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Evaluation of anticonvulsant and analgesic effects of benzyl- and benzhydryl ureides

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Abstract

The anticonvulsant effects of benzyl- and benzhydryl ureides in mice models of seizures (maximal electroshock seizure test, pentylenetetrazol test, picrotoxin-induced seizure test) and the influence on spontaneous locomotor activity has been assessed. Furthermore, the analgesic effect of ureide derivatives was studied in the hot-plate test in mice. Selected compounds were investigated for their *in vitro* interaction with adenosine receptors as well as the benzodiazepine binding site of $GABA_A$ receptors. This study demonstrated the strong anticonvulsant activity of several ureides in electrically or chemically induced seizure models, and structure-activity relationships were discussed. 1-Benzyl-3-butyrylurea (**9**) was found to be equipotent to ethosuximide in the pentylenetetrazol test with regard to the number of attacks as well as the time of the onset of seizures. The ureide **9** also revealed the highest protective activity against seizures in the other models, maximal electroshock seizure and picrotoxin test. Moreover, 1-benzyl-3-butyrylurea was not neurotoxic at doses up to 200 mg/kg. Benzylureides **8**–**10** showed affinity to the adenosine A_1 receptors at low micromolar concentrations. However, the apparent anticonvulsant activity in different seizure models does not appear to result from direct activation of adenosine A_1 receptors or $GABA_A$ receptors, respectively. In the hot-plate test, the majority of investigated compounds exhibited analgesic activity. Again, compound **9** was superior to the other substances investigated, suggesting a potential therapeutic value of that ureide derivative.

Keywords: Ureides; Anticonvulsant activity; Analgesic effect; GABA; Adenosine receptors; Benzodiazepine binding site

1. Introduction

Epilepsy is one of the most frequent neurological disorders. The basic cause of seizures is not clearly understood and the different biochemical aspects of epileptic seizures still remain unknown. Antiepileptic drugs exert their action by different mechanisms. Mainly they can influence inhibitory (GABA) or excitatory neurotransmitter (glutamic acid) systems and thus ion transports across cell membranes. While interactions of antiepileptic drugs with the different allosteric binding sites of the $GABA_A$ receptor and the resulting enhancement of inhibitory GABAergic effects is a long known mechanism (Rogawski and Löscher, 2004a), the involvement of the various adenosine receptor subtypes and their ligands in seizure disorders is still an object of discussion (Drabczyñska et al., 2004). Available antiepileptic drugs are effective in only 60–80% of epileptic patients and a number of limitations of the antiepileptic drug therapy continue to exist (Baulac, 2003). Therefore, the search for new anticonvulsant drugs is an ongoing area of investigation in medicinal chemistry (Beghi and Perucca, 1995). The present study is part of our common efforts (Mendyk et al., 2001; Jastrzębska-Więsek et al., 2003; Kiec-Kononowicz et al., 2002, 2004) in the development and synthesis of new potent anticonvulsants. The structure-activity relationships of the established antiepileptic drugs such as phenytoin, carbamazepine or phenobarbital led to the proposal of a general model for anticonvulsant activity comprising two aromatic rings usually bound to an ureide ring system. The ureide (*N*-acylurea) motif is a common structure in acyclic and

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heterocyclic compounds, such as barbituric acids and hydantoins. There are a number of publications describing the anticonvulsant activities of *N*-, *N*,*N*- and *N*,*N*′-substituted acylureas (Spielman et al., 1948; Frommel and Radouco-Thomas, 1953; Beasley et al., 1961; Kulev and Dobychina, 1963; Polezhaeva, 1966; Carraz and Emin, 1967; Zirvi and Fakouhi, 1982; Khalil and Weaver, 1990; Sobol et al., 2004). Beside their anticonvulsant activity, many of those substances show antiarrhythmic effects (Meusel and Gütschow, 2004) as well as central depressant (Mrongovius, 1975), sedative (Weiss, 1971; Mrongovius et al., 1976), antianxiety (Goldstein and Pfeiffer, 1971), local anesthetic (Chiti, 1960), or antimycotic activities (Baraldi et al., 1989). The acylurea moiety can also be found in several other drugs modulating their pharmacodynamic and -kinetic properties (*e.g.* azlocillin (Graber et al., 1981)). Moreover, an analgesic effect was described for some acylureas (Arbuzov et al., 1989). Therefore, the analgesic potential of the compounds of this study was also investigated. Herein we report the results of the examination of a series of partly novel benzyl- and benzhydryl ureides for their analgesic and anticonvulsant activities *in vivo*. Anticonvulsant activity was determined in the following mice models: picrotoxin-induced convulsions, pentylenetetrazol-induced convulsions and maximal electroshock model. In addition, effects on spontaneous locomotor activity and neurotoxicity were evaluated.

Furthermore, to characterize their anticonvulsant properties, we investigated selected compounds *in vitro* for their interaction with potential molecular targets, *i.e.* adenosine receptors and $GABA_A$ receptors. Such an investigation might also elucidate the analgesic activity of the compounds, as it has been documented that both $GABA_A$ (Mui et al., 1997) and adenosine receptors (Sawynok and Liu, 2003; Abo-Salem et al., 2004) participate in antinociception.

A link between antiepileptic activity and antinociceptive effects was proven for many established anticonvulsant drugs which have long been used in pain management, particularly in chronic neuropathic pain. For example, carbamazepine, phenytoin, lamotrigine and felbamate are clinically effective in the treatment of trigeminal neuralgia, valproate and topiramate are useful for migraine prophylaxis (Rogawski and Loscher, 2004b; Rogawski, 2006a), and gabapentin and carbamazepine as well as phenytoin are used in the treatment of post-herpetic neuralgia and diabetic neuropathy, respectively (Wiffen et al., 2005; Taylor, 1996; Collins et al., 2000). The mechanisms underlying the antinocicepitive action of antiepileptic drugs might include an enhancement of the GABAergic neurotransmission or effects on neuronal voltage-gated sodium and/or calcium channels (Rogawski and Loscher, 2004b). Whereas antiepileptic drugs are efficacious in neuropathic pain, their activity in models of acute pain is less clear. Only negligible effects of lamotrigine, felbamate and gabapentin against an acute noxious stimulus were determined in the rat tail flick test (Hunter et al., 1997). Retigabine did not show antinociceptive effects in this test (Blackburn-Munro and Jensen, 2003). Tiagabine, a GABA uptake inhibitor, produced dose-dependent antinociception in the hot-plate test in mice (Giardina et al., 1998) where lamotrigine and gabapentin were not efficacious (Laughlin et al., 2002). Voltage-gated calcium channel blockers (gabapentin and ethosuximide) and voltage-gated sodium channel blockers (phenytoin and carbamazepine) have been shown to be potent local analgesics to ameliorate acute thermal nociception. The activity of locally applied phenytoin and carbamazepine was substantially higher than that of the sodium channel-blocking local anaesthetic lidocain (Todorovic et al., 2003). Both sodium channel-blocking antiepileptic drugs are effective by virtue of the same selective block of high-frequency action-potential firing that accounts for their protective activity against seizures (Rogawski and Loscher, 2004b).

2. Materials and methods

2.1. Animals

The studies were carried out on male Albino Swiss mice (18–24 g). The animals were kept in groups of 15 mice in type III-1290 cages (26.5 × 42.0 × 15.0 cm) at a room temperature of 22 ± 2 °C, under 12/12 h light/dark cycle (light on from 7 a.m. to 7 p.m.), and had free access to water and food (standard laboratory pellets; Bacutil, Motycz, Poland) before the experiments. Each experimental group consisted of 10 animals/dose (unless stated otherwise), and all the animals were used only once. The experiments were performed between 8 a.m. and 3 p.m. Treatment of laboratory animals in the present study was in full accordance with the respective Polish and European regulations. All procedures were conducted according to Animal Care and Use Committee guidelines, and approved by the Ethical Committee of Jagiellonian University, Kraków.

2.2. Compounds

The synthetic procedures of the ureides **1**–**13** (Fig. 1) are given in the Supplementary Material. Ureides **1**–**13** were suspended in 0.5% methylcellulose (Loba-Chemie, Germany). Picrotoxin (Fluka, Germany), pentylenetetrazol (Cefarm, Poland), and ethosuximide (Ronton, ICN-Polfa Rzeszów, Poland) were dissolved in 0.9% sodium chloride (Rhone-Poulenc Rorer, France). Diazepam (Valium, Roche, France) was used as a 1% aqueous solution. Phenytoin (Phenhydan, Desitin Pharma, Switzerland) was used as a 5% solution. Appropriate amounts of the corresponding vehicles were given to the control animals. Compounds **1**–**13** and reference compounds (ethosuximide, diazepam and phenytoin) were given intraperitoneally (i.p.). Control animals received appropriate amounts of the vehicle i.p.

All chemical reagents for the synthesis were obtained from Merck (Darmstadt, Germany), Fluka (Taufkirchen, Germany), Aldrich (Steinheim, Germany) or Acros (Geel, Belgium). For detailed information on further materials and methods see Supplementary Material.

2.3. Spontaneous locomotor activity

The spontaneous locomotor activity including different types of movements such as locomotions, sniffing and grooming of a