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Preclinical activity profile of α-lactoalbumin, a whey protein rich in tryptophan, in rodent models of seizures and epilepsy

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Summary
Purpose: To evaluate the potential anticonvulsant activity of α-lactalbumin (ALAC), a whey protein rich in tryptophan (TRP) relative to other large neutral aminoacids (LNAAs), in rodent models of seizures and epilepsy.
Methods: The effects of ALAC administered per os were evaluated by standard protocols against audiogenic seizures in Genetic Epilepsy Prone Rats (GEPR-9 rats), maximal electroshock (MES)-induced seizures in rats, pilocarpine-induced seizures in mice, spontaneous chronic seizures in mice exposed to pilocarpine-induced status epilepticus (SE), and absence seizures in WAG/Rij rats. In some models, carbamazepine (CBZ) was included as an active control. Plasma TRP/LNAAs ratios were measured by GC—MS.
Results: Single doses of ALAC up to 500 or 6000 mg/kg were devoid of anticonvulsant activity in all models tested. Conversely, 5- and 12-day treatment with ALAC (250—1000 mg/kg/day) in GEPR rats reduced dose-dependently seizure scores and prolonged latency to clonus onset, with full persistence of the effect for up to 12 h. ALAC (125—500 mg/kg/day for 15 days) protected against seizures induced by 250 mg/kg pilocarpine, but was less effective against

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higher pilocarpine doses. Similarly to CBZ, ALAC (125—500 mg/kg/day for 15 days) was also effective against spontaneous seizures in the post-pilocarpine SE model. ALAC (up to 6000 mg/kg/day for 12 days) did not prevent MES-induced seizures, although it reduced the duration of tonic extension at doses between 250 and 1000 mg/kg/day. Absence seizures in WAG/Rij rats were not significantly affected by ALAC. Plasma TRP/LNAAS ratios increased 2- to 3-fold after dosing with ALAC (250 mg/kg/day) for 7 and 14 days, respectively.

Conclusions: ALAC exerts significant protective activity against seizures in animal models, the effect being especially prominent against audiogenic seizures in GEPR-9 rats, seizures induced by low-dose pilocarpine in mice, and spontaneous seizures in mice exposed to pilocarpine-induced SE. This action is likely to be mediated by increased availability of TRP in the brain, with a consequent increase in 5-HT mediated transmission.

Introduction

Serotonin (5-HT) is involved in several physiological functions in the central nervous system (CNS) such as control of appetite, sleep, memory and learning, temperature regulation, mood, behaviour, maturation of neuronal and glial cells, synaptic connections and neuronal excitability (Barnes and Sharp, 1999; Bagdy et al., 2007). As early as 1957, Bonnycastle et al. (1957) suggested that there may also be a link between 5-HT levels and epilepsy. Experimental data indicate that 5-HT has anticonvulsant properties both in experimental models of epilepsy and in humans (Bagdy et al., 2007). Likewise, a reduction in brain 5-HT concentrations leads to an increase in seizure susceptibility in animal models of epilepsy (Browning et al., 1997; Statnick et al., 1996) and, possibly, in humans (Maynert et al., 1975; Pallister, 1959). Selective 5-HT reuptake inhibitors (SSRIs), initially believed to be proconvulsant, have been subsequently reported to possess anticonvulsant properties (Favale et al., 1995, 2003; Albano et al., 2006a). In general, agents that elevate synaptic 5-HT levels, such as 5-hydroxytryptophan (5-HTP) and SSRIs, inhibit both focal (limbic) and generalized seizures (Yan et al., 1994a,b; Albano et al., 2006a). Based on this evidence, SSRIs were tested in the 1990s as potential anticonvulsants as add-on to antiepileptic drugs (AEDs) in patients with drug resistant epilepsy with promising results (Favale et al., 1995, 2003; Cupello et al., 2005; Albano et al., 2006a). Many AEDs including valproic acid, lamotrigine, carbamazepine, phenytoin and zonisamide elevate brain 5-HT levels as part of their actions (Okada et al., 1992; Dailey et al., 1996; Ahmad et al., 2005). Dailey et al. (1997) measured the effects of AEDs on brain 5-HT levels using microdialysis in genetically epilepsy prone rats (GEPRs) and they found that the anticonvulsant effects of AEDs correlated well with the increase in extracellular 5-HT concentrations.

In agreement with the role of 5-HT in epilepsy, the levels of tryptophan (TRP), an essential aminoacid and the only precursor of 5-HT in the brain, have been found to be reduced in the cerebrospinal fluid and/or plasma of some groups of patients with seizure disorders (Ko et al., 1993; Marion et al., 1985). Indeed, clinical and neurochemical evidence suggests that a decreased brain availability of TRP may play a pathogenetic role in epilepsy (Russo et al., 2009). A PET study confirmed that a decrease in brain uptake of TRP is paralleled by a decrease in brain synthesis of 5-HT (Diksic et al., 2002), which, in turn, may lead to diminished 5-HT-mediated anticonvulsant activity. Conversely, an increase in plasma TRP levels results in increased brain 5-HT synthesis. Furthermore, intravenously (i.v.) or intraperitoneally (i.p.) administered 5-hydroxy-TRP have been found to have anticonvulsant effects (Truscott, 1975; Alexander and Kopeloff, 1976), while oral TRP has not been found to be consistently effective. The lack of anticonvulsant effects of orally administered TRP could be related to inadequate intestinal absorption (Frenhani and Burini, 1999; Heuther et al., 1992).

Because TRP competes with other large neutral amino acids (LNAAs) for the transport by a specific carrier at the blood brain barrier (BBB), the cerebral uptake of TRP is enhanced by an increase in TRP/LNAAs ratio in plasma (Diksic et al., 2002). An elevation in plasma TRP/LNAA ratio can be obtained by administration of whey proteins rich in TRP and poor in other LNAAs content (Feurté et al., 2001; Markus et al., 2000). In particular, α-lactoalbumin (ALAC, Davisco Foods International, Inc., Minneapolis, MN, USA), a whey protein naturally occurring in human milk and commercially available as a food supplement, shows the highest TRP/LNAAs ratio among all quantitatively relevant food-derived proteins. Oral doses of ALAC have been found to increase significantly plasma TRP/LNAAs ratio in rodents and in humans (Yokogoshi and Wurtman, 1986; Markus et al., 2000; Feurté et al., 2001). In addition, ALAC has been found to increase brain 5-HT concentrations (Orosco et al., 2004) and to reduce sleep disturbances in rats (Minet-Ringuet et al., 2004). In a pilot study, ALAC administration was also reported to be associated with improved seizure control in patients with drug resistant epilepsy (Albano et al., 2006b; Mainardi et al., 2008). Based on these data, we considered it of interest to investigate its activity profile in various animal models of seizures and epilepsy.

Materials and methods

Animals

For experiments in mice, we used male C57BL/6 mice weighing 22—28 g (8 weeks old, Harlan Italy SRL, Correzzana, Milano, Italy), housed in groups of 8—10 per cage under stable conditions of humidity (60 ± 5%) and temperature (21 ± 2 °C). The animals were maintained on a 12 h light and 12 h dark cycle (lights on at 7:00 pm) and were allowed free access to standard laboratory chow (Teklad 2018) and tap water until the time of experiments.

For experiments in rats, GEPRs of both sexes, 12—18 weeks of age, were obtained from our breeding stock (Department of Phar-