Bioorganic & Medicinal Chemistry Letters 18 (2008) 6290–6292

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Validation of high-affinity binding sites for succinic acid through distinguishable binding of gamma-hydroxybutyric acid receptor-specific NCS 382 antipodes

Tünde Molnár [a,*], Júlia Visy [b], Ágnes Simon [a], István Moldvai [c], Eszter Temesvári-Major [c], Gábor Dörnyei [c], Erzsébet Kútiné Fekete [a], Julianna Kardos [a]

[a] Department of Neurochemistry, Chemical Research Center, Hungarian Academy of Sciences, Budapest, Hungary
[b] Department of Molecular Pharmacology, Chemical Research Center, Hungarian Academy of Sciences, Budapest, Hungary
[c] Department of Natural Organic Compounds, Chemical Research Center, Hungarian Academy of Sciences, Budapest, Hungary

ARTICLE INFO

Article history:
Received 18 July 2008
Accepted 23 August 2008
Available online 28 August 2008

Keywords:
Succinic acid
Gamma-hydroxybutyric acid (GHB)
NCS 382

ABSTRACT

Gamma-hydroxybutyric acid (GHB) binding to multiple sites for the tricarboxylic acid cycle intermediate succinic acid (SUC) has been disclosed recently. In order to better characterize these targets, distinguishable binding of GHB receptor-specific NCS 382 antipodes to [^{3}H]-SUC or [^{3}H]-GHB labelled sites in rat brain synaptic membranes was explored. Eutomer binding parameters suggest identity of the high-affinity target for SUC with a synaptic GHB receptor subtype.

Succinic acid (SUC), the intermediary metabolite of the tricarboxylic acid (TCA) cycle has a central role in the control of cellular energy supply.[1] In brain cells, oxidative metabolism of the major inhibitory neurotransmitter gamma-aminobutyric acid (GABA) is directly coupled to the TCA cycle through SUC formed from gamma-hydroxybutyric acid (GHB).[2–4] Binding interaction between SUC and [^{3}H]-GHB-labelled sites has been disclosed in synaptic membranes isolated from rat forebrain and human basal ganglia areas.[5] Moreover, GHB displaced [^{3}H]-SUC specifically bound to high- and low-affinity synaptic binding sites.[6] These findings indicate that in addition to cellular energy supply, SUC may also control synaptic activity.[6] The remarkable similarities of SUC-sensitive [^{3}H]-GHB and GHB-sensitive [^{3}H]-SUC ligand binding profiles[5,6] suggest that SUC and GHB can compete for the same synaptic recognition sites. Identity of sites, however, is called into question by the preferred binding of the GHB receptor-specific ligand 6,7,8,9-tetrahydro-5-hydroxy-5*H*-benzocyclohept-6-ylidene-acetic acid ((*rac*)-NCS 382) to the high-affinity SUC binding site.[6]

The aim of the present study was to qualify putative interaction of SUC with synaptic GHB receptor sites by comparing enantioselectivity profiles of [^{3}H]-SUC or [^{3}H]-GHB binding in rat forebrain synaptic membranes. To this end, new and known chiral compounds have been assayed for [^{3}H]-SUC or [^{3}H]-GHB binding. Design and synthesis of new chiral sets of 4-hydroxypiperidine-2-carboxylic acid derivatives (Fig. 1A) were based on the close structural similarity of (2*R*,4*R*)-4-hydroxypiperidine-2-carboxylic acid to the high-affinity GHB analogue (*R*)-3-hydroxycyclohex-1-ene-carboxylic acid ((*R*)-HOCHCA[7]; Fig. 1B). The (*R*) and (*S*) antipodes of (*rac*)-NCS 382 were prepared by a novel chiral HPLC separation method.

(*R*)-HOCHCA has been reported to have much higher binding affinity to GHB binding sites present in rat cortex preparations than GHB[7], indicating that with its OH and the COOH groups locked in a ring, its structure closely mimics the bioactive conformation of the endogenous ligand. In addition to the matching structural prerequisites, the novel 4-hydroxypiperidine-2-carboxylic acid derivatives **1–8** (Fig. 1A; *c.f.* Supplementary Scheme 1) contain two chiral centres providing chiral sets of possible GHB analogues. The derivatives have been synthesised by a combined procedure of Bio-Méga and Ciba groups.[8,9] Details of synthesis and purification steps are given as Supplementary information. To explore possible enantioselectivity profiles of GHB and SUC sites present in synaptic membranes[5,6], binding of chiral 4-hydroxypiperidine-2-carboxylic acid derivatives **1–8** to sites labelled by [^{3}H]-GHB (49.5 Ci/mM[5]) or [^{3}H]-SUC (40 Ci/mM; Sigma-Aldrich, Budapest, Hungary) have been performed.[5,6] Surprisingly, among the (4*S*) isomers, (2*R*,4*S*)-4-hydroxypiperidine-2-carboxylic acid (**6**) indicated some low binding affinity to SUC and GHB binding sites (26 ± 4% and 30 ± 14% inhibition at 100 μM, respectively). Other derivatives, structurally related to (4*R*) isomers, were all devoid of significant displacing activity ($p < 0.05$) (Supplementary, Table 1). Even

* Corresponding author. Tel.: +36 1 438 1167; fax: +36 1 438 1143.
E-mail address: tmolnar@chemres.hu (T. Molnár).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.08.083

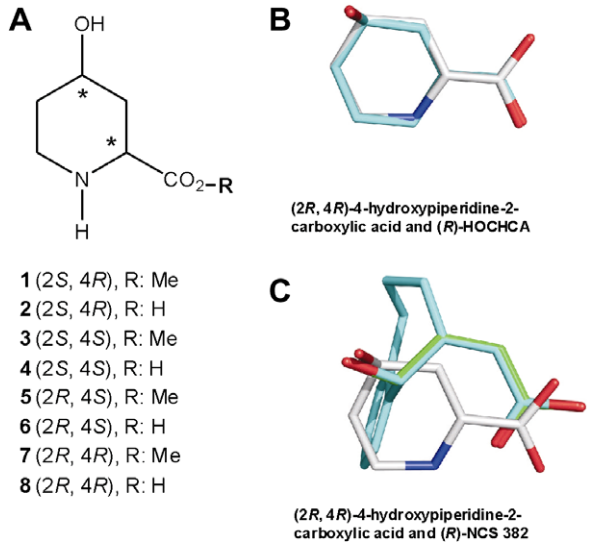

Figure 1. Structural analysis of 4-hydroxypiperidine-2-carboxylic acid derivatives. (A) Chemical structures of 4-hydroxypiperidine-2-carboxylic acid derivatives **1–8**. (B) (2*R*,4*R*)-4-hydroxypiperidine-2-carboxylic acid (grey carbon atoms) fitted on (*R*)-HOCHCA (cyan carbon atoms) using Surflex-Sim, in which a score value greater than 0.70 is generally significant in terms of similarity and indicates a functional relationship of the molecules. (C) Relative orientation of (2*R*,4*R*)-4-hydroxypiperidine-2-carboxylic acid (grey carbon atoms) and (*R*)-NCS 382 (cyan carbon atoms), after simultaneously fitting their COOH and OH groups using PyMOL 0.99 (DeLano Scientific LLC, http://www.pymol.org). Carbon atoms of the GHB moiety of (*R*)-NCS 382 are highlighted in light green. Oxygen and nitrogen atoms are coloured according to atom type.

though, the structure of (2*R*,4*R*)-4-hydroxypiperidine-2-carboxylic acid (**8**) is strikingly similar to (*R*)-HOCHCA, as indicated by a high similarity score (0.92, Surflex-Sim program[10]; Fig. 1B), these scaffolds did not prove to be efficient GHB analogues. The lack of activity may be attributed to the fact, that GHB adopts a different conformation in (2*R*,4*S*)-4-hydroxypiperidine-2-carboxylic acid derivatives and in (*R*)-NCS 382, illustrated by fitting their functional OH and COOH groups simultaneously (Fig. 1C).

In order to compare enantioselectivity profiles of SUC and GHB recognition sites, binding of HPLC separated enantiomers of (*rac*)-NCS 382 (Tocris, Bristol, UK) to synaptic sites labelled by [^{3}H]-SUC or [^{3}H]-GHB have been performed as described above. The chiral HPLC separation was performed with a system composed of a Jasco PU-980 pump, a Rheodyne 7125 injector (20 μl loop), a Jasco MD 2010 Plus UV/VIS photodiode-array detector and a ChromPass chromatographic software. Successful separation of NCS 382 enantiomers was performed on a Chiracel OJ column (250 × 4.6 mm I.D. Daicel Chemical Ind. Tokyo, Japan). The mobile phase was 85% *n*-hexane, 15% 2-propanol and 0.1% trifluoroacetic acid, flow rate was 1 mL/min. The two fractions were baseline separated having retention times of 10.1 and 12.7 min. The products of twenty runs were combined; enantiomeric purities (% ee ⩾ 97.5) were checked by the same HPLC method.

(*R*)- and the (*S*)-NCS 382 partially displaced [^{3}H]-SUC from its synaptic binding sites with IC_{50} values of 0.4 ± 0.1 μM and 74 ± 9 μM, respectively (Fig. 2A) and (*R*)-NCS 382 showed approx. 200-fold higher binding affinity to [^{3}H]-SUC labelled synaptic sites than (*S*)-NCS 382. Neither the (*R*)-NCS 382 nor the (*S*)-NCS 382 antipodes displaced specifically bound [^{3}H]-SUC completely (Fig. 2A). As a proof of the evidence, "mixed" (*rac*)-NCS 382 obtained from the resolved (*R*)- and (*S*)-NCS 382 antipodes was assayed for [^{3}H]-SUC binding as well. Maximal displacements exerted by the "mixed" (*rac*)-NCS 382 and the (*R*)-NCS 382 antipode displayed saturation approximately at the same level (17 ± 1% and 26 ± 1%, respectively; Fig. 2A). In addition, half-saturation values for the "mixed" (*rac*)-NCS 382 and (*R*)-NCS 382 were similar ($IC_{50,RS}$ = 0.5 ± 0.1 μM vs $IC_{50,R}$ = 0.4 ± 0.1 μM; Fig. 2A). These findings are consistent with our previous results[5] obtained with unresolved (*rac*)-NCS 382, and suggest that identity of the high-affinity SUC binding sites with the (*R*)-NCS 382-selective recognition site of the synaptic GHB receptor. Furthermore, we may hypothesize the existence of an NCS 382-insensitive low-affinity binding site for SUC. The existence of the presumed (*R*)-NCS 382-insensitive low-affinity SUC binding site has been challenged in measurements of [^{3}H]-GHB binding in rat forebrain synaptic membranes. Similarly to [^{3}H]-SUC binding, none of the (*R*)-NCS 382 or (*S*)-NCS 382 antipodes could totally displace [^{3}H]-GHB from its specific synaptic binding sites ($IC_{50,R}$ = 0.8 ± 0.3 μM and $IC_{50,S}$ = 93 ± 18 μM; Fig. 2B). The (*R*)-NCS 382 antipode showed approx. 115-fold higher binding affinity to [^{3}H]-GHB labelled sites than the (*S*)-NCS 382 antipode (Fig. 2B). The higher binding selectivity obtained with the (*R*) and (*S*) enantiomers (this study) compared to previously reported data[11] (*c.f.* $IC_{50,R}$ = 0.2 ± 0.01 μM vs $IC_{50,S}$ = 12.9 ± 2.3 μM) may be due to the higher homogeneity of purified synaptic membrane preparation that had been used. In contrast to the [^{3}H]-SUC binding, however, when we used "mixed" (*rac*)-NCS 382, we got total displacement of specifically bound [^{3}H]-GHB with an IC_{50} value (0.9 ± 0.2 μM; Fig. 2B) similar to our previously reported (*rac*)-NCS 382 data (*c.f.* K_i = 1.2 ± 0.2 μM[4]).

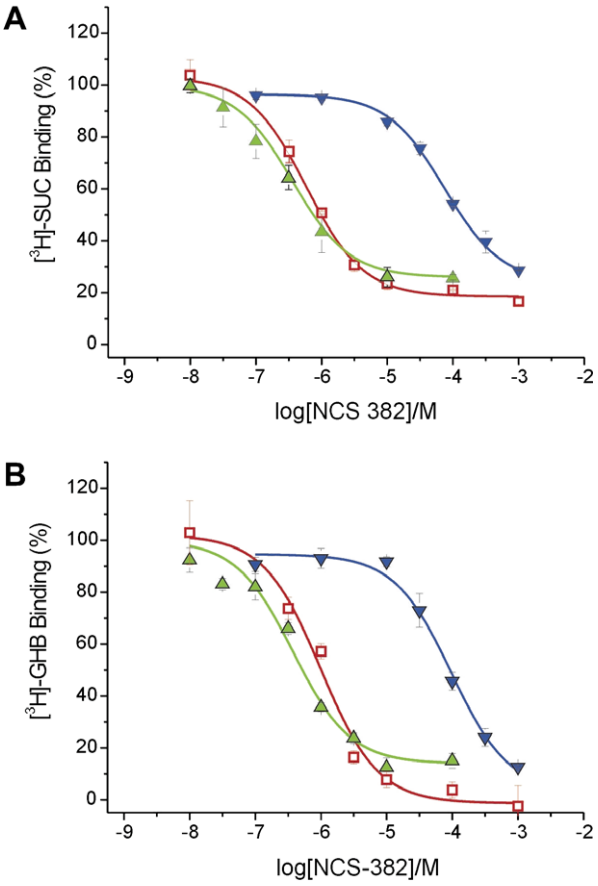

Figure 2. Displacement of specifically bound [^{3}H]-SUC (A) and [^{3}H]-GHB (B) by NCS-382 antipodes in rat forebrain synaptic membrane fractions. Symbols: (*R*)-NCS 382 (green, ▲); (*S*)-NCS 382 (blue, ▼); "mixed" (*rac*)-NCS 382 (red, □). To define non-specific binding 30 mM succinate or 1 mM GHB was used. Data are presented as means ± SD obtained from experiments with 3 different rat forebrain preparations (N = 3) performed in duplicate measurements. Displacement curves were fitted by one-site competition approximation (OriginPro 7.5).

It follows, that there may be two synaptic GHB receptor subtypes, distinguishable by NCS 382 antipodes of high enantiomeric purity, showing opposite enantioselectivity. Similar enantioselec-

tivity profiles, disclosing the (*R*)-NCS 382 antipode as the high-affinity eutomer indicate the identity of the high-affinity SUC binding site with the GHB receptor recognition site. In conclusion, these results together with our previous findings[5,6] suggest that the target of the high-affinity SUC binding may be a synaptic GHB receptor subtype.

Acknowledgments

Edit Simon-Trompler (Group for Radiosyntheses, Institute of Biomolecular Chemistry, Chemical Research Center) for the synthesis of the radioligand [^{3}H]-GHB and the financial support of grants MediChem2 (1/A/005/2004 NKFP), OTKA (T049721) and Transporter Explorer (GVOP 3.1.1-2004-05-00) are acknowledged.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.08.083.

References and notes

1. Krebs, H. A. *Annu. Rev. Biochem.* **1950**, *19*, 409.
2. Buzzi, A.; Wu, Y.; Frantseva, M. V.; Perez Velazquez, J. L.; Cortez, M. A.; Liu, C. C.; Shen, L. Q.; Gibson, K. M.; Snead, O. C. *Brain. Res.* **2006**, *23*, 15.
3. Gupta, M.; Greven, R.; Jansen, E. E.; Jakobs, C.; Hogema, B. M.; Froestl, W.; Snead, O. C.; Bartels, H.; Grompe, M.; Gibson, K. M. *J. Pharmacol. Exp. Ther* **2002**, *302*, 180.
4. Schousboe, A.; Waagepetersen, H. S. *Adv. Mol. Cell Biol* **2004**, *31*, 461.
5. Molnár, T.; Fekete, E. K.; Kardos, J.; Simon-Trompler, E.; Palkovits, M.; Emri, Zs. *J. Neurosci. Res.* **2006**, *84*, 27.
6. Molnár, T.; Barabás, P.; Héja, L.; Fekete, E. K.; Lasztóczi, B.; Szabó, P.; Nyitrai, G.; Simon-Trompler, E.; Hajós, F.; Palkovits, M.; Kardos, J. *J. Neurosci. Res* **2008**, *86*, 1566.
7. Wellendorph, P.; Høg, S.; Greenwood, J. R.; de Lichtenberg, A.; Nielsen, B.; Frølund, B.; Brehm, L.; Clausen, R. P.; Bräuner-Osborne, H. *J. Pharmacol. Exp. Ther.* **2005**, *315*, 346.
8. Gillard, J.; Abraham, A.; Anderson, P. C.; Beaulieu, L.; Bogri, T.; Bousquet, Y.; Grenier, L.; Guse, I.; Lavallée, P. *J. Org. Chem.* **1996**, *61*, 2226.
9. Skiles, J. W.; Giannousis, P. P.; Fales, K. R. *Bioorg. Med. Chem. Letters* **1996**, *6*, 963.
10. Cleves, A. E.; Jain, A. N. *J. Med. Chem.* **2006**, *49*, 2921.
11. Castelli, M. P.; Mocci, I.; Pistis, M.; Peis, M.; Berta, D.; Gelain, A.; Gessa, G. L.; Cignarella, G. *Eur. J. Pharmacol.* **2002**, *446*, 1.