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Evaluation of anxiolytic-like, anticonvulsant, antidepressant-like and antinociceptive properties of new 2-substituted 4-hydroxybutanamides with affinity for GABA transporters in mice

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ABSTRACT

Purpose: The inhibition of plasma membrane GABA transporters (GATs) is responsible for anxiolytic-like, anticonvulsant, antinociceptive and antidepressant-like effects in mice. It also influences animals' motor coordination and their sensitivity to ethanol. The aim of this study was to assess the pharmacological activity of two novel 2-substituted 4-hydroxybutanamides (BM 130 and BM 131) in some screening models. An attempt has been made to establish the relationship between the inhibition of GAT subtype and the observed *in vivo* activity.
Methods: The affinity for GAT subtypes was evaluated by means of [^{3}H]GABA uptake assay. It indicated that BM 130 inhibited GAT1 and GAT2, whereas BM 131 inhibited GAT1 and GAT3. In mice anxiolytic-like, antidepressant-like, anticonvulsant and antinociceptive properties of the test compounds were assessed. Their influence on motor coordination, locomotor activity and the ability to potentiate effects of subnarcotic doses of ethanol was also tested.
Results: Both compounds administered intraperitoneally exerted a significant anxiolytic-like effect in the four plate test with ED_{50} values 3.4 and 7.9 mg/kg, respectively. At 30 mg/kg they reduced duration of immobility in the forced swim test for 33% and 19%, respectively. They had no effect on electroconvulsive threshold or pain reactivity in the hot plate assay but they were antinociceptive in the acetic acid-induced writhing test (ED_{50} values were 12.7 and 18.6 mg/kg, respectively) and in both phases of the formalin test (ED_{50} values in the first phase were 10.2 and 2.1 mg/kg for BM 130 and BM 131, respectively). No motor adverse effects were observed in mice pretreated with the test compounds in the rotarod or chimney tests but BM 131 caused a transient but statistically significant decrease of animals' locomotor activity.
Conclusions: In mice BM 130 and BM 131 have anxiolytic-like, antidepressant-like and antinociceptive properties which can be attributed to their affinity for not only mGAT1 but also mGAT2–4.

1. Introduction

Gamma-aminobutyric acid (GABA) is the most abundant inhibitory neurotransmitter released at approximately 50% of brain synapses (Brambilla et al., 2003; Hu et al., 2004) but apart from this GABAergic mechanisms have also been found in peripheral tissues: in the stomach, pancreas, intestine, urinary bladder, kidney, uterus and other organs (Schlessinger et al., 2012). Since the GABAergic neurotransmission is responsible for a variety of inhibitory activities, such as anxiolysis, muscle relaxant effect or anticonvulsant activity, the dysfunction within this system leads to several diseases, including anxiety, seizures, neurodegenerative disorders, motion impairments, insomnia, pain, alcoholism and others (Dalby, 2003; Liu et al., 2007; Thoeringer et al., 2010).

The termination of GABA action depends *inter alia* on GABA uptake from the synaptic cleft. In this process plasma membrane GABA transporters (GATs) are involved. So far, four GAT subtypes have been identified in the mouse (m): mGAT1, mGAT2, mGAT3, and mGAT4 (Clausen et al., 2006; Hu et al., 2004; Itouji et al., 1996; Salat and Kulig, 2011; Schousboe et al., 2004). All of them are members of Na^+/Cl^--coupled transporter family which also comprises transporters for monoamines and amino acids — taurine, glycine and betaine (solute carrier 6 — SLC6 superfamily of Na^+-dependent transporters) (Conti et al., 2004; Meldrum and Rogawski, 2007; Sarup et al., 2003; Schlessinger et al., 2012; Schousboe et al., 2004; Soudijn and van Wijngaarden, 2000).

In the recent years much attention has been paid to the role of GAT in the regulation of various biological functions, including seizures (Chroscinska-Krawczyk et al., 2009), anxiety (Schmitt and Hiemke, 1999; Thoeringer et al., 2010), depression (Thoeringer et al., 2010) and nociception (Xu et al., 2008). Numerous lipophilic analogs of nipecotic acid have been developed so far and they inhibit GAT1–GAT4. Among these agents only tiagabine selectively inhibits

Abbreviations: ECT, electroconvulsive threshold; GABA, gamma aminobutyric acid; GATs, plasma membrane GABA transporters; MES, maximal electroshock seizures; MC, methylcellulose; rpm, rotations per minute.

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mGAT1, while (S)-SNAP-5114 (Fig. 1) inhibits GAT2, GAT3 and GAT4 with much higher affinity than GAT1 (Table 1) (Schousboe et al., 2004; Soudijn and van Wijngaarden, 2000).

The functional significance of GAT1 has been thoroughly investigated due to the availability of tiagabine which apart from its use as a pharmacological tool in the research is also used for the treatment of partial seizures (Chroscinska-Krawczyk et al., 2009) and chronic pain (Todorov et al., 2005) in humans. In contrast to this, the biological role of non-GAT1 subtypes is much less described (Clausen et al., 2006) and numerous agents targeting at mGAT2–4 are tested for their biological activity in animal models of seizures, anxiety, depression, pain and others. Recent studies have demonstrated that the overexpression of mGAT1 is responsible for hyperalgesic effects in mice (Hu et al., 2003), whereas in mice lacking GAT1 hypoalgesia was observed (Xu et al., 2008). Based on this knowledge, several mGAT1 inhibitors, including tiagabine were tested for their antinociceptive activity in the hot plate, tail flick, formalin and writhing tests in mice (Hu et al., 2003; Ipponi et al., 1999; Xu et al., 2008). The results of these experiments indicated that GAT1 is involved in the regulation of pain processes and pointed to the possibility of developing analgesic drugs that target GAT1. These studies also confirmed the crucial role of GAT1 in the regulation of nociceptive threshold and indicated that two GAT1-selective inhibitors, NO-711 and tiagabine have the potential for their clinical use in pain therapy (Xu et al., 2008). In addition, it was demonstrated that a peripherally located mGAT3 transporter might play a regulatory role in peripheral GABAergic mechanisms controlling numerous pathological processes, including pain (Schlessinger et al., 2012).

It was recently reported that some 2-substituted 4-hydroxybutanamides display inhibitory potency at four murine GAT subtypes in *in vitro* tests (Kulig et al., 2011). It was also shown that some of these compounds exert anticonvulsant and antinociceptive activities in mouse models of electrical seizures, acute pain (Sałat et al., 2012a,b) and tonic pain (Sałat et al., 2012b). Promising results obtained until now in our laboratory confirmed that the pharmacological activity of some derivatives from this chemical group extends far beyond the well-known therapeutic targets and this suggests that there are more opportunities for their use.

In view of this, in the present work, we report on the results from *in vivo* studies regarding the activity of 2-substituted 4-hydroxybutanamides, compounds BM 130 and BM 131. These compounds are various substituted benzylamides of 4-hydroxybutanoic acid, which in the second position have diphenylmethylpiperazine fragment. The difference between these two structures is related to the substituent in the benzyl fragment of the molecule BM 130 which is a benzylamine derivative, while BM 131 has a fluorine atom at *para*-position of the aromatic fragment. Schematic structures of these compounds and (S)-SNAP-5114, a semi-selective murine GAT4 inhibitor (Table 1) used as a reference in this research (Dalby, 2000) are presented in Fig. 1.

In vitro BM 130 and BM 131 were evaluated for their affinity for mGAT1–4. BM 130 showed inhibitory potency at mGAT1 and mGAT2, although it was not active at mGAT3 and mGAT4, whereas BM 131 inhibited mGAT1 and mGAT3 without any activity at mGAT2 and mGAT4 (Table 1).

Table 1
Median inhibitory concentration values (IC_{50} in μM) obtained for the investigated compounds in [^{3}H]GABA uptake assay.

Compound	mGAT1 uptake	mGAT2 uptake	mGAT3 uptake	mGAT4 uptake	mGAT1 NO711 binding[a]
BM 130	21.88	33.88$^{n=1}$	NA	NA	10.96
BM 131	14.45	NA	45.71	NA	14.13
(S)-SNAP-5114[b]	>300	22.00	20.0	6.6	NT
Tiagabine[c]	0.8	>100	>100	800	NT

Inhibitory potency of the compounds tested at four murine GAT 1–4. Study performed as [^{3}H]GABA uptake assay based on stably transfected HEK cells (for methodological details see Kragler et al., 2008; Kulig et al., 2011). Results are shown as mean $IC_{50} \pm$ SEM. Number of samples (n) = 3 unless otherwise stated. IC_{50} means concentration resulting in 50% inhibition of GABA uptake.
NT: not tested.
[a] % of NO711 bound to GAT1 at 100 μM concentration of tested compound; NA: not active at concentrations below 100 μM.
[b] Data from Dalby (2000).
[c] Data from Clausen et al. (2006) and Soudijn and van Wijngaarden (2000).

The inhibition of mGAT subtypes is thought to enhance the GABAergic neurotransmission within the central nervous system and in the periphery and this effect is beneficial in the treatment of anxiety, epilepsy, pain and possibly depression. In view of this, it is expected that the test compounds will show anticonvulsant, anxiolytic-like and antinociceptive activities in mouse models of seizures, anxiety and pain. Our earlier unpublished studies, as well as the literature data indicated for the activity of GAT inhibitors in screening models of depression, so the antidepressant-like activity was also investigated. Since the GABAergic neurotransmission controls motor coordination and mediates ethanol sensitivity, the influence of the test compounds on locomotor activity, motor deficits and potentiation of ethanol effects was investigated, as well. Based on these results an attempt was made to establish a relationship between the inhibition of mGAT subtype and the effect observed *in vivo*.

2. Materials and methods

2.1. Animals

The behavioral experiments were carried out at the Department of Pharmacodynamics, Pharmaceutical Faculty, Jagiellonian University in Cracow. Adult male Albino Swiss (CD-1) mice weighing 18–25 g were used in the experiments. The animals were kept in groups of 15 mice in cages at a room temperature of 22 ± 2 °C, under light/dark (12:12) cycle and had free access to food and water. The ambient temperature of the room and the humidity were kept consistent throughout all the tests. For the experiments the animals were selected in a random way and killed by cervical dislocation immediately after

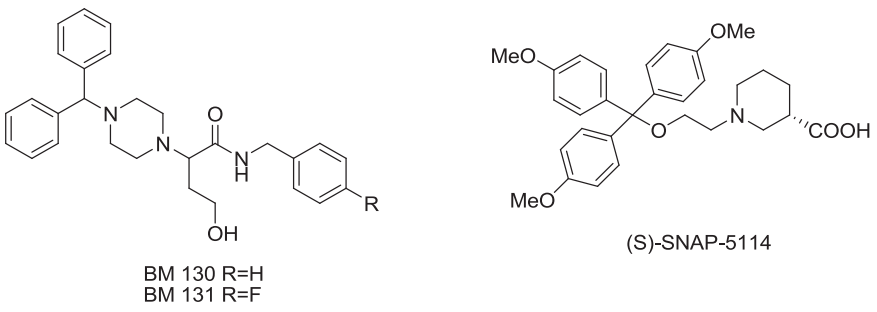

Fig. 1. Chemical structure of the test compounds: BM 130, BM 131 and (S)-SNAP-5114.