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Retigabine, a K_V7 (KCNQ) potassium channel opener, attenuates L-DOPA-induced dyskinesias in 6-OHDA-lesioned rats

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ABSTRACT

L-DOPA-induced dyskinesias (LID) represent a severe complication of long-time pharmacotherapy in Parkinson's disease that necessitates novel therapeutics. The acute and chronic effects of $K_V7.2–7.5$ channel openers (retigabine, flupirtine) on the severity of LID and parkinsonian signs were examined in comparison to the glutamate receptor antagonist amantadine (positive control) in a rat model of LID. Acute treatment with retigabine (2.5, 5 mg/kg i.p.) and flupirtine (5, 10 mg/kg i.p.) significantly reduced the severity of abnormal involuntary movements (AIM) to a comparable extent as amantadine (20, 40 mg/kg s.c.), but flupirtine delayed the disappearance of AIM. Chronic treatment with retigabine (daily 5 mg/kg i.p. over 19 days combined with L-DOPA 10 mg i.p.) did not prevent or delay the development of LID, but reduced the severity of AIM, while antidyskinetic effects of amantadine (40 mg/kg i.p.) were restricted to the first day of treatment. Retigabine caused sedation and ataxia which declined during the chronic treatment, but did not reduce the antiparkinsonian effects of L-DOPA in these experiments. Acute co-injections of retigabine (5 mg) together with L-DOPA (10 mg/kg) neither reduced the motor performance in the rotarod test nor exerted negative effects on the antiparkinsonian efficacy of L-DOPA in the block and stepping test. Nevertheless, the sedative effects of retigabine may limit its therapeutic potential for the treatment of LID. The present data indicate that K_V7 channels deserve attention in the research of the pathophysiology of dyskinesias.

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1. Introduction

Dopamine replacement with L-DOPA remains the most effective pharmacotherapy of Parkinson's disease (PD). However, motor fluctuations and L-DOPA-induced dyskinesia (LID) are common and potentially disabling complications in long-term treatment with L-DOPA (Del Sorbo and Albanese, 2008; Obeso et al., 2000). Dyskinesias are abnormal, involuntary movements, including dystonic and choreic movements, which are heterogeneous with respect to affected body parts and the time course after L-DOPA intake (Jankovic, 2005). The underlying mechanisms for LID are unclear, but there is evidence for the importance of pulsatile stimulation of postsynaptic dopamine receptors in the dopamine denervated striatum (Del Sorbo and Albanese, 2008; Grace, 2008). Once established, LID is difficult to treat. The only currently available drug with an evidence-based recommendation on efficacy for dyskinesia is amantadine (Buck and Ferger, 2010; Fabbrini et al., 2007). Beside other mechanisms, its antagonistic action at glutamate NMDA receptors has been suggested to contribute to antidyskinetic effects of amantadine (Ossola et al., 2011; Paquette et al., 2010). However, its use has been reported to be limited by the development of tolerance, rebound and psychomimetic adverse effects (Thomas et al., 2004).

An important component of the pathophysiology of LID is an increased activity of GABAergic striatal projection neurons (Deogaonkar and Subramanian, 2005; Grace, 2008). Thus, compounds which reduce the activity of striatal medium spiny neurons might exert antidyskinetic effects. Indeed, as previously shown in a genetic animal model of inborn paroxysmal non-kinesigenic dyskinesia (also termed paroxysmal dystonic choreoathetosis or paroxysmal dystonia) in which an increased striatal activity is critically involved (Richter, 2005), the $K_V7.2–K_V7.5$ potassium channel openers retigabine and flupirtine exerted striking beneficial effects (Richter et al., 2006). Although voltage-gated channels of the K_V7 family (formerly known as KCNQ or

Abbreviations: AIM, abnormal involuntary movements; ALO, axial, limb, orolingual; 6-OHDA, 6-hydroxydopamine; LID, L-DOPA-induced dyskinesias; PD, Parkinson's disease.

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M-channels) are localized on striatal medium spiny projections neurons and are important for establishing and stabilizing the resting potential of these neurons (Saganich et al., 2001; Shen et al., 2005), the effects of $K_V7.2–K_V7.5$ channel openers have yet not been examined in animal models of LID.

$K_V7.2–K_V7.5$ subunits contribute to the multimeric K_V7 channels in the brain, while $K_V7.1$ subunits are expressed in the heart (Shieh et al., 2000). Neuronal K_V7 channels were originally called "M-channels" because of their suppression by muscarinic receptor signalling. In the striatum, GABAergic 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).

With regard to the function of $K_V7.2–K_V7.5$ channels and the pathophysiological findings in dyskinesias (see above), openers of these channels might provide novel therapeutic approaches for LID. Retigabine (N-(2-amino-4-(4-fluorobenzylamino)-phenyl) carbamic acid ethyl ester), a novel antiepileptic drug with analgesic and anxiolytic effects (Korsgaard et al., 2005; Schenzer et al., 2005; Wuttke et al., 2005), activates $K_V7.2–K_V7.5$ channels at concentrations of 1–10 μM (Dost et al., 2004; Rundfeldt and Netzer, 2000). The analgesic flupirtine (Katadolon), a structural analogue (2-amino-3-carbethoxyamino-6-(4-fluorobenzylamino)-pyridine) of retigabine, was initially thought to act as a glutamate receptor antagonist but is now known to enhance $K_V7.2–K_V7.5$ channel function. Flupirtine has a lower potency than retigabine (Ilyen et al., 2002). In the present study, we examined if retigabine and flupirtine exert antidyskinetic effects and if 10,10-bis(4-pyridinylmethyl)-9(10H)-anthracenone (XE-991), a selective blocker of $K_V7.2–K_V7.5$ channels (Wang et al., 2000), worsens LID. Furthermore, the long-term effects of retigabine on LID were investigated in comparison to amantadine. Behavioural tests were undertaken to examine if antiparkinsonian effects of L-DOPA are reduced or increased by K_V7 channel openers. These investigations were carried out in a well-established rat model in which repeated treatment with L-DOPA after lesions of dopaminergic neurons with 6-hydroxydopamine (6-OHDA) mimics the symptoms of peak-dose dyskinesias, the most common type of LID (Cenci and Lundblad, 2005; Richter and Sander, 2010).

2. Material and methods

2.1. Animals

The present study was performed in female Sprague-Dawley rats, obtained by breeding pairs which were originally provided by a commercial breeder (DIMED Schönwalde GmbH, Germany). The rats were born and kept under controlled environmental conditions (23–25 °C, 50–60% humidity, 12 h light/dark cycle) and had free access to standard diet and water. All experiments were done in accordance with the European Communities Council Directive (86/609/EEC) and in compliance with the German Animal Welfare Act (G 0158/05, G 0297/08).

2.2. 6-Hydroxydopamine (6-OHDA) lesioning

All rats (body weight 200–225 g) received unilateral injections of 6-OHDA-HCl (Sigma–Aldrich, Germany) into the left medial forebrain bundle. The rats were anaesthetised (430 mg/mg chloralhydrate; 3.6%) and injections of 8 μg 6-OHDA-HCl, dissolved in 1 μl of 0.02% ascorbic acid/saline, were done at the rate of 1 μl/min at the following coordinates (in mm, relative to bregma and the dural surface): Incisor bar = −2.4 mm, AP = −4.0 mm, L = +1.2 mm and V = −7.6. After 5 min the needle was slowly retracted. Rats received the analgesic carprofen (5 mg/kg, subcutaneous, Rimadyl®, Pfizer, Germany) perioperative and for 3 days after lesion. Supplemental soft food was given until the rats regained their presurgery weight.

Amphetamine-induced rotational behaviour was determined two weeks after surgery in order to evaluate the extent of dopaminergic denervation. Rotations were monitored 30, 45, 60, 75 and 90 min for 60 s after intraperitoneal (i.p.) injection of 2.5 mg/kg D-amphetamine sulphate (Sigma–Aldrich, Germany). The threshold was 4 rotations/min for selection of 6-OHDA-lesioned rats (Lundblad et al., 2002). Considering the rotational behaviour, homogeneous treatment groups were divided for investigations of chronic drug effects (see below).

Tyrosine hydroxylase (TH) immunohistochemistry was done at the end of all experiments to verify the extent of the lesion by methods as previously described in detail (e.g., Richter et al., 2007). The optical density of striatal TH immunoreactivity was analyzed in the anterior striatum (AP 2.2 mm), two in the middle part (AP 1.2 and 0.2 mm) and one in the posterior striatum (AP − 1.1 mm). All animals included into statistical analysis of this study (for number of animals see Figure legends) showed >90% (99 ± 0.2; range: 91.6–100%) reduction of TH-fibre density in the striatum ipsilateral to the lesioned side.

2.3. Rating of the severity of AIM

L-DOPA-induced AIM were monitored according to standard procedures, as described by Lundblad et al. (2002). All recordings of dyskinesia and determinations of the severity of AIM were carried out by an experimentally blinded investigator. Briefly, rats were placed in home cages and observed for 1 min at following time points after L-DOPA/benserazide administration: 20, 50, 80, 110, 140 and 180 min. Following subtypes of AIM were scored: dyskinesia-like movements (axial, orolingual, forelimb) and locomotive activity. Axial AIM consisted of dystonic posturing or choreiform twisting of the neck and upper body (trunk) towards the side contralateral to the lesion. Orolingual AIM included empty jaw movements and contralateral tongue protrusion. Limb AIM were characterized by jerky or dystonic movements of the contralateral forelimb and/or purposeless grabbing movement of the contralateral paw. Locomotive AIM means increased locomotion with contralateral side bias. Each of these four subtypes was scored on a severity scale from 0 to 4: 1, present during less than half of the observation time (occasional); 2, present during more than half of the observation time (frequent); 3, continuous but interruptible by external stimuli; 4, continuous, severe and not suppressible by external stimuli. The AIM, affecting trunk (axial), limbs and orofacial regions, share common features to dyskinesias in humans, while locomotive AIM do not. Therefore, only the scores obtained from these three subtypes of dyskinesia were summed (ALO AIM).

2.4. Treatment regimen

2.4.1. Experiment 1: acute drug effects on AIM

Four weeks after unilateral 6-OHDA lesions, the rats received daily i.p. injections of 20 mg/kg L-DOPA over a period of 20 d for the induction of AIM (see Fig. 1A). The dose of 20 mg/kg was chosen, because common treatments vary from 6 to 50 mg/kg L-DOPA in the rat model (Lundblad et al., 2002; Ostock et al., 2011; Spinnewyn et al., 2011; Steece-Collier et al., 2003) and was found to be sufficient to induce AIM in preceding experiments. In all experiments L-DOPA was combined with the DOPA-decarboxylase inhibitor benserazide (15 mg/kg i.p.). The acute effects of the $K_V7.2–K_V7.5$ channel openers retigabine (2.5 and 5 mg/kg i.p.) and flupirtine (5 and 10 mg/kg i.p.) and the effects of the K_V7 channel blocker XE-991 (1.5 and 3 mg/kg i.p.) as well as the glutamate receptor antagonist amantadine (20 and 40 mg/kg s.c., injected 90 min prior to L-DOPA), used as a positive control, were examined in groups of 5–6 lesioned dyskinetic rats (total number 20). In a cross over design, the rats received during a drug testing week (see Fig. 1A) the active compound plus L-DOPA/benserazide (20 mg/kg and 15 mg/kg) and three days later vehicle plus L-DOPA/benserazide (control trail). The following week after a drug testing session, L-DOPA was administered alone twice a week in order to wash out the drug but maintain dyskinesia. Then, the next compound or dose was tested in the following week, including drug and vehicle trials together with L-DOPA (see above). Each rat received a maximum of two active compounds at two dosages, i.e. a maximum of four drug trials and four vehicle injections. In order to examine if XE-991 aggravates AIM, rats with lower AIM scores were used for these experiments. The injection volume of vehicle or drug solutions was 1 ml/kg. As described above, the AIM were determined according to a score system 20, 50, 80, 110, 140 and 180 min after L-DOPA injections. Rats which exhibited a total ALO AIM score <10 over the whole monitoring period after L-DOPA/vehicle treatment were omitted.

In addition, the observed side effects such as ataxia (mild, slightly widened hind base; moderate, impaired gait with widened hindlimbs; severe, inability to walk, hindlimbs extended caudally) or hypolocomotion (slight, reduced spontaneous locomotion; moderate, absence of spontaneous locomotion, but inducible by external stimuli; severe, locomotion not inducible) were noted during this period.

2.4.2. Experiment 2: chronic drug effects on AIM

Two weeks after surgery, the rats were divided into 6 homogeneous treatment groups considering their rotational behaviour after induction with amphetamine, as described above (Fig. 1B). Three weeks later, 5 groups received daily i.p. injections of drug or vehicle (injection volume 2 ml/kg) in combination with L-DOPA (injection volume 1 ml/kg) over a period of 19 d (sufficiently long to induce AIM as observed in the acute drug experiments, see above): L-DOPA + vehicle, L-DOPA + amantadine (20 mg/kg), L-DOPA + amantadine (40 mg/kg), L-DOPA + retigabine (2.5 mg/kg) or L-DOPA + retigabine (5 mg/kg). In view of relatively low antidyskinetic potency of amantadine under the chosen treatment regime with 20 mg/kg in the acute studies, the dose of L-DOPA was lowered in the chronic experiments to 10 mg/kg, as commonly used in recent studies (e.g., Walsh et al., 2010). L-DOPA was always