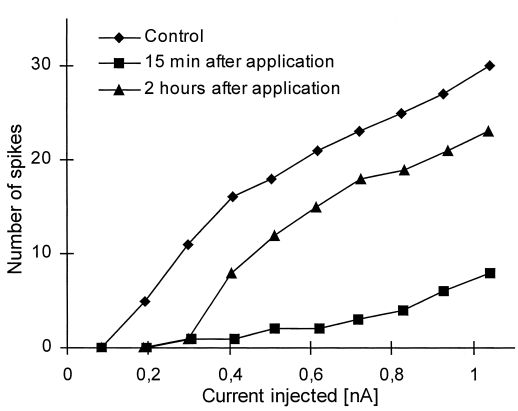

Fig. 2. Effect of different current injection strengths on the number of action potentials induced in a sample cell. Diamonds: response of the cell prior to drug application (control); squares: response 15 min after start of retigabine application; triangles: partial recovery two hours after application of retigabine (100 μM) for 2 min.

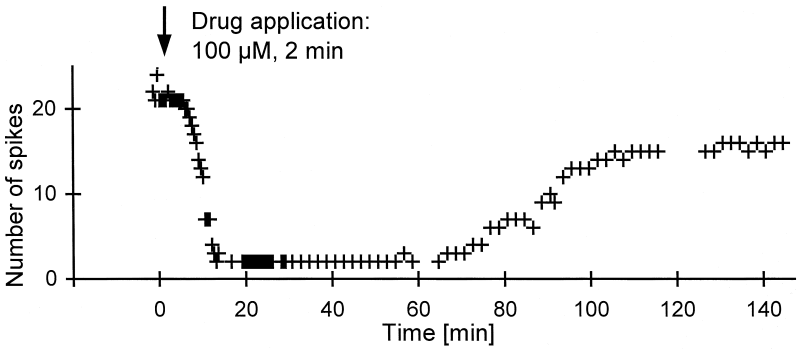

Fig. 3. Time course of the effect of 100 μM retigabine applied for 2 min on repetitive firing in a sample cell. The action potentials were elicited by 1 s long depolarising current injections of 0.6 nA repeated every 30–120 s during 150 min. The current strength was selected to elicit 20–30 spikes/s under control conditions.

The average input resistance measured at resting membrane potential by hyperpolarizing current pulses was 67.1 $\pm$ 7.0 MΩ ($n = 6$). Following the application of retigabine it decreased not significantly by 3.8 $\pm$ 2.8% ($n = 6$; $P = 0.20$). However, when membrane resistance was measured in the same cells close to the firing-threshold by depolarising pulses or constant current injection, membrane resistance decreased significantly by 37.3 $\pm$ 8.1% ($n = 5$, $P = 0.05$).

3.3. Effects on EPSPs and IPSPs

In order to test whether retigabine affects glutamatergic and/or GABAergic synaptic transmission we tested the drug on compound excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials/currents (IPSP/Cs) in a total of 13 neurones. Compound synaptic potentials were evoked by electrical stimulation via a bipolar insulated stimulation electrode placed in the lateral entorhinal cortex. A typical experiment for the investigation on EPSPs is illustrated in Fig. 4a. Neither the early nor the late component of the EPSP were significantly altered by the drug applied with 100 μM for 2 min. The peak EPSP amplitude remained stable for the observation period of 120 min (see Fig. 4b). After 120 min, the fast EPSP amplitude amounted to 105.7 $\pm$ 5.1% of the initial amplitude ($n = 7$, $P = 0.56$).

Monosynaptic IPSP/Cs were evoked by nearby stimulation in the presence of AMPA/kainate- and NMDA-re-

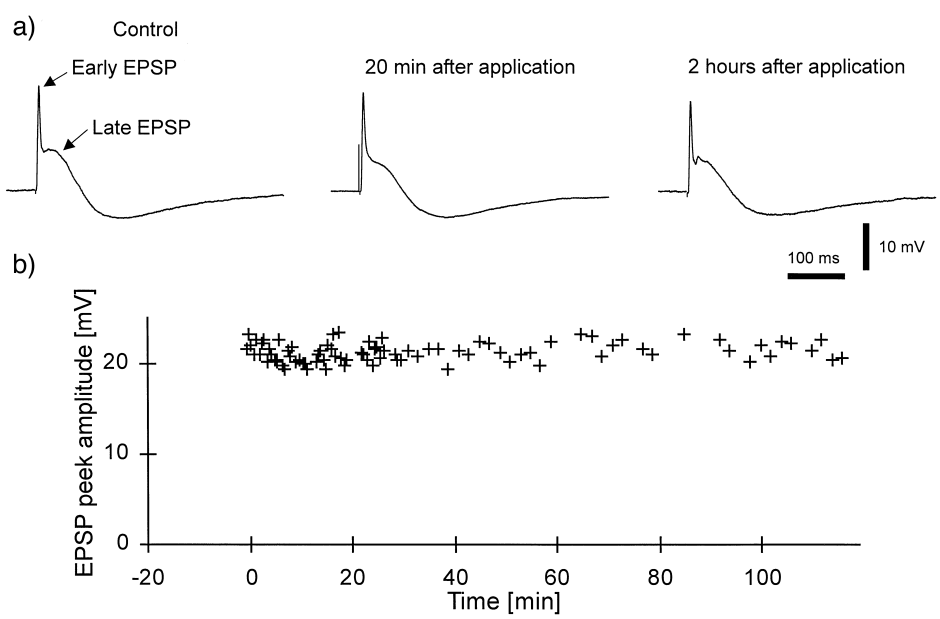

Fig. 4. (a) Sample traces of the effect of retigabine on compound EPSP/IPSP sequences in a neuron of the medial entorhinal cortex evoked by electrical stimulation of the lateral entorhinal cortex. (b) Compound EPSP/IPSP sequences were repetitively evoked by stimulation of the lateral entorhinal cortex every 30–120 s during 120 min. The plot shows the amplitude of fast EPSP component over more than 100 min after application of 100 μM retigabine for 2 min (drug application started at 0). Note that retigabine did not induce a significant effect on the peak EPSP amplitude.

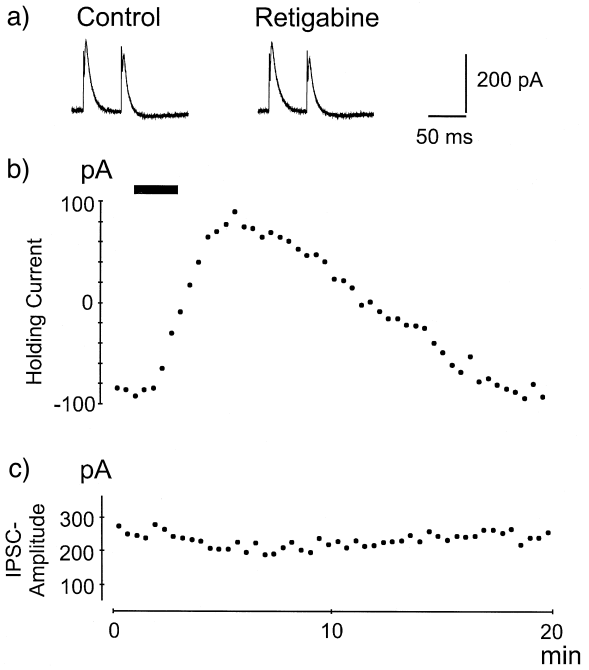

Fig. 5. Effects of retigabine on monosynaptic IPSCs evoked by paired stimulation (stimulus interval 50 ms) close to the recorded neurone. The recording was obtained with NBQX and APV. Slow IPSCs were blocked by QX-314 inside the recording electrode. Retigabine was applied for 2 min. (a) Sample traces prior to application of retigabine and 15 min after the start of drug administration. (b) Holding current necessary to compensate for the outward current induced by retigabine. In the displayed sample cell, retigabine induced an outward current of up to 180 pA. (c) Peak IPSC amplitude monitored over the course of the experiment. Note that retigabine did not induce a significant effect on the IPSC amplitude.

ceptor antagonists (NBQX and (±)-APV, respectively). Typical recordings taken prior to drug application and 15 min after application of retigabine (100 μM) for 2 min are shown in Fig. 5a. Neither in current clamp nor in voltage clamp there was a significant effect on the amplitude and decay time constant of the IPSP/Cs. Over the observation period of 20 min the IPSC amplitude remained stable (Fig. 5c). After 120 min, the amplitude amounted to 101.7 ± 2% ($n = 6$, $P = 0.39$). During these experiments a small hyperpolarization was often noted. This was corrected either by current injection in current clamp experiments or by obtaining the results under voltage clamp. The holding current necessary to clamp one sample cell during the observation period of 20 min is displayed in Fig. 5b.

4. Discussion

The main findings of the present paper are that retigabine reduced the number of action potentials evoked by depolarising current injection, hyperpolarized neurones and caused an outward rectification near threshold for action potential induction. There was no significant effect on either EPSPs nor on IPSPs at the concentrations tested.

Retigabine is a novel anticonvulsant currently undergoing phase II clinical trials for treatment of epilepsy. There is need for the development of new anticonvulsants since about 25–30% of epilepsy patients do not get seizure free with presently available drugs (Löscher and Schmidt, 1994; Pledger and Schmidt, 1994). Only a minority of these patients profit from epilepsy surgery (Wieser, 1996). The available data suggest that retigabine may have properties beyond those of presently available drugs. Thus it was effective in two models of convulsant activity which do not respond to clinically employed standard anticonvulsants. Thus, retigabine blocked late recurrent discharges evoked by lowering of Mg^{2+} (Rundfeldt et al., 1997). These discharges depend on facilitated activation of NMDA receptors, augmented transmitter release and an increase in neuronal excitability. These discharges have been shown to be resistant to valproic acid, phenytoin, carbamazepine and phenobarbital (Dreier and Heinemann, 1990; Zhang et al., 1995). Similarly, retigabine could block epileptiform discharges evoked by combined application of 4-aminopyridine, a blocker of A, D and a sub-portion of delayed rectifier K^+currents and bicuculline, a competitive blocker of $GABA_A$ receptors (Yonekawa et al., 1995; Armand et al., 1999). Although the predictive value of these epilepsy models for clinical practice is still open, the fact that in these tests retigabine is superior to clinically employed anticonvulsants is promising.

Retigabine was moreover effective in a wide number of epilepsy models including the kindling epilepsy (Tober et al., 1996). This model is considered to be predictive for drug effects in partial epilepsies with focal origin. The most frequent form of partial epilepsies is temporal lobe epilepsy. The entorhinal cortex is one of the most seizure prone areas within temporal lobe structures. While lowering of Mg^{2+}, application of $GABA_A$ receptor antagonists and application of 4-aminopyridine induce typically only short recurrent discharges in the hippocampal slice, these treatments induce prolonged seizure like events in the entorhinal cortex and neighbouring structures such as the temporal neocortex and the subiculum. It was therefore of particular interest to test the effects of retigabine on neuronal behaviour in entorhinal cortex cells.

Our data indicate that retigabine shares with phenytoin, carbamezepine and valproate effects on repetitive firing (Macdonald and McLean, 1986). Significant effects on synaptic currents were not seen in entorhinal cortex cells during application of retigabine. This was somewhat unexpected since retigabine was shown to potentiate GABA induced currents in rat cortical neurones (Rundfeldt et al., 1995). However, two factors can help explaining this result. Perfusion of the slices with 100 μM retigabine for 2 min does not result in an actual concentration of 100 μM at the proposed site of action, i.e., the GABAergic synapse. Indeed, full equilibration with a drug in an interface chamber can be reached in slices only after 40–80 min perfusion (Zhang et al., 1995). Thus, the locale concentration of

retigabine may not have been high enough to significantly augment IPSP/Cs. Our experiments do not exclude that retigabine can also augment synaptic inhibition if applied for more prolonged periods or in higher concentrations, but compared to the other effects observed in this study, the modulation of GABA induced currents seems to be less sensitive and may therefore contribute only to a small extend to the anticonvulsant activity. The second possibility may be that retigabine interacts only with GABA receptor subunits, which are not expressed in the brain region under investigation. To clarify this, further studies using GABA receptor subunits expressed in *Xenopus* oocytes are under way.

In our experiments, retigabine causes a net hyperpolarisation of neuronal membrane-potentials. Such effects were not reported for carbamazepine, valproate or phenytoin. Indeed, it was previously shown that retigabine opens K^+ channels in neuronal cells (Rundfeldt, 1997, 1999), a property so far not shown for any other anticonvulsant. Several lines of evidence can be found that the opening of K^+ channels is a promising strategy for new anticonvulsant drug therapy (Doupnik et al., 1995; Sah, 1996; Meldrum, 1997). In both animal models of epilepsy and epileptic patients, mutations of K^+ channels were reported as possible cause (Patil et al., 1995; Beck et al., 1996; Janigro et al., 1997; Charlier et al., 1998); an in vivo virus mediated transfection of CNS neurones with a voltage gated K^+ channel resulted in a reduction in seizure susceptibility (Kirkby et al., 1996). The K^+ opening effect of retigabine may therefore contribute to the anticonvulsant properties of this agent. We also observed an outward rectification induced by retigabine which leads to an increase in the threshold for generation of action potentials. Such an effect can be explained by a drug induced facilitated activation of voltage dependent outward currents. The underlying mechanism may be the K^+ channel opening effect of retigabine, however other mechanisms like a reduction of persistent sodium currents (as shown for valproic acid, Taverna et al., 1998) and other anticonvulsants, can also cause an outward rectification. Further experiments are required to resolve this question.

References

Alonso, A., Klink, R., 1993. Differential electroresponsiveness of stellate and pyramidal-like cells of medial entorhinal cortex layer II. J. Neurophysiol. 70, 128–143.

Armand, V., Rundfeldt, C., Heinemann, U., 1999. Effects of retigabine (D-23129) on different patterns of epileptiform activity induced by 4-aminopyridine in rat entorhinal cortex hippocampal slices. Naunyn-Schmiedeberg's Arch. Pharmacol. 359, 33–39.

Beck, H., Blumcke, I., Kral, T., Clusmann, H., Schramm, J., Wiestler, O.D., Heinemann, U., Elger, C.E., 1996. Properties of a delayed rectifier potassium current in dentate granule cells isolated from the hippocampus of patients with chronic temporal lobe epilepsy. Epilepsia 37, 892–901.

Charlier, C., Singh, N.A., Ryan, S.G., Lewis, T.B., Reus, B.E., Leach, R.J., Leppert, M., 1998. A pore mutation in a novel KQT-like potassium channel gene in an idiopathic epilepsy family. Nat. Genet. 18, 53–55.

Dailey, J.W., Cheong, J.H., Ko, K.H., Adams-Curtis, L.E., Jobe, P.C., 1995. Anticonvulsant properties of D-20443 in genetically epilepsy-prone rats: prediction of clinical response. Neurosci. Lett. 195, 77–80.

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

Dreier, J.P., Heinemann, U., 1990. Late low magnesium-induced epileptiform activity in rat entorhinal cortex slices becomes insensitive to the anticonvulsant valproic acid. Neurosci. Lett. 119, 68–70.

Dreier, J.P., Heinemann, U., 1991. Regional and time dependent variations of low magnesium induced epileptiform activity in rat temporal cortex. Exp. Brain Res. 87, 581–596.

Du, F., Whetsell, W.O., Abou-Khalil, B., Blumenkopf, B., Lothman, E.W., Schwarcz, R., 1993. Preferential neuronal loss in layer III of the entorhinal cortex in patients with temporal lobe epilepsy. Epilepsy Res. 16, 223–233.

Gloveli, T., Schmitz, D., Empson, R.M., Dugladze, T., Heinemann, U., 1997. Morphological and electrophysiological characterization of layer III cells of the medial entorhinal cortex of the rat. Neuroscience 77, 629–648.

Janigro, D., Gasparini, S., D'Ambrosio, R., McKhann, G. II, DiFrancesco, D., 1997. Reduction of K^+ uptake in glia prevents long-term depression maintenance and causes epileptiform activity. J. Neurosci. 17, 2813–2824.

Jones, R.S.G., 1993. Entorhinal–hippocampal connections: a speculative view of their function. Trends Neurosci. 16, 58–64.

Jones, R.S.G., 1994. Synaptic and intrinsic properties of neurons of origin of the perforant path in layer II of the rat entorhinal cortex in vitro. Hippocampus 4, 335–353.

Jones, R.S.G., Heinemann, U., 1988. Synaptic and intrinsic responses of medial entorhinal cortical cells in normal and magnesium-free medium in vitro. J. Neurophysiol. 59, 1476–1497.

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 effect of D-23129, a new experimental anticonvulsant drug, on neurotransmitter amino acids in the rat hippocampus in vitro. Epilepsy Res. 22, 167–173.

Kirkby, R.D., Johns, D.C., Marban, E., Lawrence, J.H., Rogawski, M.A., 1996. Adenovirus encoding a voltage-gated potassium channel attenuates picrotoxin-induced hippocampal epileptiform discharge in vivo. Soc. Neurosci. Abstr. 22, 2086.

Löscher, W., Schmidt, D., 1994. Strategies in antiepileptic drug development: is rational drug design superior to random screening and structural variations? Epilepsy Res. 17, 95–134.

Macdonald, R.L., McLean, M.J., 1986. Anticonvulsant drugs: mechanisms of action. Adv. Neurol. 44, 713–736.

Meldrum, B.S., 1997. Identification and preclinical testing of novel antiepileptic compounds. Epilepsia 38 (Suppl. 9), S7–S15.

Patil, N., Cox, D.R., Bhat, D., Faham, M., Myers, R.M., Peterson, A.S., 1995. A potassium channel mutation in weaver mice implicates membrane excitability in granule cell differentiation. Nat. Genet. 11, 126–129.

Pledger, G.W., Schmidt, D., 1994. Evaluation of antiepileptic drug efficady. A review of clinical trial design. Drugs 48, 498–509.

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.

Rundfeldt, C., 1997. The new anticonvulsant retigabine (D-23129) acts as an opener of K^+ channels in neuronal cells. Eur. J. Pharmacol. 336, 243–249.

Rundfeldt, C., 1999. Characterization of the K^+ channel opening effect

of the anticonvulsant retigabine in PC12 cells. Epilepsy Res. 35, 99–107.

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

Rundfeldt, C., Armand, V., Heinemann, U., 1997. The anticonvulsant D-23129 (retigabine) is effective in a hippocampal slice model of drug resistant epilepsy. Soc. Neurosci. Abstr. 23, 2162.

Sah, P., 1996. Ca^{2+}-activated K^{+} currents in neurones: types, physiological roles and modulation. Trends Neurosci. 19, 150–154.

Stanton, P.K., Jones, R.S.G., Mody, I., Heinemann, U., 1987. Epileptiform activity induced by lowering extracellular $[Mg^{2+}]$ in combined hippocampal-entorhinal cortex slices: modulation by receptors for norepinephrine and *N*-methyl-D-aspartate. Epilepsy Res. 1, 53–62.

Steward, O., Scoville, S.A., 1976. Cells of origin of entorhinal cortical afferents to the hippocampus and fascia dentata of the rat. J. Comp. Neurol. 169, 347–370.

Taverna, S., Mantegazza, M., Franceschetti, S., Avanzini, G., 1998. Valproate selectively reduces the persistent fraction of Na^{+} current in neocortical neurons. Epilepsy Res. 32, 304–308.

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.

Walther, H., Lambert, J.D.C., Jones, R.S.G., Heinemann, U., Hamon, B., 1986. Epileptiform activity in combined slices of the hippocampus, subiculum and entorhinal cortex during perfusion with low magnesium medium. Neurosci. Lett. 69, 156–161.

Wieser, H.G., 1996. Epilepsy surgery. Baillieres Clin. Neurol. 5, 849–875.

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.

Zhang, C.L., Dreier, J.P., Heinemann, U., 1995. Paroxysmal epileptiform discharges in temporal lobe slices after prolonged exposure to low magnesium are resistant to clinically used anticonvulsants. Epilepsy Res. 20, 105–111.