Antipsychotic-Like Effect of Retigabine \([N-(2\text{-}Amino\text{-}4-(fluorobenzylamino)\text{-}phenyl)\text{carbamic Acid Ester}], a \text{ KCNQ}\ \text{Potassium Channel Opener, via Modulation of Mesolimbic Dopaminergic Neurotransmission}

Florence Sotty, Trine Damgaard, Liliana P. Montezinho, Arne Mørk, Christina K. Olsen, Christoffer Bundgaard, and Henriette Husum

Departments of Neurophysiology (F.S., L.P.M., A.M.), In Vivo Neurobiology (T.D., C.K.O., H.H.) and Discovery Absorption, Distribution, Metabolism and Excretion (C.B.), H. Lundbeck A/S, Valby, Denmark

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ABSTRACT

Dopaminergic (DAergic) neurons in the ventral tegmental area express both KCNQ2 and KCNQ4 channels, which opening is expected to decrease neuronal excitability via neuronal hyperpolarization. Because psychotic symptoms are believed to be associated with an increased excitability of dopamine (DA) cells in the mesencephalon, KCNQ channels might represent a new potential target for the treatment of psychosis. The aim of our study was to investigate the antipsychotic-like potential of KCNQ channel opening via modulation of neuronal activity within the mesolimbic DAergic system. We report that retigabine \([N-(2\text{-}amino\text{-}4-(fluorobenzylamino)\text{-}phenyl)\text{carbamic acid ester}], a \text{ KCNQ}\ \text{opener, dose-dependently reduced basal DA firing rate and more potently suppressed burst firing activity in the ventral tegmental area, whereas XE-991 [10,10\text{-}bis(pyridinylmethyl)-9(10\text{H})\text{-}anthracenone], a selective KCNQ blocker, induced opposite effects. In addition, retigabine prevented d-amphetamine-induced DA efflux in the nucleus accumbens and d-amphetamine-induced locomotor hyperactivity. In contrast, XE-991 potentiated both the locomotor hyperactivity and DA efflux evoked by d-amphetamine. These data strongly suggest that the activation of KCNQ channels attenuates DAergic neurotransmission in the mesolimbic system, particularly in conditions of excessive DAergic activity. In a model predictive of antipsychotic activity, the conditioned avoidance response paradigm, retigabine was found to inhibit avoidance responses, an effect blocked by coadministration of XE-991. Furthermore, retigabine was found to significantly inhibit the hyperlocomotor response to a phencyclidine (PCP) challenge in PCP-sensitized animals, considered as a disease model for schizophrenia. Taken together, our studies provide evidence that KCNQ channel openers represent a potential new class of antipsychotics.

KCNQ channels (also named K\(_an\text{7}\)) are voltage-dependent potassium channels composed of homo- and heterotetrameric complexes of five different KCNQ subunits (KCNQ1–5, K\(_an\text{7.1–7.5}\)). With the exception of KCNQ1 channels, KCNQ channels are strongly expressed in neuronal tissue, including neocortex and hippocampus (Jentsch, 2000). Their activation is responsible for the generation of the M current, an inhibitory K\(^+\) current regulating repetitive action potential discharges, therefore modulating neuronal excitability (Delmas and Brown, 2005). Reduction in KCNQ channel activity as a result of genetic mutation in KCNQ genes has been associated with epilepsy, progressive hearing loss (Jentsch, 2000), or, more recently, bipolar disorder (Borsotto et al., 2007). Several attempts have been made to find pharmacological KCNQ modulators as a way to treat central nervous system diseases linked to hyperexcitability. Retigabine was the first KCNQ opener to be identified (Rostock et al., 1996) and is now in clinical phase III development for the treatment of epilepsy.

Recent evidence from neuroanatomical and pharmacological studies also points toward a role of KCNQ channels in the control of mesencephalic dopaminergic (DAergic) systems (Kharkovets et al., 2000; Cooper et al., 2001; Mikkelsen, 2000). This work was supported by the Fundação para a Ciência e Tecnologia, Portugal [Grant SFRH/BPD/18389/2004]. Article, publication date, and citation information can be found at http://jpet.aspetjournals.org. doi:10.1124/jpet.108.146944.

ABBREVIATIONS: retigabine, \([N-(2\text{-}amino\text{-}4-(fluorobenzylamino)\text{-}phenyl)\text{carbamic acid ester}], DAergic, dopaminergic; DA, dopamine; VTA, ventral tegmental area; XE-991, 10,10\text{-}bis(pyridinylmethyl)-9(10\text{H})\text{-}anthracenone; CAR, conditioned avoidance response; PCP, phencyclidine, 1-(1-phenylcyclohexyl)piperidine hydrochloride; CV ISI, coefficient of variation of the interspike interval; ANOVA, analysis of variance; LSD, least significant difference.
2004; Hansen et al., 2006, 2007; Koyama and Appel, 2006). Retigabine was shown to inhibit dopamine (DA) cell firing activity in midbrain slices, block increased DA efflux induced by DA reuptake blockade in the striatum, and inhibit locomotor hyperactivity induced by various psychostimulants (Hansen et al., 2006, 2007). There is also evidence that somatodendritic dopamine D₂ receptors are functionally coupled to KCNQ channels in DA neurons and that the modulation of DAergic activity by D₂ autoreceptors would involve the activation of KCNQ2 and/or KCNQ4 subunits (Ljungstrom et al., 2003). Abnormal regulation of DA neurons in the ventral tegmental area (VTA) is believed to be involved in the etiology of psychotic symptoms. Mesolimbic DA neurons exhibit two types of activity. First, burst firing activity is responsible for transient or "phasic" DA release and is dependent on intrinsic membrane properties and glutamatergic inputs. Second, tonic, regular firing activity of DA neurons is responsible for extrasynaptic or "tonic" levels of DA and is dependent on GABAergic tone in the VTA (Grace, 1991; Floresco et al., 2003). An exacerbated bursting activity of DA neurons is believed to underlie the hyperresponsive state of the mesolimbic DAergic system that is thought to be associated with psychotic symptoms (Grace, 1991). Therefore, KCNQ channel openers may represent an attractive target as a way to restore a normal level of DAergic activity within the basal ganglia, thereby improving psychotic symptoms.

The aim of this study was to evaluate the potential benefit of KCNQ channel opening as a new antipsychotic target, using preclinical models predictive of antipsychotic-like activity. The role of KCNQ channels in the regulation of the mesolimbic DAergic activity and its behavioral consequences were studied in the rat, using retigabine, and the KCNQ channel blocker, XR-991, as pharmacological tools. Presynaptic DA activity was evaluated using in vivo electrophysiology and microdialysis. To evaluate the impact of the induced changes at the postsynaptic level, the d-amphetamine-induced locomotor hyperactivity paradigm was used. To further evaluate the antipsychotic-like potential of KCNQ channel opening, the conditioned avoidance response (CAR) and the subchronic phencyclidine (PCP) sensitization models, predictive of antipsychotic efficacy, were used.

Materials and Methods

Drugs

Retigabine (synthesized at H. Lundbeck A/S, Valby, Denmark) was dissolved in 10% 2-hydroxypropyl-β-cyclodextrine. XR-991 (Tocris Bioscience, Bristol, UK), PCP (synthesized at H. Lundbeck A/S), and d-amphetamine ([+]-α-methylphenylethylamine sulfate; Unikem A/S, Copenhagen, Denmark) were dissolved in 0.9% sodium chloride.

Animals

All experimental animals were allowed to acclimatize to the Lundbeck animal facilities for a minimum of 5 days before experimentation. The animals were kept under a 12-h day/night cycle (lights on at 6:00 AM) in a temperature (21 ± 2°C)- and humidity (60 ± 10%)-controlled environment. Unless otherwise specified, the rats were kept in groups of two rats per cage (Macrolon type III) with free access to rat chow and tap water, ad libitum. All experiments were carried out in accordance with the ethical rules of the Danish Committee and Use of Laboratory Animals.

In Vivo Electrophysiology

Animals and Surgery. In brief, adult male Wistar rats (Harlan, The Netherlands) were anesthetized with an initial intraperitoneal injection of chloral hydrate (400 mg/kg). A femoral vein catheter was then inserted for additional anesthetic injections (100 mg/kg) and drug administration. Animals were then mounted in a stereotaxic frame, the skull was exposed, and a hole (0.5 × 0.5 cm) was drilled above the ventral tegmental area.

Electrophysiological Recordings. Extracellular single-cell recordings were performed using electrodes pulled from glass capillaries and filled with 2% Pontamine Sky Blue in 2 M NaCl. The tip of the electrode was broken under microscopic control, yielding an impedance of 2.0 to 8.0 MΩ at 135 Hz. The electrode was then lowered into the VTA, using a hydraulic microdrive, at the following coordinates according to Paxinos and Watson (1996): AP, 5.5 to 5.0 mm posterior to bregma; and L, 0.5 to 0.9 mm lateral to the midline. Extracellular action potentials were amplified, discriminated and monitored on an oscilloscope and an audiomonitor. Discriminated spikes were collected and analyzed using Spike 2 software (Cambridge Electronic Design Limited, Cambridge, UK) on a personal computer-based system connected to a CED 1401 interface unit (Cambridge Electronic Design Limited). Presumed DA neurons were typically found 7.0 to 8.5 mm beneath the brain surface and were characterized by: 1) a slow and irregular firing pattern (0.5–10 Hz) and 2) triphasic action potentials with a predominant positive component, a negative component followed by a minor positive component, with an overall duration >2.5 ms (Grace and Bunney, 1983).

We investigated the effect of cumulated doses of the KCNQ channel opener, retigabine (range, 0.3–6.0 mg/kg i.v.; 0.3–1.0 ml/kg), and the KCNQ channel blocker, XR-991 (range, 0.5–2.0 mg/kg i.v.; 0.5–1.0 ml/kg), each injection being separated at least by 3 min, on: 1) the basal firing rate of DA cells, 2) the proportion of action potentials occurring in bursts (defined as the occurrence of two consecutive spikes with an interspike interval of less than 80 ms and the termination of a burst defined as the occurrence of an interspike interval exceeding 160 ms) (Grace and Bunney, 1983), and 3) the coefficient of variation of the interspike interval (CV ISI) defined as the ratio between the average interspike interval and the S.D. of the interspike interval × 100.

Data Analysis and Statistics. Drug effects were assessed by comparing the mean firing rate calculated from the 2- to 3-min period immediately before the first drug administration (baseline) with the mean firing rate calculated from at least 60 s at the maximal drug effect. Similar analysis was performed using the percentage of spikes in bursts. Data were analyzed statistically by a one-way ANOVA followed by Fisher’s LSD post hoc test. A p value less than 0.05 was considered significant.

In Vivo Microdialysis

Surgery and Microdialysis in Freely Moving Rats. Adult male Sprague-Dawley rats from Charles River Laboratories (Les Oncins, France) were used. Two days before the experiments, rats were anesthetized with 2 ml/kg Hypnorm/Dormicum (fentanyl citrate, 0.079 mg/ml; fluanisone, 2.5 mg/ml; midazolam, 1.25 mg/ml), and intracerebral guide cannulas (CMA/12) were stereotactically implanted into the brain to allow the positioning of the dialysis probe tip in the nucleus accumbens [coordinates according to Paxinos and Watson (1996): 1.7 mm anterior to bregma; lateral, 0.7 mm; 6.0 mm ventral to dural]. Anchor screws and acrylic cement were used for fixation of the guide cannulas. The body temperature of the animals was monitored by a rectal probe and maintained at 37°C. The rats were allowed to recover from surgery for 2 days and housed individually in cages.

On the day of the experiment, a microdialysis probe (CMA/12, 0.5-mm diameter, 2-mm length) was inserted through the guide cannula. The probes were connected via a dual channel swivel to a microinjection pump. Perfusion of the microdialysis probe with fil-

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tered Ringer’s solution (145 mM NaCl, 3 mM KCl, 1 mM MgCl₂·6H₂O, 1.2 mM CaCl₂·2H₂O, pH 7.4) was initiated shortly before the insertion of the probe into the brain and continued for the duration of the experiment at a constant flow of 1 μl/min. After 180 min of stabilization, the experiments were initiated. A 20-min sampling regime was used throughout the experimental period. Time-points were corrected for lag time of the perfusate from the microdialysis site to the probe outlet. Retigabine (10 mg/kg s.c., 2.5 ml/kg) or XE-991 (1 mg/kg i.p., 2.5 ml/kg) were administered 30 and 5 min, respectively, before d-amphetamine (0.5 mg/kg s.c., 2.5 ml/kg) or vehicle (0.9% sodium chloride, 2.5 ml/kg), and eight samples were further collected. The dialysates were stored at 80°C until DA determination by high-performance liquid chromatography.

Measurement of DA in the Dialysates. Aliquots (20 μl) were injected onto the high-performance liquid chromatography column by a refrigerated microsampler system, consisting of a syringe pump, a multicolumn injector, and a temperature regulator. DA and metabolites were separated by reverse phase liquid chromatography (ESA Inc., Chelmsford, MA; ODS; 150 μm) using a mobile phase consisting of 90 mM NaH₂PO₄, 50 mM sodium citrate, 1.7 mM 1-octanesulfonic acid, 50 mM EDTA, and 8% acetonitrile, pH 4.0, at a flow rate of 0.5 ml/min. Electrochemical detection was accomplished using a coulometric detector, potential set at E1 = −75 mV and E2 = 350 mV; guard cell = 350 mV (Coulochem II; ESA Inc.). Data were collected and analyzed using Chromelone Clinet 6.30 software (Diaxone Corporation, Sunnyvale, CA).

Data Analysis and Statistics. Three consecutive microdialysis samples were taken as baseline levels and set at 100%. Changes in DA were expressed as percentage of baseline within the same animal. Data are presented as means ± S.E.M. Statistical analysis was performed by two-way ANOVA, followed by post hoc Bonferroni's test/ Tukey test. The level of statistical significance level was set at p < 0.05.

Basal and d-Amphetamine-Induced Locomotor Hyperactivity

Animals. Adult male Wistar rats (Harlan) housed three per cage were used.

Experimental Conditions. The experiments were run under normal light conditions in an undisturbed room. The experimental cage was placed in a U-frame equipped with four infrared light sources and photocells. The light beams crossed the cage 4 cm above the bottom of the cage, which was covered by a thin layer of wood chip bedding material. Recording of a motility count required the interruption of two adjacent light beams, thus avoiding counts induced by stationary movements of the rat. Locomotor activity was recorded every 5 min for a period of 2 h and compiled into a total measure of light beam crossings. The experimental cage was replaced with a clean cage in between experiments.

Retigabine (subcutaneously, 2.5 ml/kg) and XE-991 (intraperitoneally, 1 ml/kg) injections took place in the home cage 30 min and 5 min before d-amphetamine/saline administration, respectively. Immediately after the administration of d-amphetamine (0.5 mg/kg s.c., 2.5 ml/kg) or saline, the rat was placed in the test box, and the recording of locomotor activity was begun.

Statistical Analysis. Statistical analysis of differences in total light beam crossings after retigabine treatment of d-amphetamine-challenged rats was carried out using a one-way ANOVA with post hoc Bonferroni’s t test. For the analysis of effects of XE-991 and d-amphetamine, a two-way ANOVA was used. In the event of a significant interaction, post hoc Tukey test was employed. A probability level of 0.05 was considered significant.

Conditioned Avoidance Response Model

Animals. Adult male Wistar rats (Harlan) were used. The animals were kept on a restricted amount of food pellets to keep them at 80% of their free-feeding weight.

Experimental Conditions. Conditioned avoidance behavior was assessed using automated shuttle boxes (42 × 16 × 20 cm, ENV-010M; MED Associates, St. Albans, VT), each placed in a sound-attenuated chamber. The boxes were subdivided into two compartments by a partition with one opening. The position of the animal and movement from one compartment to the other was detected by eight photocells sensitive to infrared light on each side of the dividing wall. Rats were trained to move into the adjacent compartment (response), within 10 s upon presentation of the conditioned stimuli (tone and light), to avoid the appearance of the unconditioned stimulus, a 0.5 mA scrambled electric shock in the grid floor of 10 s in maximal duration. The following behavioral variables were recorded: avoidance (response to conditioned stimuli within 10s) and escape failures (failure to respond to the unconditioned stimuli within 10 s).

Rats were habituated to the shuttle box 3 min before each test session. During training, each test session consisted of 30 trials with intertrial intervals varied at random between 20 and 30 s. Training was carried out 5 days/week until the animals showed avoidance in >80% (24 trials) of the trials on 3 consecutive days. All experimental sessions consisted of 10 trials with intertrial intervals varied at random between 20 and 30 s.

The drug test was preceded by a pretest (the day before). A period of at least 3 days was allowed to elapse between drug injections to prevent cumulative drug effects. Vehicle-treated groups and drug-treated groups were run concurrently. Retigabine (20 mg/kg s.c., 5 ml/kg) was either administered alone (20 mg/kg s.c.) at various pretreatment times (1–20 h) or in combination (10 mg/kg s.c., 30 min) with XE-991 (1 mg/kg i.p., 5 ml/kg, 5 min).

Data Analysis and Statistics. Drug effects were assessed by comparing mean number of avoidances and analyzed by two-way ANOVA, followed by Student-Newman-Keuls Method comparison (treatments or treatment and pretreatment time). In addition, data were expressed as percentage inhibition of avoidance compared with preceding pretest, with each animal serving as its own control.

Subchronic PCP Sensitization Model

Animals. Adult male Lister hooded rats (Charles River Laboratories) were used.

Subchronic PCP Treatment. Rats were dosed intraperitoneally with PCP (5 mg/kg, n = 30) or saline (1 ml/kg, n = 29) twice daily (7:00 AM and 7:00 PM) for 7 days, followed by a 7-day drug-free period.

Locomotor Hyperactivity Induced by a PCP Challenge. The test conditions for this experiment were identical to those listed used for the assessment of d-amphetamine-induced/baseline locomotor activity. The animals were placed individually in the test cage for 1 h before injection of retigabine or vehicle (2.5 ml/kg s.c.). Thirty minutes later, a challenge dose of PCP (5 mg/kg i.p.) or vehicle (2.5 ml/kg) was administered. Locomotor activity was measured in 5-min intervals throughout this dosing paradigm and for 2 h after the PCP challenge administration. Only data for the period between 60 and 120 min post-PCP challenge administration are shown.

Data Analysis and Statistics. Differences in total light beam crossings during the habituation period between treatment groups were evaluated by means of one-way ANOVA (subchronic vehicle or PCP). Differences between treatment groups during the period from 60 to 120 min after the challenge dose of PCP or vehicle were separately evaluated by two-way ANOVA for animals subchronically treated with vehicle or PCP, respectively. In the event of a significant interaction, post hoc Tukey test was used.

Plasma and Brain Exposure Analysis

Blood samples and brains were collected to determine plasma (nanograms per milliliter) and brain (nanograms per gram) levels of retigabine after intravenous (in vivo electrophysiology experiments) or subcutaneous (CAR model) administration. Retigabine concentrations were determined in harvested EDTA plasma and brain homog-
enantiomers using turboflow chromatography (dual column, focus mode; Thermo Fisher Scientific, Waltham, MA) followed by tandem mass spectrometry detection (Sciex API-3000 MS; Applied Biosystems, Foster City, CA) (Sanchez and Kreilgaard, 2004). Brain homogenate was prepared by homogenizing the whole brain with 70% acetonitrile [1:4 (v/v)], followed by centrifugation and collection of the supernatant. Samples of plasma and brain homogenate were prepared by adding an equal amount of 10% methanol with internal standard included (escitalopram), and after centrifugation (6000g; 5°C, 20 min), 10 µl was injected into the turboflow system.

Results

Effect of Retigabine and XE-991 on DA Cell Firing Activity in the VTA. Retigabine (0.3–6.0 mg/kg i.v., cumulated doses) induced a significant decrease of the mean firing rate of DAergic neurons compared with vehicle [F(5,25) = 11.457, p < 0.001] (Fig. 1). A significant decrease was already achieved at 2.0 mg/kg (81.1 ± 5.1% of baseline firing rate, p < 0.05). Higher doses of retigabine further decreased the mean firing rate of DAergic cells [67.1 ± 6.7 and 60.6 ± 0.7% of baseline firing rate at 4.0 (p < 0.001) and 6.0 (p < 0.001) mg/kg, respectively]. In addition, retigabine significantly reduced the percentage of spikes in bursts [F(5,21) = 10.757, p < 0.001]. The dose-dependent effect reached approximately 40, 15, and 13% of the baseline percentage of spikes in bursts after 2.0 (p < 0.001), 4.0 (p < 0.001), and 6.0 (p < 0.001) mg/kg retigabine, respectively. A significant effect on burst firing activity was further confirmed by the significant dose-dependent decrease in the CV ISI [F(5,20) = 11.981, p < 0.001] (Fig. 1). Analyses of plasma and brain levels of retigabine confirmed dose-dependent exposure 3 to 5 min postintravenous administration (Table 1).

XE-991 (0.5–2.0 mg/kg i.v., cumulated doses) induced a significant, dose-dependent increase of the mean firing rate of DAergic neurons compared with vehicle [F(3,23) = 4.937, p = 0.010] (Fig. 2). A significant increase was achieved at 1.0 (p < 0.05) and 2.0 (p < 0.01) mg/kg (122.3 ± 5.1 and 137.3 ± 12.9% of baseline firing rate, respectively). In addition, XE-991 induced a significant increase in the percentage of spikes in bursts [F(3,19) = 11.457, p < 0.001]. The dose-dependent increase reached 307 ± 44 and 481 ± 91% of baseline percentage of spikes in bursts, respectively, after 1.0 (p < 0.05) and 2.0 (p < 0.001) mg/kg. A significant effect on burst firing activity was further confirmed by the significant dose-dependent increase in the CV ISI [F(3,19) = 7.594, p = 0.002] (Fig. 2).

Effect of Retigabine and XE-991 on Basal and d-Amphetamine-Induced DA Efflux in the Nucleus Accumbens. Basal levels of DA in nucleus accumbens were 21.3 ± 3.6, expressed as femtomoles per 20-µl dialysates (mean ± S.E.M., n = 15, not corrected for the in vitro dialysis probe recovery). Administration of d-amphetamine (0.5 mg/kg s.c.) induced a significant increase in extracellular DA, reaching the peak value at 60 min (Fig. 3, a and b). When retigabine (10 mg/kg s.c.) was administered 30 min before d-amphetamine, the evoked increase in DA efflux was partially prevented, reaching 221 ± 27 versus 375 ± 45% of baseline levels 60 min after the d-amphetamine challenge [F(1,105) = 11.61, p < 0.001] (Fig. 3a). Administration of retigabine alone did not affect the levels of DA in the nucleus accumbens up to 170 min postinjection (Fig. 3a). Conversely, when XE-991 (1 mg/kg i.p.) was administered 5 min before d-amphetamine, the evoked increase in DA efflux was significantly enhanced, reaching 530 ± 79 versus 399 ± 44% in the d-amphetamine-treated animals 60 min postinjection [F(2,131) = 71.88, p < 0.0001] (Fig. 3b). Administration of XE-991 alone did not affect the levels of DA the in nucleus accumbens up to 165 min postinjection (Fig. 3b).

Effect of XE-991 and Retigabine on Basal and d-Amphetamine-Induced Locomotor Hyperactivity. Two-way analysis of variance showed a significant effect of factors d-amphetamine [F(1,55) = 13.96, p < 0.001] and XE-991 [F(2,55) = 85.40, p < 0.001] in that both compounds dose-dependently stimulated locomotor activity recorded for a period of 2 h after drug administration. A significant interaction was noted [F(2,55) = 3.59, p < 0.05], and post hoc analysis showed that administration of XE-991 (1 mg/kg i.p.) 5 min before d-amphetamine significantly potentiated the locomotor hyperactivity induced by 0.5 (p < 0.01) and 2.0 (p < 0.001) mg/kg d-amphetamine but not 0.25 mg/kg d-amphetamine (Fig. 4).

As shown in Fig. 5A, the prior administration of retigabine significantly reduced the d-amphetamine (0.5 mg/kg s.c.)-induced locomotor hyperactivity [F(2,27) = 8.15, p < 0.001]. The highest dose of retigabine (5.0 mg/kg) abolished the hyperactivity (p < 0.001), whereas the lower doses of retigabine (1.25 and 2.5 mg/kg s.c.) did not significantly reduce the induced locomotor response. In contrast, retigabine did not significantly reduce basal locomotor activity until doses of 10 mg/kg s.c. [F(1,35) = 13.6, p < 0.001, Fig. 5b].

Effect of Retigabine and XE-991 in the Conditioned Avoidance Response Model. Retigabine (5 and 10 mg/kg s.c.) administered 30 min before testing in the CAR paradigm dose-dependently inhibited number of avoidance [F(2,47) = 3.50, p = 0.039; Fig. 6]. Post hoc analysis showed that retigabine (10 mg/kg, p = 0.009) significantly inhibited avoidance response, an effect reversed by administration of XE-991 (1 mg/kg i.p.) 5 min before retigabine (p < 0.001) (Fig. 6). There was no induction of escape failures (data not shown).

Retigabine (20 mg/kg s.c.) administered 1 to 20 h before testing in the CAR paradigm significantly inhibited avoidance response [F(5,90) = 10.2, p < 0.001, Fig. 7], reaching significance up to 5 h of pretreatment (p = 0.012). There was no induction of escape failures (data not shown). Analysis of the plasma levels of retigabine showed a maximum exposure 1 h after administration. Approximately 50% of the peak plasma levels of retigabine were still present after 4 h (Fig. 7).

Effect of Retigabine on Basal and PCP-Induced Locomotor Hyperactivity after Subchronic PCP Administration. Basal locomotor activity recorded during the habituation phase of the experiment did not significantly differ between groups treated subchronically with PCP or vehicle (data not shown). It is interesting that the induced locomotor activity response during the period from 60 to 120 min after a short-term PCP/vehicle challenge was markedly dependent on the pretreatment of the animals. Thus, in both vehicle-pretreated animals and particularly so in PCP-pretreated animals, a short-term PCP challenge significantly increased locomotor activity by 258 and 875%, respectively [F(1,23) = 14.17 and 52.6, respectively, p < 0.001] (Fig. 8). It is interesting that retigabine pretreatment only significantly reduced this increase in induced locomotor activity in animals that were subchronically treated with PCP [F(1,23) = 8.13, p = 0.002], whereas it had no significant effect in control.
Fig. 1. Effect of retigabine (0.3–6.0 mg/kg i.v., cumulated doses) on the mean firing rate (a and c), firing pattern (b), and percentage of spikes in bursts (d) of DA neurons recorded from the VTA. a, firing rate histogram of a typical DA neuron recorded from the VTA; b, action potentials from the same DA neuron as shown in a after vehicle or retigabine treatment; c to e, histograms showing effects of different doses of retigabine on the firing rate (c), percentage of spikes in burst (d), and CV ISI (e) from all recorded DA neurons (n = 5). Note that retigabine dose-dependently decreased the firing rate, burst firing, and CV ISI of DA cells and a higher potency to inhibit burst firing than the mean firing rate. Data were analyzed statistically by a one-way ANOVA followed by Fischer’s LSD post hoc test (*, p < 0.050; **, p < 0.010; ***, p < 0.001).
animals (Fig. 8). Post hoc analysis showed that both doses of retigabine (5 and 10 mg/kg) significantly, and at equal efficacy, impaired the PCP challenge-induced locomotor hyperactivity ($p < 0.001$) compared with vehicle treatment.

**Discussion**

The aim of the present study was to evaluate the potential benefit of KCNQ channel opening as a new antipsychotic target, using preclinical models predictive of antipsychotic-like activity. We showed that the KCNQ channel opener, retigabine, decreased both the mean firing rate and burst firing activity of VTA DA neurons in vivo, whereas the KCNQ channel blocker XE-991 induced opposite effects. In addition, retigabine prevented $d$-amphetamine-induced DA efflux in the nucleus accumbens and the induced locomotor hyperactivity. In contrast, XE-991 potentiated both DA efflux and locomotor hyperactivity evoked by $d$-amphetamine. Our data strongly suggest that opening of KCNQ channels attenuates DAergic neurotransmission in the mesolimbic system, particularly in conditions of excessive DAergic activity. The antipsychotic-like potential of KCNQ channel opening was further strengthened by data from a predictive behavioral model of antipsychotic efficacy, the CAR paradigm, and a disease model of schizophrenia, the subchronic PCP model. Taken together, our studies provide evidence that KCNQ channel openers represent a potential new class of antipsychotics.

Retigabine is a KCNQ channel opener also reported to potentiate GABAergic currents in cortical neurons (Rundfeldt and Netzer, 2000). However, the concentrations needed to activate KCNQ channels were 30-fold lower than those potentiating GABA currents. In addition, we showed that retigabine induced a variety of effects either opposite to those induced by XE-991, a selective KCNQ blocker, or prevented by XE-991 pretreatment. Taken together, these observations suggest that the effects of retigabine observed in our studies were probably exclusively attributable to KCNQ channel modulation.

We found that retigabine inhibited the basal firing rate of DA neurons in the VTA of anesthetized rats. Our results are in accordance with in vitro studies showing a similar inhibitory effect of retigabine in midbrain slices (Hansen et al., 2006). It is interesting that we report for the first time that retigabine also, and more potently, inhibited burst firing activity in vivo. In contrast, XE-991 increased the basal firing rate and promoted burst firing activity in DA neurons, thus confirming the outcome from a recent study using a computational approach mimicking DA neuron electrophysiological characteristics (Bonjean et al., 2007). This is a very interesting finding because excessive burst firing activity in DA neurons has been suggested to contribute to psychotic symptoms (Grace, 1991). Our data suggest that modulating KCNQ channels would have a greater impact on the firing pattern than on the firing rate of DA neurons. As suggested in the literature, burst firing in DA neurons would be more potent to release DA at the terminal level than regular, tonic firing activity (Suaud-Chagny et al., 1992). Thus, modulation of KCNQ channels is expected to have modest effects on tonic DA release and marked effects on phasic DA release. The dialysis data reported herein showed that retigabine prevented the $d$-amphetamine-evoked increase in extracellular DA levels in the nucleus accumbens, whereas basal levels of DA were not significantly affected. As expected, XE-991 potentiated $d$-amphetamine-induced increase in DA efflux without significantly changing basal DA efflux. Even though $d$-amphetamine-induced DA efflux is not directly dependent on burst firing in DA neurons, our findings support that retigabine is more potent to regulate excessive and, by extrapolation, pathological DA neurotransmission. We report a lack of effect of retigabine or XE-991 on basal DA efflux measured in the nucleus accumbens, whereas our electrophysiological data suggest the existence of a basal tone exerted by KCNQ channels on DA neuronal activity. A similar discrepancy has also been reported by Hansen et al. (2006, 2007). One possible explanation for this apparent inconsistency may rely on a different sensitivity of DAergic systems in anesthetized (electrophysiology) compared with conscious (microdialysis) animals (Kelland et al., 1989; Hamilton et al., 1992). More importantly, Floresco et al. (2003) reported that increasing burst firing activity of DA neurons in the VTA did not significantly enhance accumbal DA levels as measured by microdialysis, unless DA reuptake mechanisms were blocked. In accordance, our data showing a more profound effect of KCNQ modulation on DA burst firing activity rather than firing rate support a primary effect of KCNQ channels in the modulation of phasic DA activity.

Reversal of $d$-amphetamine-induced locomotor hyperactivity, which depends on the blockade of postsynaptic DA receptors in the nucleus accumbens, is a very commonly used model to predict antipsychotic-like efficacy (Arnt, 1995). We found that locomotor hyperactivity induced by $d$-amphetamine was prevented and potentiated by retigabine and XE-991, respectively, at doses that did not significantly reduce basal locomotor activity. In addition, the dose of retigabine effective to prevent $d$-amphetamine-induced hyperlocomotor activity was reported to not affect roterod performances in rats (Nielsen et al., 2004), further ruling out a potential confounding effect on motor function. Our findings are in agreement with those reported by Hansen et al. (2007) showing that retigabine potently blocked the acute hyperactivity induced by other psychostimulants. In our approach, we also studied the effect of retigabine in PCP-sensitized rats. The subchronic PCP model is believed to be a model with good face and construct validity with regard to positive symptoms in schizophrenia (Jentsch and Roth, 1999). Short-term PCP would reduce cortical GABAergic function via blockade of NMDA receptors (Homayoun and Moghaddam, 2007) and indirectly activate DAergic neurotransmission within the mesolimbic system (Jentsch and Roth, 1999). In fact, short-term PCP increases both the firing rate and burst firing activity of VTA DA neurons (French, 1988) and DA efflux in the nucleus accumbens, the latter effect being responsible for the locomotor hyperactivity (Steinpreis and Salamone, 1993). In our study, we found a sensitization to the locomotor effect

<table>
<thead>
<tr>
<th>Retigabine</th>
<th>Plasma Level</th>
<th>Brain Level</th>
<th>B/P Ratio</th>
</tr>
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<tbody>
<tr>
<td>0.3 mg/kg i.v.</td>
<td>216 ± 73.9</td>
<td>391 ± 83.7</td>
<td>2.0 ± 0.24</td>
</tr>
<tr>
<td>2.0 mg/kg i.v.</td>
<td>685 ± 126.4</td>
<td>3851 ± 1047.5</td>
<td>6.4 ± 2.51</td>
</tr>
<tr>
<td>6.0 mg/kg i.v.</td>
<td>1736 ± 90.2</td>
<td>11566 ± 2050.3</td>
<td>6.7 ± 1.17</td>
</tr>
</tbody>
</table>

TABLE 1
Plasma and brain levels of retigabine 3 to 5 min after intravenous administration
All doses were achieved using the same cumulated schedule as used in the electrophysiological studies.
Fig. 2. Effect of XE-991 (0.5–2.0 mg/kg i.v., cumulated doses) on the mean firing rate (a), firing pattern (b), and percentage of spikes in bursts (c) of DA recorded from the VTA. a, firing rate histogram of a typical DA neuron recorded from the VTA; b, action potentials from the same DA neuron as shown in a after vehicle or XE-991 treatment; c to e, histograms showing effects of different doses of XE-991 on the firing rate (c), percentage of spikes in burst (d), and CV ISI (e) from all recorded DA neurons (n = 5–6). Note that XE-991 dose-dependently increased the firing rate, burst firing, and CV ISI of DA cells and a higher potency to increase burst firing than the mean firing rate. Data were analyzed statistically by a one-way ANOVA followed by Fischer’s LSD post hoc test (*, p < 0.050; **, p < 0.010; ***, p < 0.001).
of PCP in animals subchronically treated with PCP. This phenomenon is believed to rely on an increased sensitivity of the mesolimbic DAergic system to afferent stimulation, leading to an overactive DAergic neurotransmission (Nabeshima et al., 1987; Jentsch et al., 1998), consistent with the “DA hyperactivity” hypothesis of schizophrenia. In the subchronic PCP model, the hyperlocomotor response to a short-term PCP challenge is accentuated and prolonged. Studying the time period from 60 to 120 min post-PCP challenge offered an optimal pharmacological window because the hyperactivity in vehicle-pretreated animals wears off while being still significantly elevated in PCP-pretreated animals. In these con-
Fig. 5. Locomotor activity in rats after pretreatment with d-amphetamine (0.5 mg/kg) (a) or saline (b) and increasing doses of retigabine (0–5 mg/kg). One-way ANOVA multiple comparisons procedure show that retigabine (5 mg/kg) significantly reduced locomotor hyperactivity induced by d-amphetamine (0.5 mg/kg); ***, p < 0.001 (a). Furthermore, baseline locomotor activity is only significantly reduced by retigabine at higher doses (10 mg/kg); ***, p < 0.001 (b). Dotted reference lines, average level of light beam crossings in vehicle-vehicle-pretreated animals.

Fig. 6. Dose response of retigabine alone and in combination with XE-991 on CAR behavior in rats. Retigabine was administered subcutaneously 30 min before test and XE-991 5 min before retigabine. Values are mean ± S.E.M. number of avoidances. Statistical analyses were performed using a two-way ANOVA followed by post hoc Student-Newman-Keuls test. **, p < 0.01.
ditions, retigabine (5 and 10 mg/kg) significantly attenuated the markedly aggravated PCP-induced hyperactivity in PCP-pretreated rats, whereas these doses did not significantly reduce PCP-induced hyperactivity in vehicle-pretreated rats. In contrast to our data, Hansen et al. (2007) previously reported that retigabine potently blocked the locomotor response to a short-term PCP challenge. However, they used a higher dose of PCP, a different rat strain, and recorded nonhabituated locomotor activity, which probably explains the different effects of retigabine. Taken together, our findings imply that modulation of KCNQ channels influences the psychotic-like behavior elicited by psychostimulants. Different mechanisms underlie the effect of various psychostimulants on DAergic neurotransmission, but ultimately, they all increase DA levels in the nucleus accumbens. Although the mechanism of action of these drugs is believed to rely on mechanisms occurring at the terminal level, the increased DA output is able to activate long feedback loops to the VTA, thereby modulating DA cell firing activity (Shi et al., 2000). Because KCNQ channels are expressed by DA neurons in the VTA (Kharkovets et al., 2000; Cooper et al., 2001; Hansen et al., 2006), the effect of retigabine on d-amphetamine-induced increase in DA neurotransmission may involve a modulatory role of KCNQ channels on DA cell firing activity. Alternatively, KCNQ channels may also act at the terminal level in the nucleus accumbens to modulate DA neurotransmission via presynaptic (Martire et al., 2007) or postsynaptic mechanisms (Cooper et al., 2001; Shen et al., 2005). In addition to DA systems, KCNQ channels are also expressed by other non-DA neurons throughout the brain, and their modulation has been shown to affect several neurotransmitter systems, including noradrenergic and serotonergic pathways (Hansen et al., 2008). Because the locomotor responses to d-amphetamine and CAR are sensitive to pharmacological manipulations affecting both serotonergic and noradrenergic systems (Auclair Darraaq et al., 1998; Linnér et al., 2002; et al., 2004), a possible contributing effect of KCNQ channel modulation on other monoaminergic systems than DA cannot be entirely excluded.

Further strengthening the antipsychotic potential of KCNQ opening, we report here for the first time that retigabine potently suppressed CAR behavior in rats, a model predictive of antipsychotic activity highly dependent on mesocorticolimbic DAergic transmission (Koob et al., 1984; Wa-

Fig. 7. Effect of retigabine (top) at various pretreatment times. Values are mean ± S.E.M. number of avoidances. Statistical analyses were performed using a two-way ANOVA with post hoc Student-Newman-Keuls test; *, p < 0.05; ***, p < 0.001. Effect of retigabine and corresponding plasma levels (bottom) at various time points. Data are expressed as mean ± S.E.M. percentage avoidances compared with the preceding pretest, with each animal serving as their own control and exposure levels, respectively.
The suppressive effect of retigabine observed in our study is likely to result from an attenuation of DAergic neurotransmission induced by KCNQ channel opening. These data fit well with the suppressive effect of retigabine on d-amphetamine-induced hyperactivity, although the doses of retigabine tested in the two models were quite different. However, it is also very likely that the two models do not rely on disturbances in DAergic neurotransmission to the same extent. In this respect, it is not surprising that retigabine is more potent in the CAR model than its effect in CAR, which may be explained by a low dissociation rate.

In conclusion, our data suggest that KCNQ channels modulate excessive, pathological DAergic neurotransmission in the mesolimbic system and, therefore, may represent a potential new antipsychotic target. All available antipsychotic drugs share the common feature of blocking D₂ receptors. By doing so, they block the impact of an overactive DAergic system, which may attenuate their clinical efficacy. It is interesting that retigabine reverses haloperidol-induced increases in DA neuronal firing and c-fos expression in the striatum (Mikkelsen, 2004; Hansen et al., 2006). By attenuating an overactive DAergic system, retigabine may offer a better efficacy profile. Moreover, this new mechanism of action may provide, by avoiding postsynaptic D₂ receptor blockade, a potential benefit in terms of extrapyramidal symptoms liability.

**Fig. 8.** Effect of retigabine on PCP-stimulated locomotor activity in PCP-sensitized rats. In vehicle-pretreated rats (left side), a short-term challenge with PCP caused a modest increase in locomotor activity (\( p < 0.05 \)) that was not significantly inhibited by retigabine pretreatment. In PCP-pretreated rats (right side), locomotor activity was markedly increased by a PCP challenge (\( +++, p < 0.001 \)). It is interesting that both doses of retigabine (5 or 10 mg/kg) significantly reduced this response (\( p < 0.001 \)).

**Acknowledgments**

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**References**


