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RESEARCH PAPER

Antidystonic effects of K_v7 (KCNQ) channel openers in the *dt*^{sz} mutant, an animal model of primary paroxysmal dystonia

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Background and purpose: Mutations in neuronal K_v7 (KCNQ) potassium channels can cause episodic neurological disorders. Paroxysmal dyskinesias with dystonia are a group of movement disorders which are regarded as ion channelopathies, but the role of K_v7 channels in the pathogenesis and as targets for the treatment have so far not been examined.
Experimental approach: In the present study, we therefore examined the effects of the activators of neuronal $K_v7.2/7.3$ channels retigabine (5, 7.5, 10 mg kg^{-1} i.p. and 10, 20 mg kg^{-1} p.o.) and flupirtine (10, 20 mg kg^{-1} i.p.) and of the channel blocker 10,10-bis(4-pyridinylmethyl)-9(10H)-anthracenone (XE-991, 3 and 6 mg kg^{-1} i.p.) in the *dt*^{sz} mutant hamster, a model of paroxysmal dyskinesia in which dystonic episodes occur in response to stress.
Key results: Retigabine (10 mg kg^{-1} i.p., 20 mg kg^{-1} p.o.) and flupirtine (20 mg kg^{-1} i.p.) significantly improved dystonia, while XE-991 caused a significant aggravation in the *dt*^{sz} mutant. The antidystonic effect of retigabine (10 mg kg^{-1} i.p.) was counteracted by XE-991 (3 mg kg^{-1} i.p.).
Conclusions and Implications: These data indicate that dysfunctions of neuronal K_v7 channels deserve attention in dyskinesias. Since retigabine and flupirtine are well tolerated in humans, the present finding of pronounced antidystonic efficacy in the *dt*^{sz} mutant suggests that neuronal K_v7 channel activators are interesting candidates for the treatment of dystonia-associated dyskinesias and probably of other types of dystonias. The established analgesic effects of K_v7 channel openers might contribute to improvement of these disorders which are often accompanied by painful muscle spasms.
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Keywords: channelopathy; dystonia; ion channels; movement disorders; paroxysmal dyskinesias; potassium

Abbreviations: XE-991, 10,10-bis(4-pyridinylmethyl)-9(10H)-anthracenone

Introduction

Voltage-gated K^+ channels are critical for establishing and stabilizing the resting potential of neurons. Throughout this probably most diverse class of ion channels, mutations in $K_v7.2$ or $K_v7.3$ containing channels of the K_v7 family (formerly known as KCNQ or M-channels) are associated with the pathophysiology of hereditary paroxysmal disorders, such as epilepsy and episodic ataxia (Lawson, 2000). $K_v7.2$–$K_v7.5$ subunits contribute to the multimeric K_v7 channels in the brain, whereas $K_v7.1$ is found in the heart and in epithelial tissue (Shieh *et al*., 2000; Robbins, 2001). Neuronal K_v7 channels were originally called 'M-channels' because of their suppression by muscarinic receptor signalling. In the striatum (the main input structure of the basal ganglia), medium spiny projection neurons express $K_v7.2$, $K_v7.3$ and $K_v7.5$ as well as muscarinic M1 receptors (Saganich *et al*., 2001; Shen *et al*., 2005). K_v7 channels are potent regulators of the excitability of medium spiny neurons at up-state potentials and they are modulated by intrastriatal cholinergic interneurons (Shen *et al*., 2005). As K_v7 channels can thereby influence the discharge rate and firing pattern of striatal projection neurons, we hypothesize that these channels are interesting targets for the treatment of basal ganglia disorders, such as Parkinson's disease and the dystonic syndrome, including dystonia-associated dyskinesias.

Dystonia is a neurological syndrome characterized by sustained, sometimes painful, muscle contractions frequently causing twisting or repetitive movements and abnormal postures. The etiologies and the clinical spectrum of dystonias are wide (Fahn *et al*., 1998; Saunders-Pullman

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and Bressman, 2005). Paroxysmal dyskinesias are a group of episodic movement disorders that include dystonia. There are four major types of paroxysmal dyskinesias differentiated by the precipitating and exacerbating factors: paroxysmal non-kinesigenic dyskinesia (induced by stress and caffeine; see below), paroxysmal kinesigenic dyskinesia (induced by sudden movements), exertion-induced and hypnogenic paroxysmal dyskinesias (Jankovic and Demirkiran, 2002; Nardocci *et al*., 2002). These episodic disorders have been suggested to be related to ion channelopathies, such as dysfunctions of potassium channels (Ptacek, 1997). There is evidence that abnormal neuronal activity within the basal ganglia, including the striatum where $K_v7.2$ and $K_v7.3$ channels are localized, plays a critical role in dyskinesias and in the dystonic syndrome in humans (Lombroso and Fischman, 1999; Albin, 2005). However, the underlying mechanisms are unknown and are probably heterogeneous in various forms of dyskinesias as well as of dystonia (Nardocci *et al*., 2002).

Animal models of inborn generalized dystonia with basal ganglia dysfunction, helpful for preclinical drug testing and examination of the pathophysiology of these often intractable movement disorders, are restricted to the *dt*^{sz} mutant hamster (Richter and Löscher, 1998; Raike *et al*., 2005). In this inbred line of mutant hamsters, the motor disturbances are transmitted by a recessive gene. The *dt*^{sz} mutant hamster shows the clinical and pharmacological characteristics of a type of hereditary dyskinesias in humans, that is, the idiopathic paroxysmal non-kinesigenic dyskinesia, briefly paroxysmal dystonia (for review see Richter and Löscher, 1998; Nardocci *et al*., 2002; Richter, 2005). In this movement disorder, episodes of generalized dystonia (the predominant feature) last up to several hours and can be provoked by stress. Paroxysmal dystonia responds poorly to medical therapy (Nardocci *et al*., 2002; Richter, 2005). Although the pathophysiolgy of paroxysmal dystonia is unknown, there is strong evidence that hyperactivity of striatal projection neurons is critically involved in paroxysmal dystonia as well as in other types of dystonia (Gernert *et al*., 2000; Vitek and Giroux, 2000).

In view of the expression of $K_v7.2$–$K_v7.5$ channels in striatal projection neurons and several lines of evidence that enhanced striatal output is involved in the dystonic syndrome (Richter and Löscher, 1998; Vitek and Giroux, 2000), openers of these K^+ channels might provide novel therapeutic approaches for dyskinesias, such as for paroxysmal dystonia. In the present study, we therefore examined the effects of retigabine and flupirtine on the severity of dystonic episodes in the *dt*^{sz} mutant hamster. Retigabine (*N*-(2-amino-4-(4-fluorobenzylamino)-phenyl) carbamic acid ethyl ester) is a positive modulator of $K_v7.2$–$K_v7.5$ channels at concentrations of 1–10 μM (Rundfeldt and Netzer 2000a, b; Dost *et al*., 2004). It is a novel antiepileptic drug with analgesic and anxiolytic effects (Korsgaard *et al*., 2005; Schenzer *et al*., 2005; Wuttke *et al*., 2005). A structural analogue of retigabine, the analgesic flupirtine (2-amino-3-carbethoxyamino-6-(4-fluorobenzylamino)-pyridine), also enhances $K_v7.2$–$K_v7.5$ channel function, although with lower potency (Ilyen *et al*. 2002). In the present study, we investigated if retigabine and flupirtine may improve dystonia and whether the effects of these K_v7 channel openers can be antagonized by XE-991 (10,10-bis(4-pyridinylmethyl)-9(10H)-anthracenone) which is known to block $K_v7.2$–$K_v7.5$ channels (Wang *et al*., 2000).

Methods

Animals

The *dt*^{sz} mutant hamsters (Syrian golden hamsters), used in the present experiments, were obtained by selective breeding as described in detail elsewhere (Richter and Löscher, 1998). The animals were born and kept under controlled environmental conditions (23–25°C, 50–60% humidity, 13 h light/11 h dark cycle) with free access to standard Altromin 7204 diet and water. When drugs were given orally, food was withheld for 2 h before the experiments. All experiments were carried out in the morning (08:30–12:00) at controlled temperatures (23–25°C). The experiments were carried out in compliance with the German Animal Welfare Act (G 016/05; Reg A 0216/05).

Induction of dystonic episodes and severity-score of dystonia

As reported previously in detail (for reviews see Richter and Löscher, 1998, 2002; Richter, 2005), the *dt*^{sz} mutant hamster exhibits long-lasting dystonic episodes which can be provoked by mild stress, such as handling. For drug testing, dystonic attacks can be reproducibly induced by a triple stimulation technique (Richter and Löscher, 1998), that is, stressful stimuli consisting of (1) taking the animal from its home cage and placing it on a balance, (2) intraperitoneal (i.p.) injection of isotonic saline (or of drugs, see below) or oral administration of isotonic saline via pharyngeal cannulation (or of retigabine, see below) and (3) placement of the animal in a new plastic cage. After this procedure, *dt*^{sz} hamsters develop a sequence of generalized abnormal movements and postures. Dystonia is the predominant symptom in these animals. The severity of dystonia can be rated by the following score system (Richter and Löscher, 1998): stage 1, flat body posture; stage 2, facial contortions, rearing with forelimbs crossing, disturbed gait with hyperextended forepaws; stage 3, hyperextended hindlimbs so that the animals appear to walk on tiptoes; stage 4, twisting movements and loss of balance; stage 5, hindlimbs hyperextended caudally; stage 6, immobilization in a twisted, hunched posture with hind- and forelimbs tonically extended forward. After reaching the individual maximum stage the hamsters recover within 2–5 h. The individual maximum stage of dystonia is usually reached within 3 h after the hamsters were placed into the new cage. Therefore, the animals have to be observed for 3 h after the induction of dystonic attacks to determine the individual maximum stage reached after administrations of vehicle (for pre- and post-drug control recordings) or of drugs.

In the present study, all animals were examined for the presence of dystonia after weaning at the age of 21 days by the triple stimulation procedure, including injections of saline. Dystonia shows an age-dependent time course with a maximum of the severity of dystonia at an age of 30–42 days (Richter and Löscher, 1998). All groups of mutant hamsters

used for investigations were repeatedly tested by triple stimulations (injections of saline) every 2–3 days after weaning until the severity of dystonia and latencies to the different stages were reproducible. The drug experiments were carried out at an age of the maximum severity of dystonia (in 30–42-day-old hamsters).

Drug experiments

The effects of the $K_v7.2$–$K_v7.5$ channel openers retigabine (5, 7.5 and 10 mg kg^{-1} i.p. and 10 and 20 mg kg^{-1} *per os* (p.o.)) and flupirtine (10 and 20 mg kg^{-1} i.p.) and the effects of the K_v7 channel blocker XE-991 (3 and 6 mg kg^{-1} i.p.) were examined in groups of 6–10 male and female *dt*^{sz} hamsters. Dystonic attacks were induced by the procedure of triple stimulation, as described above, but instead of saline the active compound was given (5 ml kg^{-1} i.p. or p.o.). Pre- and post-drug control trials with the vehicle (5 ml kg^{-1} saline i.p. or p.o.) were undertaken 2–3 days before and 2–3 days after drug testing in the same animals. As the individual maximum stage of dystonia is usually reached within 3 h, the hamsters were observed for 3 h after triple stimulation. During this period the severity of dystonia, the latencies to the different stages and behavioural side effects were noted. The severity scores (and the latencies to stages) were recorded without knowledge of the treatments of the animals. A second person who had prepared the solutions, observed the animals for behavioural effects. The side effects were not quantified, but locomotor activity and ataxia were determined according to a score system, as described previously (Löscher and Richter, 1994). Animals which differed in their individual maximum dystonic stage by more than two scores between pre-drug and post-drug controls were omitted from evaluation.

Statistics

Significant differences in severity of dystonia and in the latencies to onset of dystonia (latency to stage 2; see Table 1) between control trials (pre- and post-drug) and drug trial in the same group of animals were calculated by the Friedman test and, if there was a significant difference (at least $P<0.05$), the Wilcoxon's signed rank test for paired replicates was used *post hoc* to determine which pairs were different.

Drugs

The K_v7 channel openers retigabine and its structural analogue flupirtine were provided by Elbion (Radebeul, Germany). XE-991 was purchased from Tocris (Bristol, UK). All compounds were freshly dissolved in saline before the experiments. The doses of these drugs were chosen on the basis of previous experiments in rodents (Schwarz *et al*., 1996; Blackburn-Munro and Jensen, 2003; Dost *et al*., 2004; Nielsen *et al*., 2004; Korsgaard *et al*., 2005).

Results

As shown in Figure 1, retigabine exerted a dose-dependent improvement of dystonia after i.p. injections. At a dose of 10 mg kg^{-1}, retigabine significantly suppressed the progression of dystonia (see first and second hour after administration) and significantly reduced the maximum severity (see third hour after injection), whereas a lower dose of 7.5 mg kg^{-1} only tended to reduce the severity as indicated by a significant decrease of the severity which was restricted to the second hour after injection. At a dose of 5 mg kg^{-1}, retigabine failed to exert any significant effects on the severity of dystonia. A complete prevention was observed in one hamster treated with 10 mg kg^{-1}. Retigabine increased the latency to onset of dystonia at a dose of 7.5 mg kg^{-1}, whereas 5 and 10 mg kg^{-1} merely tended to delay the onset of dystonic episodes (Table 1). Behavioural effects were a moderate to unequivocal hypolocomotion (sometimes interrupted by short lasting periods of increased locomotor activity) and ataxia within the first hour after administration. During the first 5 min after injection of 10 mg kg^{-1}, the hamsters showed writhed postures, probably caused by abdominal pain.

Table 1 Effects of the K_v7 channel openers retigabine and flupirtine and of the K_v7 channel blocker XE-911 on the latency to onset of dystonia

Dose (mg kg^{-1}) (route of administration)	*Latency to onset (min)*			*(n)*
	Pre-drug	*Drug*	*Post-drug*	
Retigabine				
5.0 (i.p.)	6.9±2.4	27.1±17.7	4.0±1.1	9
7.5 (i.p.)	6.0±1.1	11.8±1.0*	5.0±1.2	6
10.0 (i.p.)	3.7±0.6	7.1±3.0	4.4±1.2	9
10.0 (p.o)	2.0±0.6	4.9±2.9	5.1±1.9	7
20.0 (p.o.)	4.0±0.6	9.6±2.0	3.1±0.7	10
Flupirtine				
10.0 (i.p.)	6.0±0.7	6.9±1.6	4.6±0.9	8
20.0 (i.p.)	1.8±0.3	13.8±3.9*	4.1±0.8	10
XE-991				
3.0 (i.p.)	6.1±1.2	3.6±0.3	7.5±0.9	8
6.0 (i.p.)	2.5±0.5	1.3±0.4	2.8±0.6	10
XE-991 (3.0 i.p.) + retigabine (10.0 i.p.)	3.4±0.8	15.7±1.9*	3.3±0.9	7

Abbreviations: i.p., intraperitoneally; p.o., *per os*.
Latency to onset was determined as the time to the first unequivocal signs of the dystonic attacks (stage 2). Data are shown as means ±s.e. of the number of animals indicated (*n*). Significant differences from pre-drug and post-drug controls are marked by asterisks (*$P<0.05$).

The abdominal adverse effects after i.p. injections of retigabine prompted us to examine the effects of retigabine after oral administration. As shown in Figure 2, retigabine significantly reduced the severity of dystonia at an oral dose of 20 mg kg^{-1}, whereas oral administration of 10 mg kg^{-1} failed to exert antidystonic effects. At both oral doses, retigabine did not exert significant effects on the latency to onset of dystonia (Table 1). In contrast to the observations after i.p. injections, retigabine did not exert severe side effects at the oral doses of 10 and 20 mg kg^{-1}. Two hamsters treated with 10 mg kg^{-1} p.o. showed a moderate reduction of the locomotor activity. At a higher dose of 20 mg kg^{-1} p.o., seven animals exhibited moderate ataxia and five hamsters showed moderate hypolocomotion during the first hour after administration.

As shown in Figure 3, flupirtine did not exert significant antidystonic effects at a dose of 10 mg kg^{-1} i.p. At a higher

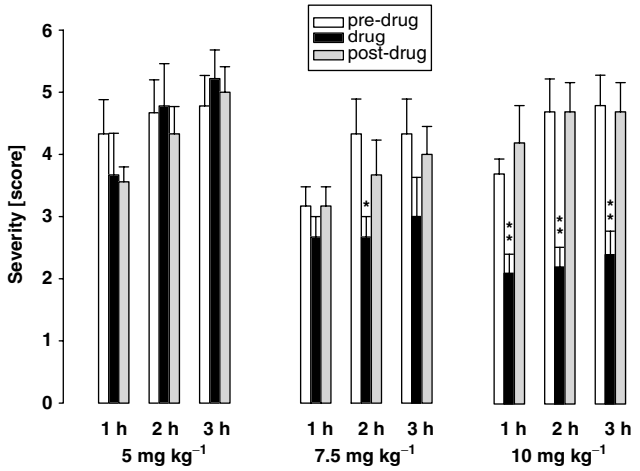

Figure 1 Effect of retigabine on severity of dystonia in mutant hamsters after i.p. injections of 5.0, 7.5 and 10 mg kg^{-1}. The white bars in each set of three bars indicate the control values obtained 2 days before drug administration (pre-drug control). The black bar refers to the day of drug administration in the same animal groups. The grey bars in each set of three bars indicate the control values obtained 2 days after drug administration (post-drug control). The individual maximum severity of dystonia is usually reached within 3 h after induction of dystonia by triple stimulation including the injection of drugs or vehicle. The figure shows the average of the maximum individual severity scores of dystonia reached within the first, second and third hour post-injection of isotonic saline (control trials) or of retigabine, reflecting the progression of dystonia in *dt*^{sz} hamsters during control recordings and after treatment with the active compound. Asterisks indicate significant reduction of dystonia in comparison to the pre- and post-drug control (*$P<0.05$, **$P<0.01$). Data are shown as means + s.e. (number of animals: see Table 1).

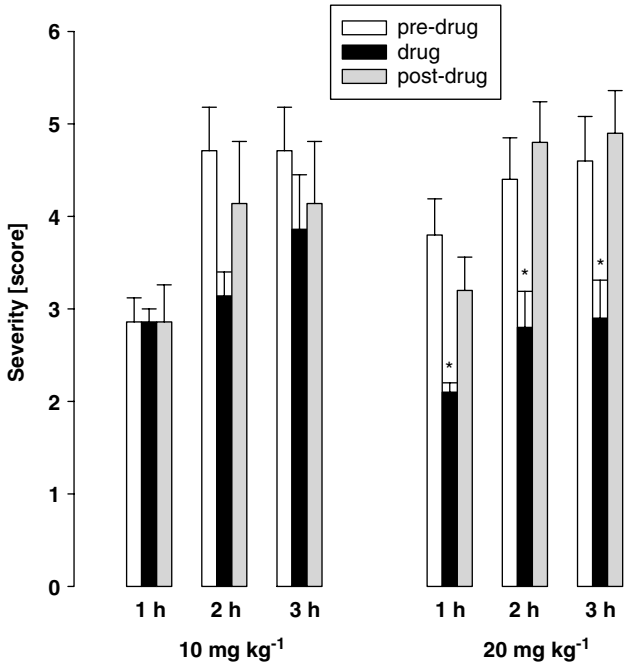

Figure 2 Effect of retigabine on severity of dystonia in mutant hamsters after oral administration of 10 and 20 mg kg^{-1}. The white bars in each set of three bars indicate the control values obtained 2 days before drug administration (pre-drug control). The black bar refers to the day of drug administration in the same animal groups. The grey bars in each set of three bars indicate the control values obtained 2 days after drug administration (post-drug control). The figure shows the average of the maximum individual severity scores of dystonia reached within the first, second and third hour after oral administration via a pharyngeal cannulation of isotonic saline (control trials) or of retigabine. Asterisks indicate significant reduction of dystonia in comparison to the pre- and post-drug control (*$P<0.01$). Data are shown as means + s.e. (number of animals: see Table 1). For further explanations see Figure 1 legend.

dose of 20 mg kg^{-1} i.p., flupirtine delayed the progression of dystonia (first and second hour), reduced the maximum severity (third hour) and increased the latency to onset of dystonia, indicating a fast onset of action (Figure 3, Table 1). Adverse effects were a moderate hypolocomotion and ataxia within the first hour after administration of 10 mg kg^{-1}. At a dose of 20 mg kg^{-1}, flupirtine caused a more marked ataxia (lasting up to 90 min) and an unequivocal hypolocomotion (5–15 min after injection) followed by hyperlocomotion (15–60 min after injection).

The K_v7 channel blocker XE-991 caused an aggravation of dystonia, that is, increased the maximum severity of dystonia at doses of 3 and 6 mg kg^{-1} i.p. (Figure 4). The latency to onset of dystonia tended to be decreased after treatment with XE-991 (Table 1). Two out of eight animals exhibited moderate to unequivocal hyperlocomotion and moderate ataxia (up to 180 min) and marked initial facial contortions 10–20 min after administration of 3 mg kg^{-1}. All hamsters, which were treated with the higher dose exhibited facial contortions, salivation and increased defecation. Furthermore, unequivocal hyperlocomotion and ataxia were observed during the first hour after administration. XE-991 (3 mg kg^{-1} i.p.) injected 10 min after retigabine (10 mg kg^{-1} i.p.) counteracted the antidystonic effect of retigabine (see Figure 4), whereas the latency to onset of dystonia was unexpectedly increased after combined treatment (Table 1).

Discussion

The present data demonstrate for the first time antidystonic effects of the K_v7 (KCNQ) channel activators retigabine and flupirine, whereas the channel blocker XE-991 aggravated the dystonic syndrome and counteracted the beneficial effects of retigabine in the *dt*^{sz} mutant. These data suggest that dysfunctions of K_v7 channels may be an important mechanism in the dystonia-associated disorders. Antidystonic efficacy was found at well-tolerated doses of retigabine (at least after oral administration) and of flupirtine in mutant hamsters. The antiepileptic retigabine and analgesic flupirtine are also known to be well tolerated in humans (Herrmann *et al*., 1993; Fatope, 2001). As shown for retigabine, tolerability is good in humans when titrated up to its therapeutic dose range (600–1200 mg per day). No tolerance, dependence or withdrawal potential has been reported, although adverse effects can include mild dizziness and somnolence (Blackburn-Munro *et al*., 2005).

Dystonic symptoms may be managed to a certain extent with a combination of treatments, however often with only moderate beneficial effects and at the expense of severe side effects (Jankovic, 2004). Therapy of secondary dystonias aims to treat the underlying condition, whereas in idiopathic

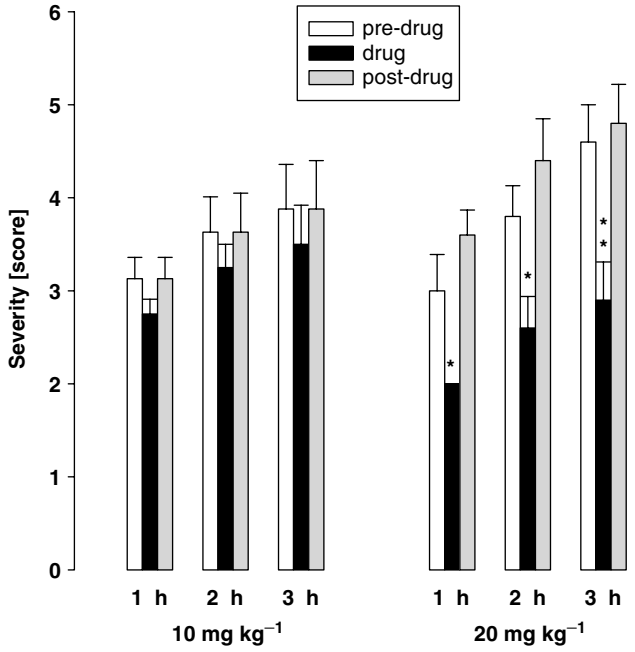

Figure 3 Effect of flupirtine on severity of dystonia in mutant hamsters after i.p. injections of 10 and 20 mg kg^{-1}. The white bars in each set of three bars indicate the control values obtained 2 days before drug administration (pre-drug control). The black bar refers to the day of drug administration in the same animal groups. The grey bars in each set of three bars indicate the control values obtained 2 days after drug administration (post-drug control). The figure shows the average of the maximum individual severity scores of dystonia reached within the first, second and third hour post-injection of isotonic saline (control trials) or of flupirtine. Asterisks indicate significant reduction of dystonia in comparison to the pre- and post-drug control (*$P<0.05$, **$P<0.01$). Data are shown as means + s.e. (number of animals: see Table 1).

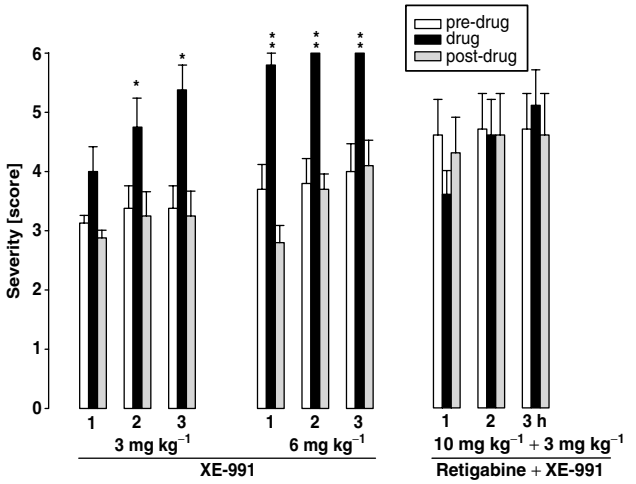

Figure 4 Effect of the K_v7 channel blocker XE-991 on severity of dystonia in mutant hamsters after i.p. injections of 3 and 6 mg kg^{-1} alone or of 3 mg kg^{-1} 10 min after administration of retigabine (10 mg kg^{-1} i.p.). The effects of retigabine alone (10 mg kg^{-1} i.p.) are shown in Figure 1. The white bars in each set of three bars indicate the control values obtained 2 days before drug administration (pre-drug control). The black bar refers to the day of drug administration in the same animal groups. The grey bars in each set of three bars indicate the control values obtained 2 days after drug administration (post-drug control). The figure shows the average of the maximum individual severity scores of dystonia reached within the first, second and third hour post-injection of isotonic saline (control trials) or of XE-991, reflecting the progression of dystonia in *dt*^{sz} hamsters during control recordings and after treatment with the active compound. Asterisks indicate significant increase of the severity of dystonia in comparison to the pre- and post-drug control (*$P<0.05$, **$P<0.01$). Data are shown as means + s.e. (number of animals: see Table 1).

dystonias the treatment is merely symptomatic, designed to improve motor function and to relieve associated pain (Jankovic, 2004). The K_v7 channel openers retigabine and flupirtine are effective against neuropathic or muscle-mediated pain (Herrmann *et al*., 1993; Dost *et al*., 2004; Nielsen *et al*., 2004), a mode of action relevant to the treatment of dystonia-associated painful muscle spasms. Although paroxysmal dystonia occurs in the absence of epileptogenic EEG changes in patients and in the *dt*^{sz} mutant (Gernert *et al*., 1998; Nardocci *et al*., 2002), nocturnal dyskinesias and paroxysmal kinesiogenic dyskinesias can coexist with epilepsy in the same individual or family (Guerrini, 2001; Du *et al*., 2005). In these epilepsy-associated dyskinesias, the well-known anticonvulsant efficacy of retigabine may contribute to beneficial effects.

$K_v7.2$ and $K_v7.3$ channels are expressed in striatal medium spiny neurons, that is, GABAergic projection neurons of the striatum. These channels have been reported to be potent regulators of the excitability of striatal projection neurons (Shen *et al*., 2005). Despite different primary defects in various types of dystonias and dyskinesias, there are possibly common mechanisms leading to dystonic disturbances. There is evidence that the dystonic syndrome in patients with idiopathic dystonia as well as hereditary dyskinesias is associated with increased striatal activity leading to reduced basal ganglia output (Vitek and Giroux, 2000; Gernert *et al*., 2000; Albin, 2005). Thus, K_v7 channel openers may be effective in various types of dystonias. In movement disorders which are regarded as basal ganglia disorders, it is obvious to focus on the effects of K_v7 channel activators in basal ganglia nuclei, but $K_v7.2$, $K_v7.3$ and $K_v7.5$ channels are expressed in several brain regions (Saganich *et al*., 2001). The antidystonic effects of retigabine and flupirtine could be also mediated by their depressant effects on spinal motor neurons or on motor neurons (Devaux *et al*., 2004; Rivera-Arconada and Lopez-Garcia, 2005). Interestingly, $K_v7.5$ is also expressed in skeletal muscle (Lerche *et al*., 2000; Schroeder *et al*., 2000). Therefore, the effects of KCNQ2-5 channel openers should be further investigated after intrastriatal microinjections in the *dt*^{sz} mutant in ongoing studies to clarify if the antidystonic efficacy of retigabine and flupirtine is mediated by their action on striatal neurons.

In view of antidystonic effects of GABA-potentiating drugs in the *dt*^{sz} hamster (Richter, 2005), it has to be noted that retigabine potentiates the action of GABA (Sills *et al*., 2000; Rundfeldt and Netzer, 2000b). However, its antidystonic effect is probably mainly mediated by the opening of K_v7 channels because flupirtine also exerted antidystonic effects. Furthermore, the channel blocker XE-991 worsened dystonia and counteracted the antidystonic effects of retigabine. Previous studies in the *dt*^{sz} mutant hamsters have shown a reduced density of striatal GABAergic interneurons, but not

of cholinergic interneurons (Gernert *et al*., 2000; Hamann *et al*., 2005, 2006; Sander *et al*., 2006). Decreased GABAergic inhibition and a shift towards cholinergic activation of striatal projection neurons (medium spiny neurons) may be important for the enhanced activity of striatal output in the dt^{sz} mutant. In line with this suggestion, GABA-potentiating drugs ameliorated dystonia, whereas cholinergic drugs such as pilocarpine aggravated dystonic episodes in the dt^{sz} model (Richter and Löscher, 1998; Richter, 2005). Interestingly, anticholinergic medications have been found to be the most useful in the treatment of generalized and segmental dystonias in humans, but patients require often very high doses (Jankovic, 2004). Increased cholinergic tone can result in a reduction of K_v7 channel opening in medium spiny neurons, increasing their excitability (Shen *et al*., 2005). Pharmacological activation of K_v7 channels seems to be therefore an interesting therapeutic approach for basal ganglia diseases, which are related to increased striatal output.

In summary, the present study demonstrated significant antidystonic effects of retigabine and flupirtine at well-tolerated doses in an animal model of paroxysmal dystonia. In addition to antidystonic effects, $K_v7.2/K_v7.3$ channel openers are known to exert analgesic efficacy. Therefore, K_v7 channel openers are interesting candidates for the treatment of various types of dyskinesia, which are often associated with painful muscle-spasms. The present data should initiate investigations on the efficacy of $K_v7.2$–$K_v7.5$ channel openers in other types of dystonias and dyskinesias. Studies on the efficacy against levodopa-induced dyskinesias are underway.

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Conflict of interest

The authors state no conflict of interest.

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