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Neuropharmacological profile of FrPbAII, purified from the venom of the social spider *Parawixia bistriata* (Araneae, Araneidae), in Wistar rats

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Abstract

The aims of the present study were to investigate the anticonvulsant activity and behavioral toxicity of FrPbAII using freely moving Wistar rats. Moreover, the effectiveness of this compound against chemical convulsants was compared to that of the inhibitor of the GABAergic uptake, nipecotic acid. Our results show that FrPbAII was effective against seizures induced by the i.c.v. injection of pilocarpine (ED_{50}=0.05 μg/animal), picrotoxin (ED_{50}=0.02 μg/animal), kainic acid (ED_{50}=0.2 μg/animal) and the systemic administration of PTZ (ED_{50}=0.03 μg/animal). The anticonvulsant effect of FrPbAII differed from that of nipecotic acid in potency, as the doses needed to block the seizures were more than 10 folds lower. Toxicity assays revealed that in the rotarod, the toxic dose of the FrPbAII is 1.33 μg/animal, and the therapeutic indexes were calculated for each convulsant. Furthermore, the spontaneous locomotor activity of treated animals was not altered when compared to control animals but differed from the animals treated with nipecotic acid. Still, FrPbAII did not induce changes in any of the behavioral parameters analyzed. Finally, when tested for cognitive impairments in the Morris water maze, the i.c.v. injection of FrPbAII did not alter escape latencies of treated animals. These findings indicate that the novel GABA uptake inhibitor is a potent anticonvulsant with mild side-effects when administered to Wistar rats.

Keywords: Spider venom compounds; FrPbAII; GABA uptake; Anticonvulsants

Introduction

The importance of developing compounds that inhibit GABAergic uptake is immense (Dalby, 2000, 2003). The temporary blockade of GABA transporters leads to a raise on GABA synaptic concentration and, thus, potentiates inhibitory neurotransmission, which is mostly represented by GABAergic synapses in the mammalian Central Nervous System (CNS) (Andersen et al., 2001; White et al., 2002; Beleboni et al., 2004a). GABA uptake inhibitors have been widely used as pharmacological tools to elucidate many aspects of the complex neural mechanisms in which GABA is involved (Schousboe et al., 2004). Furthermore, consistent literature has pointed that some of these drugs exert potent anticonvulsant effects in many animal models, and their analogues were subsequently demonstrated to possess clinical efficacy (Suzdak and Jensen, 1995; White et al., 2002). In this respect, the specific GABA uptake blocker, tiagabine, was approved for the adjunctive treatment of epilepsy, controlling partial seizures with or without generalization (Kwan et al., 2001; White et al., 2002; LaRoche and Helmers, 2004).

However, clinical treatment of epilepsy, which consists of keeping under control unprovoked and recurrent seizures, still demands novel alternative agents, as the existing anticonvulsants (AEDs) fail to treat all types of epilepsies (Meldrum, 1997; Jallon, 1997; Löscher, 1998; Moldrich et al., 2003). Also, chronic treatment with AEDs often induces a great set of side-effects that limit therapy, such as: sedation, cognitive impairment, lethargy,

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drug–drug interactions and teratogenesis (Löscher, 1998; Andersen et al., 2001; Raza et al., 2001; Villetti et al., 2001). In the light of these facts, GABA uptake blockers appear as potential candidates to anticonvulsants, as these compounds act over a physiologically released GABAergic concentration, thus inducing milder side-effects (Dalby, 2000; Andersen et al., 2001).

Novel compounds are either derived from previously known molecules that are modified in order to alter intrinsic properties or discovered from natural sources. Among the formers, the venoms of arthropods have long been investigated since their molecules exert selective activity over mammalian CNS structures (Beleboni et al., 2004b; Mellor and Usherwood, 2004).

In the search for anticonvulsant compounds targeting GABAergic transmission, our laboratory purified the compound 2-amino-5-ureidopentanamide (FrPbAII, M_r=174 Da) from the venom of the social spider *Parawixia bistriata*. Initially, data using this highly hydrophilic molecule revealed a consistent inhibitory effect on GABA uptake in cortical synaptosomes from rat brains. Moreover, when injected via i.c.v. in freely moving Wistar rats, FrPbAII exerts a potent anticonvulsant effect, blocking seizures induced by i.c.v.-administered bicuculline (Cairrão et al., 2002). Recently, Beleboni et al. (2006) presented the chemical structure of FrPbAII, and suggested its action mechanisms using synaptosomes. In this regard, the main mode of action FrPbAII is probably due to inhibition of GABA and glycine transporters, since this compound appears to have no activity over ion channels, GABA receptors, GABA release, GABA transaminase or reverse GABAergic transport. Still, the neuroprotective activity of FrPbAII was also assayed using an experimental model of glaucoma. In this case, pre-treatment with FrPbAII inhibits cell death in the retinas of rats submitted to both ischemia and ischemia followed by reperfusion (Beleboni et al., 2006).

In the present work, we describe the anticonvulsant activity of FrPbAII in a variety of chemical models of epilepsy, comparing its efficacy with that of nipecotic acid, a selective GAT1 inhibitor, and the benzodiazepine, diazepam. In addition, the effects of acute i.c.v. administration of FrPbAII over motor activity in the open field and rotarod were investigated. Finally, rats treated with FrPbAII were tested in the Morris water maze, in order to assess deficits in learning and memory.

Material and methods

This work was approved by the Ethics Committee for Experimental Animals at the University Campus that follows the Guidelines of the Brazilian College of Animal Experimentation; Guiding Principles for Research involving Animals and Human Beings; American Physiological Society and Ethical Guidelines for investigations of Experimental Pain in Conscious Animals. Also, every effort was made to avoid unnecessary stress and pain to the experimental animals.

Animals and surgery

Male Wistar rats (220–250 g) from the animal housing of the University Campus of Ribeirão Preto were used in the assays. The animals were kept in pairs in wire-mesh cages in a room with a 12-h dark/light cycle (lights on at 7:00 a.m.) with food and water ad libitum.

All animals were anesthetized with sodium thiopental 40 mg/kg (Cristalia, Brazil) for stereotaxic implantation of a stainless steel guide cannula (10 mm) in the right lateral ventricle. The coordinates used were 0.9 mm posterior to bregma, 1.6 mm lateral from midline and 3.4 mm ventral from the surface of the skull according to the atlas of Paxinos and Watson (1986). The cannula was fixed to the skull with dental acrylate and was sealed with a stainless steel wire to avoid obstruction. The animals were then allowed to rest for 5–7 days to recover from surgery.

FrPbAII and drugs

FrPbAII was obtained as described in details by Beleboni et al. (2006). Molecular mass spectral analyses of compound were performed on a Quattro-LC instrument from Micromass (Manchester, UK). The chemical structure of FrPbAII is shown in Fig. 1.

In order to induce seizures chemically, picrotoxin (Research Biochemical Incorporates, USA), pilocarpine (Fluka, USA) and kainic acid (Sigma, USA), were all injected via i.c.v., and pentylenetetrazole (PTZ) (Sigma, USA) was injected by subcutaneous (s.c.) route. Finally, in order to compare the anticonvulsant effects of FrPbAII, nipecotic acid (Sigma, USA), injected via i.c.v., and diazepam (Sanofi-Synthelabo, Brazil), injected via i.p., were used.

Anticonvulsant screening

The doses of all convulsants were calculated in previous experiments as searched for the dosage producing convulsions in 97% of the animals (CD_{97}) (data not shown).

Before injections, the animals were placed in the open field for 10 min. Rats were divided in groups (n=8) and each group was treated with either FrPbAII (0.03, 0.15 and 0.3 μg/animal), nipecotic acid (12 μg/animal) or saline 15 mM (all i.c.v., route) 10 min before the administration of the convulsing drugs (picrotoxin 21 mg/mL; pilocarpine 2.4 mg/mL; kainic acid 0.8 mg/mL — i.c.v. route and PTZ 85 mg/kg, injected via s.c.). The injection volume for all drugs via i.c.v. was 3 μL in a period of 1 min, while PTZ was injected in a volume of 0.2 mL in the loose fold of the neck.

After administration of the convulsants, animals were placed in the arena (open field) and filmed by 20 min. At the end of the filming, the rats were packed in individual cages until total recovery.

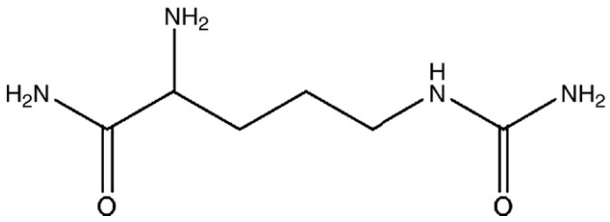

Fig. 1. Chemical structure of FrPbAII according to Beleboni et al. (2006).