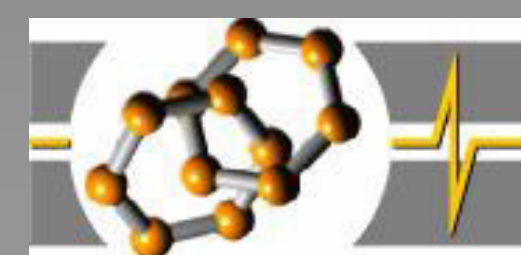


Succinate-sensitive and GABA_BR-independent gamma-hydroxybutyrate binding sites in brain synaptic membranes

Tünde Molnár¹, Erzsébet Kútiné Fekete¹, Julianna Kardos¹, Edit Simon-Trompler², Miklós Palkovits³, Zsuzsa Emri¹

¹Department of Neurochemistry and ²Group for Radiosyntheses, Institute of Biomolecular Chemistry, Chemical Research Center, HAS, ³Laboratory of Neuromorphology, Semmelweis University and HAS



contact person: Tünde Molnár, tmolnar@chemres.hu

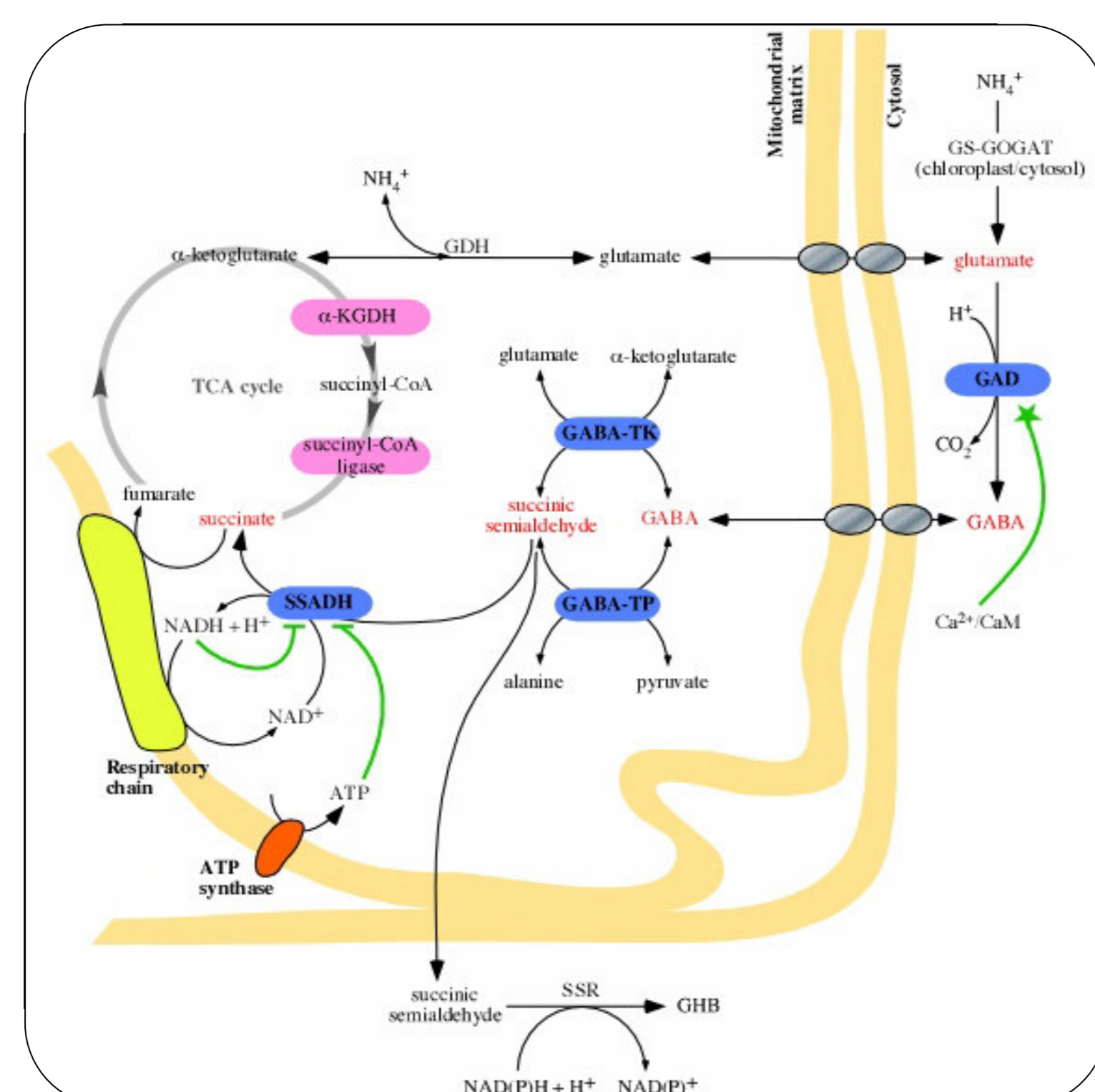


Introduction

Gamma-hydroxybutyrate (GHB) is a naturally occurring metabolite of γ -aminobutyric acid (GABA) in brain. It is produced from glutamate and metabolise via succinate (Schouboe and Waagepetersen, 2004). GHB administration causes a number of pharmacological and physiological actions including slow-wave sleep, absence epilepsy and coma (Wong et al. 2003). Moreover, GHB becomes popular as an illicit „club drug” (Wong et al. 2004).

The *nucleus accumbens* (NA) is responsible for the development of reward properties of different drugs. The *globus pallidus* (GP) is the motoric effector area of the NA (Mogenson et al., 1983). In many brain areas GHB acts on its own receptor and also binds to GABA_B receptor (Mathivet et al., 1997).

A synaptic receptor for γ -hydroxybutyrate interacting succinate has been disclosed in purified rat forebrain, human NA and GP subcellular fractions.



Methods

Subcellular fractionation of rat forebrain and human tissue samples: Rats were kept and used in accordance with the European Council Directive of 24 November 1986 (86/609/EEC) and with the Hungarian Animal Act, 1998 and associated local guidelines. Human brain tissue samples were obtained from the Human Brain Tissue Bank, Budapest. All of the procedures were approved by the Regional Committee of Science and Research Ethics at Semmelweis University, Budapest. Purified synaptosomal and synaptic membrane sub-cellular fractions have been prepared as described (Molnár et al., 2006).

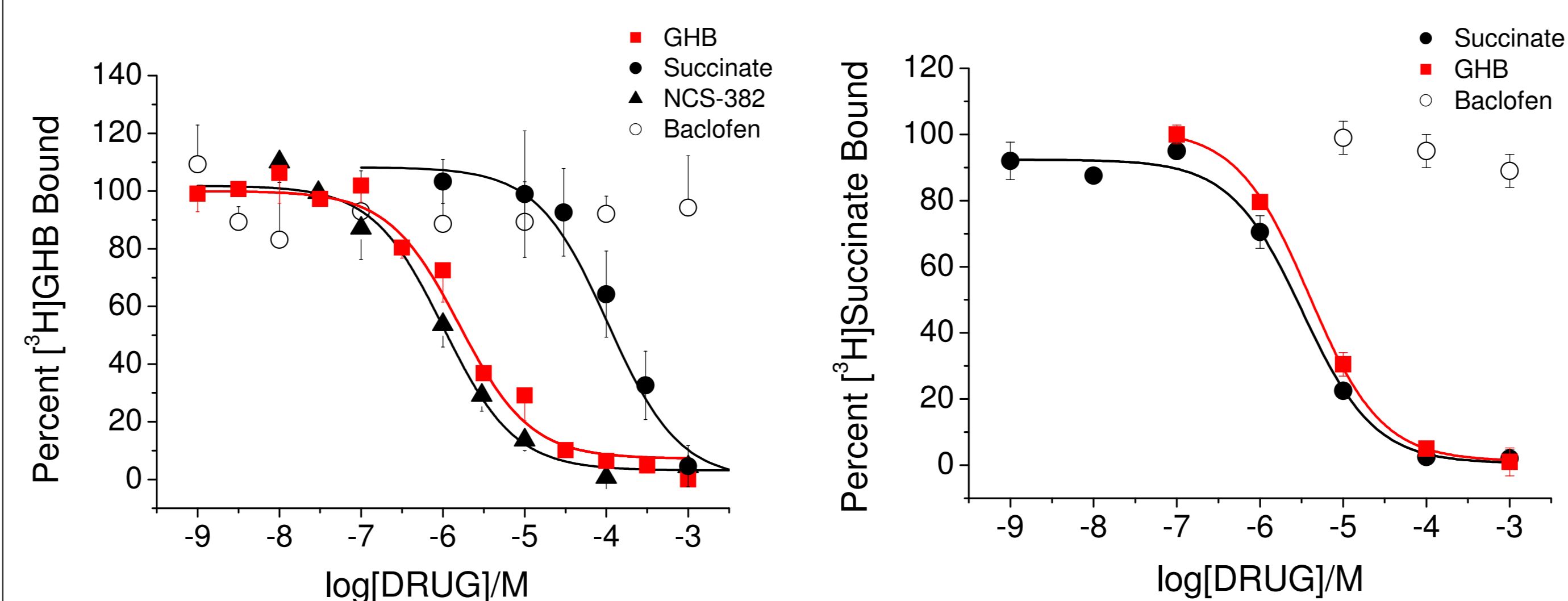
Preparation of human NA and GP tissue samples: Human brains were taken from persons died without any known neurodegenerative diseases. Brains were removed from the skull after 2-6 h post mortem delay, sliced, frozen and kept on -70°C until microdissection. The micropunch technique was applied to dissect NA and GP samples from the coronal brain sections (Palkovits, 1973). To isolate synaptic membrane fraction, deep-frozen (-80°C) samples were thawed and subjected to the steps described as above.

[³H]GHB and [³H]Succinate binding to synaptic membrane fraction have been performed as described previously (Molnár et al., 2006).

Data represent means of 3-8 experiments performed in duplicate measurements for each incubation.

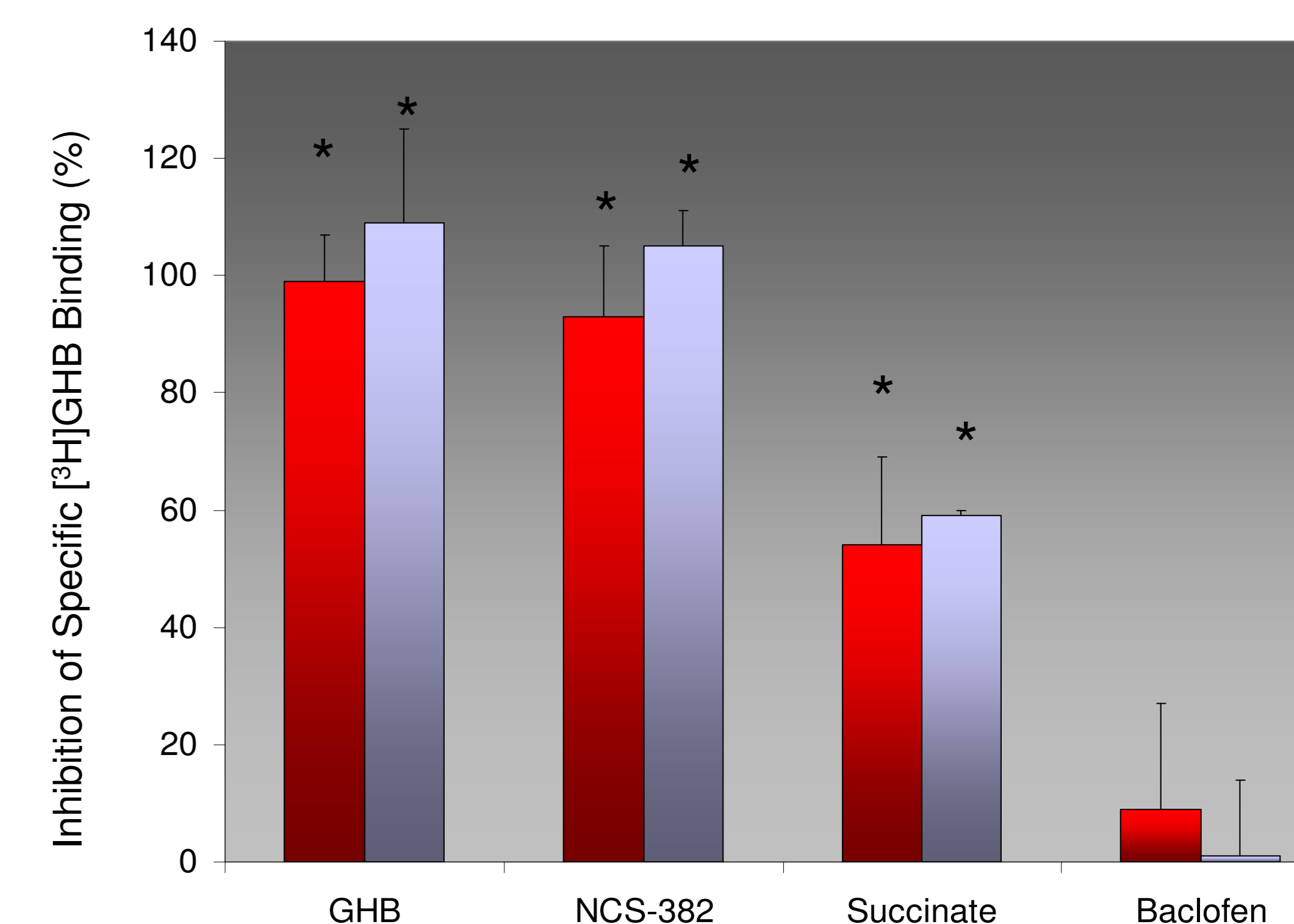
Results

Rat forebrain



Comparison of [³H]GHB (left) and [³H]Succinate (right) binding in rat synaptic membrane fraction isolated from rat brain tissue containing cortex, striatum and NA. Inhibition constants for [³H]GHB displacements by GHB, NCS-382 and succinate were $K_{i,GHB}=2.5\pm 0.3 \mu\text{M}$, $K_{i,NCS-382}=1.2\pm 0.2 \mu\text{M}$, $K_{i,succinate}=212\pm 66 \mu\text{M}$, respectively. GABA_BR agonist baclofen was ineffective ($K_{i,baclofen}>1 \text{ mM}$). Inhibition constants for [³H]Succinate displacements were $K_{i,GHB}=2.1\pm 1.3 \mu\text{M}$, $K_{i,succinate}=2.9\pm 0.9 \mu\text{M}$ and $K_{i,baclofen}>1 \text{ mM}$. Data are presented as means \pm SD (N=3). Data fitted one-site approximation (OriginPro7.0). The inhibition constants (K_i) were calculated according to the equation $K_i=IC_{50}/[1+(c^*)/K_D]$ where IC_{50} is the half-saturation concentration of the inhibitor and c^* is the concentration of the radioligand (10 nM).

Human NA and GP



Pharmacological characterisation of GHB binding sites in human NA (red) and GP (blue) samples. Effects of 100 μM GHB, NCS-382, succinate and baclofen on [³H]GHB binding are presented. Data are expressed as percent inhibition of specific binding and shown as means \pm SD from N=3 experiments. *P> 0,05

Conclusions

- Existence of a synaptically localized succinate-sensitive GHB binding site different from GABA_B receptor in rat forebrain membrane has been disclosed.
- The synaptic GHB binding site is present in human basal ganglia areas *nucleus accumbens* and *globus pallidus*.
- We suggest the presence of a GHB receptor within the synapse that binds succinate.

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