2-Phosphonomethyl-pentanedioic acid (glutamate carboxypeptidase II inhibitor) increases threshold for electroconvulsions and enhances the antiseizure action of valproate against maximal electroshock-induced seizures in mice

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Abstract

This study examined the effect of 2-(phosphonomethyl)-pentanedioic acid (2-PMPA), a potent and selective inhibitor of glutamate carboxypeptidase II (GCP II), an enzyme releasing glutamate and *N*-acetyl-aspartate from synaptical terminals, on the electroconvulsive threshold in mice. Moreover, the influence of 2-PMPA on the anticonvulsant activities of four conventional antiepileptic drugs (carbamazepine, phenobarbital, phenytoin and valproate) was evaluated in the maximal electroshock-induced seizure test in mice.

Results indicated that 2-PMPA (at a dose range of 50–200 mg/kg, i.p.) raised the electroconvulsive threshold in mice dose-dependently. Linear regression analysis of dose–response relationship between the doses of 2-PMPA and their corresponding threshold values allowed the calculation of threshold increasing dose by 20% (TID_{20}), which was 109.2 mg/kg. Moreover, 2-PMPA administered i.p. at a constant dose of 150 mg/kg (the dose increasing the threshold for electroconvulsions) enhanced significantly the anticonvulsant action of valproate, by reducing its median effective dose (ED_{50}) from 281.4 to 230.1 mg/kg ($P < 0.05$). In contrast, 2-PMPA at the lower dose of 100 mg/kg (i.p.) had no impact on the antiseizure activity of valproate in the maximal electroshock-induced seizure test. Likewise, 2-PMPA at 100 and 150 mg/kg did not affect the antiseizure action of carbamazepine, phenobarbital and phenytoin against maximal electroshock-induced seizures in mice. Additionally, none of the combinations investigated between 2-PMPA (150 mg/kg, i.p.) and carbamazepine, phenobarbital, phenytoin and valproate (at their ED_{50} values) produced motor coordination impairment in the chimney test. Pharmacokinetic evaluation of interaction between 2-PMPA and valproate revealed that 2-PMPA at 150 mg/kg selectively increased total brain concentrations of valproate, remaining simultaneously without any effect on free plasma concentrations of valproate, indicating a pharmacokinetic nature of observed interaction in the maximal electroshock-induced seizures in mice.

Based on our preclinical data, it may be concluded that 2-PMPA possesses a seizure modulating property by increasing the electroconvulsive threshold. The reduction of glutamate neurotransmission in the brain, as a consequence of inhibition of GCP II activity by 2-PMPA, was however insufficient to enhance the anticonvulsant activity of conventional antiepileptic drugs, except for valproate, whose antiseizure action against maximal electroconvulsions was potentiated by 2-PMPA. Unfortunately, the favourable interaction between 2-PMPA and valproate was associated with a pharmacokinetic increase in total brain valproate concentrations.

1. Introduction

Overwhelming evidence indicates that imbalance between inhibitory (γ-aminobutyric acid [GABA], adenosine) and excitatory (glutamate, aspartate) neurotransmitters in the

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central nervous system (CNS) is a main cause underlying seizure initiation, propagation and amplification in both, experimental and clinical conditions (Bradford, 1995; Dudek and Spitz, 1997; Meldrum, 2000; Parsons et al., 1998; Pearl and Gibson, 2004; Sherwin, 1999; Treiman, 2001). At present, modern therapeutic approaches to control seizure attacks in epileptic patients are based on: (1) the administration of drugs enhancing the inhibitory neurotransmission in the brain through the direct stimulation of $GABA_A$ receptors and inhibition of GABA metabolism, providing in consequence an increase in GABA content in the brain; (2) the administration of drugs blocking voltage-dependent neuronal Na^+ channels; or (3) the application of drugs that reduce the excitatory neurotransmission either via blocking ionotropic and metabotropic glutamate receptors or through the blockade of glutamate synthesis and its liberation from neurons and glia (Coyle, 1997; Löscher and Schmidt, 2004). The latter strategy, to reduce glutamate content in the extracellular (synaptic) space in neurons through the inhibition of its release from synaptical storage forms, has recently been proposed as a new therapeutic target in numerous CNS diseases (including epilepsy), in which glutamate plays an important pathophysiological role (Meldrum, 2000; Parsons et al., 1998).

Relatively recently, extensive experimental research has focused on inhibition of the metabolism of N-acetyl-aspartyl-glutamate (NAAG), an endogenous neuropeptide, which is considered as a physiological storage form for glutamate in the CNS. Noticeably, NAAG is present in the brain in millimolar (0.5–2.7 mM) concentrations (Pouwels and Frahm, 1997), and it is released from neurons and glia after Ca^{2+}-dependent depolarization (Neale et al., 2000; Tsai et al., 1990). In the brain, NAAG is hydrolyzed by the neuropeptide glutamate carboxypeptidase II (GCP II, EC 3.4.17.21), also known as N-acetylated-α-linked-acidic dipeptidase (NAALADase), localized on neuronal and glial surfaces, to liberate N-acetyl-aspartate (NAA) and glutamate (Cassidy and Neale, 1993a,b; Robinson et al., 1987; Stauch et al., 1989). In physiological conditions, GCPII terminates the neurotransmitter action of NAAG by releasing glutamate (Coyle, 1997; Stauch et al., 1989).

Accumulating evidence indicates that the inhibition of GCP II activity associated with blockade of metabolic degradation of NAAG to glutamate and NAA reduces neuronal damage caused by glutamate excitotoxicity, providing simultaneously neuroprotective effects in experimental models of ischemia (Fuhrman et al., 1994; Jackson et al., 1996; Slusher et al., 1992; Tortella et al., 2000). So, the inhibition of GCP II may be a potential therapeutic target for neurological disorders in which excessive amino acid transmission is pathogenic (Neale et al., 2000, 2005).

With respect to NAAG, it has been found experimentally that this endogenous neuropeptide at 20 μM acts as an N-methyl-D-aspartate (NMDA) receptor antagonist (Bergeron et al., 2005) and, simultaneously, it has NMDA receptor agonist activity, when administered at 300–666 μM (Burlina et al., 1994; Puttfarcken et al., 1993; Sekiguchi et al., 1992; Trombley and Westbrook, 1990; Westbrook et al., 1986). Hence, NAAG may

be regarded as a neurotransmitter and/or neuromodulator with mixed agonist/antagonist properties at NMDA receptors. Additionally, NAAG activates pre-synaptic subtype 3 metabotropic glutamate receptors (mGlu3) (Wroblewska et al., 1993, 1997). Extensive neurochemical studies have documented that NAAG, as the mGlu3 receptor agonist, decreases stimulus-evoked glutamate and dopamine release from neurons (Cartmell and Schoepp, 2000), contributing to the reduction of excitatory neurotransmission in the brain.

Previously, it has been reported that 2-(phosphonomethyl)-pentanedioic acid (2-PMPA), a potent and selective GCP II inhibitor (Jackson et al., 1996), increased NAAG and decreased extracellular glutamate levels in both in vitro and in vivo models of cerebral ischemia (Slusher et al., 1999). Extensive molecular studies have revealed that 2-PMPA is highly selective for GCP II with no apparent affinity for numerous neuronal receptors, ion channels, transporters and enzymes including several glutamatergic sites such as NMDA, α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA), kainate, metabotropic glutamate receptors and glutamate transporters (Slusher et al., 1999).

It is hypothesized that selective inhibition of GCP II could attenuate the direct excitotoxic glutamate neurotransmission (Neale et al., 2005; Slusher et al., 1999) and, simultaneously, the accumulation of NAAG in the brain would potentiate the reduction of excitatory neurotransmission through the activation of mGlu3 receptors (Orlando et al., 1997; Wroblewska et al., 1998). Indeed, in animal studies, 2-PMPA has been shown to decrease extracellular glutamate and provide neuroprotection, which was correlated with glutamate decrease and an increase in NAAG content in the brain (Neale et al., 2000, 2005; Slusher et al., 1999). Undoubtedly, both mechanisms, the decreased content of glutamate in the extracellular (synaptic) space of neurons, which considerably reduces the excitatory neurotransmission at all glutamatergic receptors, and the increased NAAG concentration, which decreases indirectly glutamate release by activation of mGlu3 receptors, could attenuate the overstimulation of both ionotropic and metabotropic receptors for glutamate, contributing to seizure suppression (Meldrum, 2000; Löscher and Schmidt, 2004).

Despite the advanced knowledge of pathophysiological processes related to seizure initiation, propagation and amplification in the brain as well as a great number of antiepileptic drugs approved for the therapy in epilepsy, there are still about 30% of epileptic patients inadequately controlled with current frontline antiepileptic drugs in monotherapy (Kwan and Brodie, 2000a,b). To suppress the epileptic attacks and provide the patients with a state of seizure freedom, clinicians and researchers are obliged to search for an efficacious antiseizure therapy based on a clinical use of antiepileptic drugs with novel mechanisms of action, different from those of current frontline antiepileptic drugs, or the application of rational polytherapy with two or more antiepileptic drugs. The combinations of antiepileptic drugs are rationally preselected taking into account their molecular mechanisms of action and results from preclinical studies, verifying directly on animals the antiseizure potential