

Proton activates synaptic membrane site for the intermediary metabolite succinate

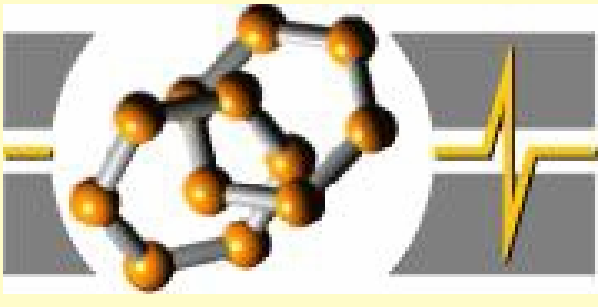
Tünde Molnár¹, Péter Barabás¹, László Héja¹, Erzsébet Kútiné Fekete¹, Edit Simon-Trompler², Bálint Lasztóczy¹, Miklós Palkovits³ and Julianna Kardos¹

¹Department of Neurochemistry and ²Group for Radiosyntheses, Institute of Biomolecular Chemistry, Chemical Research Center, Hungarian Academy of Sciences

³Neuromorphological and Neuroendocrine Research Laboratory, Hungarian Academy of Sciences and Semmelweis University Budapest, Hungary



Contact person: Tünde Molnár; tmolnar@chemres.hu



Introduction

In all living cells, C₄-dicarboxylic acid derivative succinate (SUC) is essential in respiration. The representative value of succinate in human plasma is 40 microM (Krebs, 1950). It is perhaps not surprising that, reflecting the presence of pyruvate carboxylase, astrocytes but not neurons release appreciable amounts of TCA cycle intermediates including succinate (Westergaard et al., 1994). A synaptic receptor for gamma-hydroxybutyric acid (GHB) interacting succinate has been disclosed in purified rat forebrain synaptic and human *nucleus accumbens* (NA) subcellular fractions, but the molecular identity of this recognition site was not determined (Molnár et al., 2006). These lines of evidence raised the hypothesis on the possible identity of succinate-sensitive GHB receptor and synaptic recognition site for succinate. The purpose of the present study was to identify the possible synaptic recognition site for succinate, and to differentiate binding and transport interactions.

Methods

Preparation of human NA tissue samples: Human brains were taken from persons died without any known neurodegenerative diseases. Brains were removed from the skull after 1-6 h post mortem delay, sliced, frozen and kept on -70°C until microdissection. The micropunch technique was applied to dissect NA samples from the coronal brain sections (Palkovits, 1973).

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

[³H]Succinate uptake in synaptosomal fraction was performed as [³H]GABA uptake (Héja et al., 2004). The incubation solution contained 100 nM (10 nM labeled + 90 nM unlabeled [³H]Succinate). Data are expressed as means ± SD and were analyzed using one-way analysis of variances (ANOVA, OriginPro 7.5).

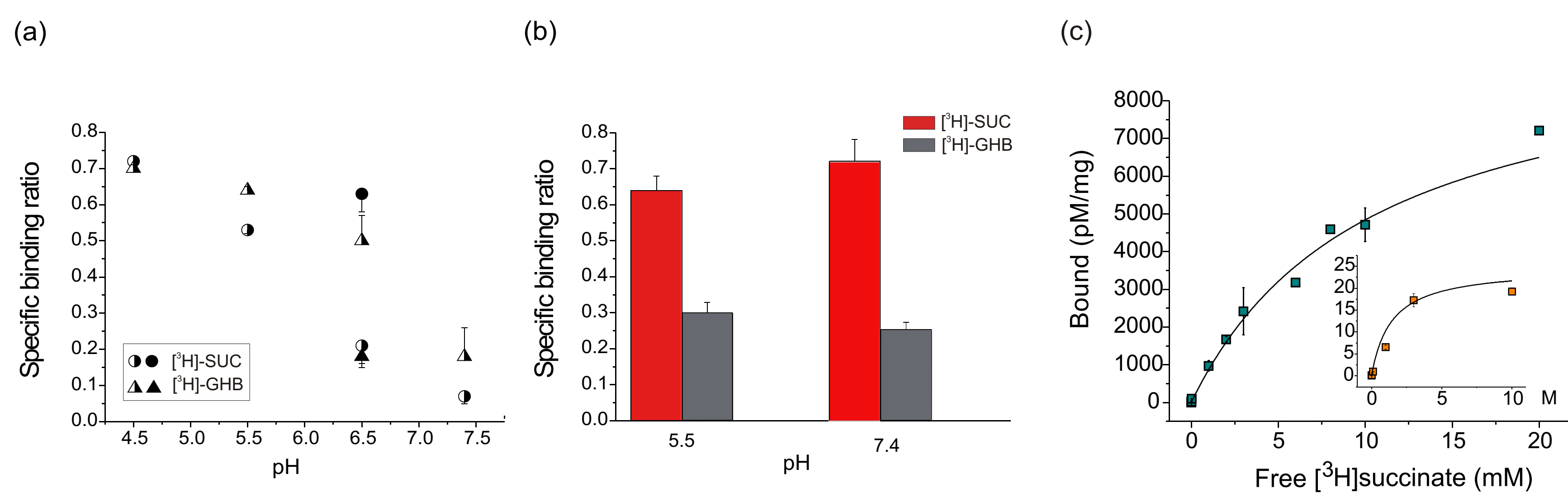
For [¹⁴C]Succinate release experiments rat NA punches were loaded with 200 microM [¹⁴C]Succinate for 30 min at 37 °C. All other conditions and materials were used as described previously at Barabás et al., 2002. To avoid metabolism of [¹⁴C]Succinate, 1 mM 3-nitropropionic acid was present throughout the release experiment. Fractional release data from individual punches were normalized to a first order exponential curve obtained by fitting the pre-stimulus and the last 3 washout values (OriginPro 7.5).

References

Barabás et al. (2002) Light induced changes in glutamate release from isolated rat retina is regulated by cGMP. *J. Neurosci. Res.* 67: 149-155.
Héja et al., (2004) Novel secoergoline derivatives inhibit both GABA and glutamate uptake in rat brain homogenates: synthesis, in vitro pharmacology and modeling. *J. Med. Chem.* 47:5620-5629.
Krebs (1950) Chemical composition of blood plasma and serum. *Annu. Rev. Biochem.* 19: 409-430.
Molnár et al. (2006) Metabolic GHB precursor succinate binds to gamma-Hydroxybutyrate receptors: Characterization of human basal ganglia areas nucleus accumbens and globus pallidus. *J. Neurosci Res* 84: 27-36.
Palkovits (1973) Isolated removal of hypothalamic or other brain nuclei of the rat. *Brain Res* 59: 449-450.
Westergaard et al., (1994) Release of alpha-ketoglutarate, malate and succinate from cultured astrocytes: possible role in amino acid neurotransmitter homeostasis. *Neurosci. Lett.* 176: 105-109.

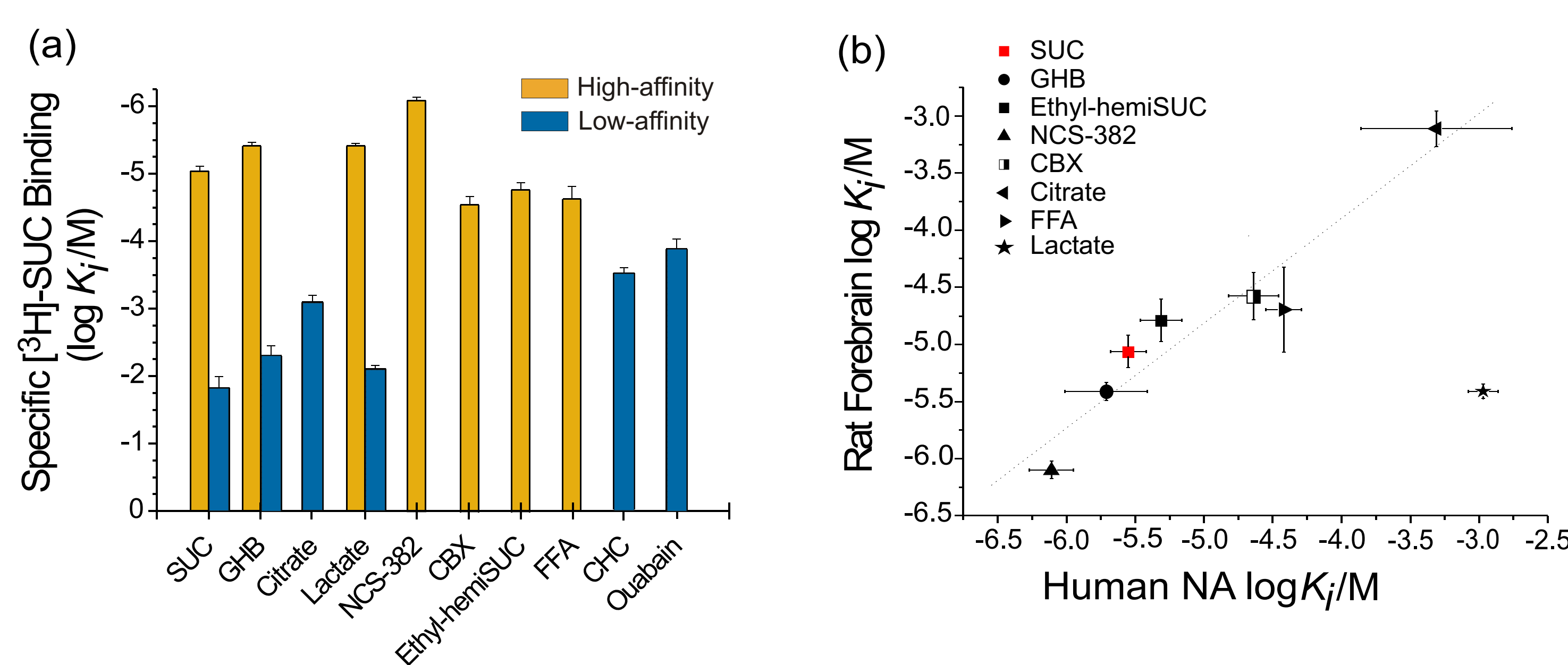
Results

Characterization of [³H]-SUC binding to rat forebrain synaptic membrane fractions



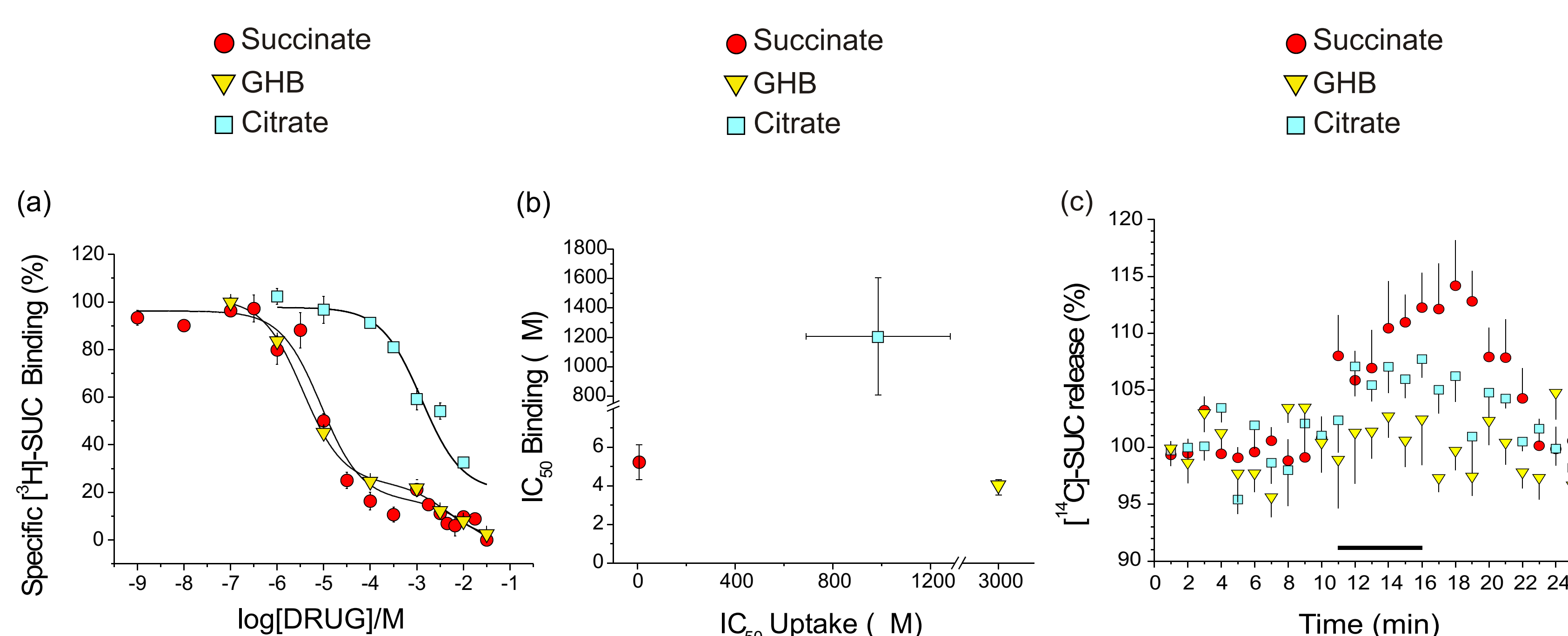
a) Effects of pH_i on the specific binding ratio (1-NS/T) of the total (T) of 10 nM [³H]-SUC (circle) or [³H]-GHB (triangle) binding in different sub-cellular fractions isolated from rat forebrain. Half-filled symbols: synaptic plasma membrane vesicles; filled symbols: synaptic plasma membrane vesicles enriched in junctional complexes. b) Effects of pH_i on specific binding ratio of [³H]-SUC and [³H]-GHB binding to rat forebrain mitochondrial fractions. c) Saturation of [³H]-SUC binding sites in rat forebrain synaptic membrane fractions at pH 5.5, 4°C. Orange symbols: high-affinity binding site (K_d = 1.4 ± 0.6 microM, B_{max} = 25 ± 3 pM/mg protein); blue symbols: low-affinity binding site (K_d = 10.4 ± 0.6 mM, B_{max} = 9.9 ± 1.9 nM/mg protein). Data are presented as means ± SD obtained from duplicate measurements. N=3.

Pharmacological characterization of [³H]-SUC binding sites in rat forebrain and human NA synaptic membrane fractions



a) Ligand binding profile of specific [³H]-SUC binding (10 nM) in synaptic membrane sub-cellular fraction isolated from the rat forebrain at pH 5.5. The equilibrium inhibition constants (K_i) values were calculated by fitting one-, or two-site approximations (OriginPro 7.5) of displacement data. The K_i values of succinate, GHB, the GHB receptor antagonist NCS-382, ethyl-hemisuccinate, lactate and citrate, the gap-junction blocker carbenoxolone (CBX), flufenamic acid (FFA), the MCT1 blocker alpha-cyano-hydroxycinnamic acid (CHC) and the Na⁺/K⁺ ATPase blocker ouabain are shown. b) Comparison of inhibitory constants (K_i) obtained from the displacement of [³H]-SUC specifically bound in synaptic membrane fractions of the rat forebrain or the human *nucleus accumbens* at pH 5.5. Data represents averages obtained from duplicate measurements of binding repeated in 2-8 experiments using different preparations. Data are expressed as mean ± SD. Correlational linear was fitted using OriginPro 7.5. R = 0.96 ± 0.03.

Comparison of the effects of carboxylic acid intermediates on the binding, uptake and release processes of SUC



(a) Specific [³H]-SUC binding in rat forebrain synaptic membrane sub-fractions at pH 5.5, 4°C. (b) Specific binding (pH 5.5, 4°C) vs. uptake (pH 7.4, 4°C) of [³H]-SUC in rat forebrain synaptic membrane and synaptosomal sub-fractions, respectively. Duplicate measurements of binding and uptake were repeated in three experiments using different preparations. Inhibitory constants (K_i) derived are expressed as means ± SD. (c) [¹⁴C]-SUC release from rat *nucleus accumbens* punches superfused with artificial cerebrospinal fluid (ACSF) containing 1 mM 3-nitropropionic acid. Application of 2 mM SUC (N=6) or citrate (N=6) or GHB (N=5) is indicated by the horizontal bar. Symbols and error bars indicate means ± SD of the normalized fractional release data obtained in measurements of [¹⁴C]-SUC release from *nucleus accumbens* punches isolated from 3-5 rats.

Conclusion

Existence of an external pH-dependent, synaptically localized high-, and low-affinity binding sites for the intermediary metabolite succinate has been shown in human *nucleus accumbens* and rat forebrain. These succinate targets probably differ from the synaptic or mitochondrial succinate transporters. Ligand-binding profiles suggest the existence of two distinguishable binding processes. The high- and the low-affinity sites may possibly be linked to different synaptic membrane targets, i.e. some sort of connexin (hemi)channel and proton-dependent MCT, respectively.

Acknowledgement

Financial support of grants Albert Apponyi (MEC_07 NKTH-KPI), MediChem2 (1/A/005/2004 NKFP) And for Transporter Explorer (GVOP 3.1.1-2004-05-00) are acknowledged.