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Determination of a potent non-competitive AMPA receptor antagonist in rat brain

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Abstract

N-acetyl-1-(*p*-chlorophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline derivative (PS3Ac) has been determined in brain tissues by high performance liquid chromatography (HPLC) coupled with a diode array detection. In a previous paper we presented a validation method for detecting PS3Ac and its metabolites in plasma samples after intraperitoneal administration to Wistar rats. In the present paper, we report the results of the determination of PS3Ac and its *N*-deacetyl (PS3) and *O*-demethyl (PS3OH) metabolites, in the brain after extraction based on a polymeric matrix with a high hydrophilic–lipophilic balance, using Oasis cartridges. The chromatographic separation was performed in an octadecylsilica stationary phase at 25 °C using a mixture of 10 mM potassium dihydrogen orthophosphate (pH 2.24) and acetonitrile in ratio of 30:70 (v/v) as mobile phase, with a flow rate of 0.8 ml/min. The method exhibited a large linear range from 0.05 to 2 μg/ml for all studied compounds ($n = 6$). In the within-day assay ($n = 4$), the accuracy ranged from 87.5% determined with 0.05 μg/ml of PS3 to 110.1% determined with 0.2 μg/ml of PS3OH. In the between-day assay the coefficient of variation ranged from 2.4 determined with 0.05 μg/ml of PS3 to 9.7 determined with 0.2 μg/ml of PS3OH. The extraction efficiency ranged from 77.8% for PS3OH at 0.2 μg/ml to 94.3 for PS3Ac at 0.5 μg/ml. The limit of detection for all the tetrahydroisoquinoline derivatives ranged around 50 ng/ml. The method proved to be highly sensitive and specific to determinate PS3Ac and its metabolites and has been successfully applied to value their concentrations in brain matrix over the time.

Keywords: AMPA receptor antagonist; SPE-HPLC; Tetrahydroisoquinoline; Brain

1. Introduction

Selective non-NMDA (*N*-methyl-D-aspartic acid) receptor antagonists, *i.e.*, 2-amino-3(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) and kainate have been proposed as a useful treatment of various disorders such as epilepsy, ischaemia, and Parkinson's disease [1,2]. Indeed, different neurological disorders have been linked to excessive activation of excitatory amino acid (EAA) receptors.

The 2,3-benzodiazepines (2,3-BZs) represent an important class of selective AMPA/kainate receptor antagonists [3,4].

GYKI 52466 (1-(4′-aminophenyl)-4-methyl-7,8-methylenedioxy-2,3-benzodiazepine), at first, was pharmacologically differentiated from classical 1,4 and 1,5-benzodiazepines for its muscle relaxant and anticonvulsant properties, acting as a highly selective non-competitive antagonist at AMPA/kainate receptor site and showing no affinity for the benzodiazepine receptors (BZRs) at GABA (γ-aminobutyric acid) complex [5,6].

Successively other 2,3-BZs, chemically similar to GYKI 52466 (Fig. 1), were synthesised in our laboratories and proved to possess anticonvulsant activity in various experimental models of seizures [7,8]. It has been demonstrated that these 2,3-benzodiazepine derivatives are non-competitive antagonists at the AMPA/kainate receptor and do not affect NMDA and GABA receptor-mediated responses [8,9].

Particularly, we studied an extensive series of 7,8-dimethoxy-2,3-benzodiazepines, such as [1-(4-aminophenyl)-3,5-dihydro-7,8-dimethoxy-4H-2,3-benzodiazepin-4-one] (CFM-2, Fig. 1), demonstrating a marked anticonvulsant activity higher than that of GYKI 52466 [10] and comparable to that of a 3-*N*-acetyl-1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-4,5-dihydro-2,3-benzodiazepine, *i.e.*, Talampanel (Fig. 1),

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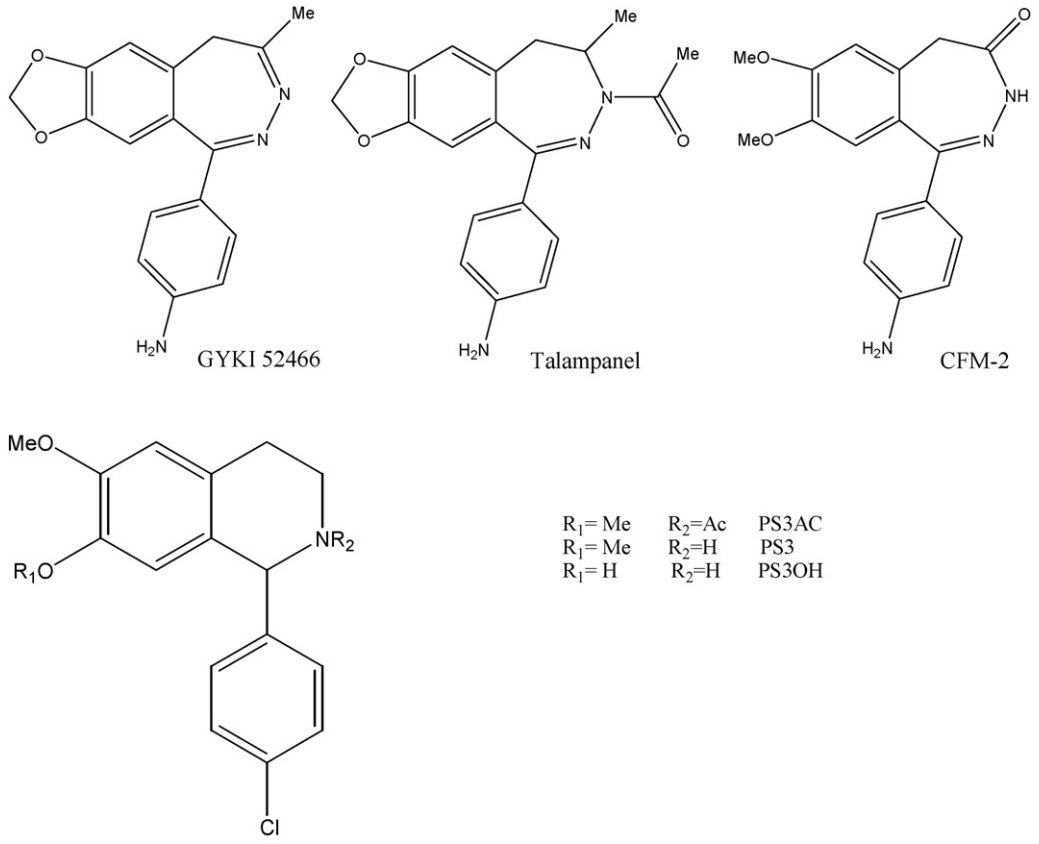

Fig. 1. Structures of GYKI 52466, Talampanel, CFM-2 and tetrahidroisoquinoline derivatives.

which aroused great interest as anticonvulsant agent and whose phase II/III clinical trials are under way [13].

In previous studies, we also reported a pharmacokinetic study of an extensive series of 7,8-dimethoxy-2,3-benzodiazepines, such as compound CFM-2, proposed as selective ligands for AMPA receptor (AMPAR) [11,12].

More recently, by using molecular modelling approaches, we have proposed a pharmacophore model of negative allosteric modulators of AMPAR which suggested us the synthesis of *N*-acetyl-1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines which might satisfy the structural requirements for AMPAR binding [14]. Indeed, *N*-acetyl-1-(*p*-chlorophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (PS3Ac, Fig. 1), was the most active compound in this novel series of anticonvulsant agents which has been demonstrated to possess markedly increased activity over CFM-2, Talampanel and GYKI 52466 [15].

Recently, we reported an analytical study of the pharmacokinetic profile of *N*-acetyl-1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline derivative in Wistar rats, showing that PS3Ac is scarcely bio-transformed into two different metabolites such as *N*-deacetyl and *O*-demethyl derivatives, PS3 and PS3OH, respectively (Fig. 1). All the metabolites are more polar than the parent compound and the study suggests that at least for the analysed time point, metabolism had a limited effect.

Moreover, those results have been cited recently by some of us and confirmed in a study where PS3Ac has been labelled with carbonium-11 and tritium and evaluated as a potential ligand for *in vivo* imaging of AMPA receptor using positron emission tomography (PET) [16].

The goal of the present study was the assessment of the metabolic process in the brain, as determined in plasma, to confirm that the observed pharmacological anticonvulsant activity could be attributed exclusively to the inoculated drug PS3Ac.

With this aim, we have developed an assay suitable for simultaneous determination of tetrahydroisoquinoline derivatives in rat brain by using high performance liquid chromatography (HPLC) with diode array (DAD) detection.

The studied compounds have been isolated from brain samples by solid-phase extraction (SPE). The proposed method has been validated according to International Conference on Harmonisation (ICH) guidelines [17].

2. Experimental

2.1. Chemicals and standards

Tetrahydroisoquinoline derivatives PS3Ac and PS3 were synthesised in our laboratories as previously described [14]. A different synthetic procedure was employed to obtain PS3OH, *i.e.*, 1-(*p*-chlorophenyl)-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline [16]. Stock solutions (1 mg/ml) of all studied compounds were prepared in methanol. Working solutions were made by dilution with methanol and were used to prepare aque-