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Short communication

γ-Hydroxybutyric acid and baclofen decrease extracellular acetylcholine levels in the hippocampus via $GABA_B$ receptors ☆

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Abstract

The effect of γ-hydroxybutyric acid (GHB) and baclofen, a $GABA_B$ receptor agonist, on extracellular hippocampal acetylcholine levels was studied in freely moving rats by microdialysis. GHB (200 and 500 mg/kg, i.p.) reduced in a dose-dependent manner, extracellular hippocampal acetylcholine concentrations and this effect was prevented by the $GABA_B$ receptor antagonist (2*S*)(+)-5,5-Dimethyl-2-morpholineacetic acid (SCH 50911), at the dose of 20 mg/kg (i.p.), while the putative GHB receptor antagonist 6,7,8,9-Tetrahydro-5-hydroxy-5*H*-benzocyclohept-6-ylideneacetic acid (NCS 382) was ineffective. Similar to GHB, the $GABA_B$ agonist baclofen (10 and 20 mg/kg, i.p.) produced a dose-related reduction in extracellular acetylcholine concentrations which was prevented by SCH 50911. These findings indicate that GHB-induced reduction of hippocampal acetylcholine release is mediated by $GABA_B$ receptors and support a possible involvement of hippocampal $GABA_B$ receptors in the control of cognitive processes and in the claimed amnesic effect of GHB intoxication. © 2001 Published by Elsevier Science B.V.

Keywords: Microdialysis; Baclofen; GHB (γ-hydroxybutyric acid); SCH 50911; Acetylcholine; Hippocampus; $GABA_B$ receptor

1. Introduction

γ-Hydroxybutyric acid (GHB) is a naturally occurring metabolite of γ-aminobutyric acid (GABA) which, when given to animals, produces a number of pharmacological effects including sedation, sleep and anaesthesia (Maitre, 1997). Animal studies have shown that GHB is self-administered in mice (Martellotta et al., 1998) and rats (Colombo et al., 1995) and possesses rewarding properties (Martellotta et al., 1997). GHB has been clinically used as hypnotic and anaesthetic agent (Laborit et al., 1962) and in the treatment of narcolepsy (Lammers et al., 1993). More recently, GHB has been proposed in the pharmacotherapy of alcoholism (Gallimberti et al., 1989, 1992) and opiate addiction (Gallimberti et al., 1993). Moreover, several papers and warning reports by U.S. agencies (National Institute of Drug Abuse) indicate that GHB is largely abused by humans and that GHB intoxication is associated with amnesia (Galloway et al., 1997; Leshner, 1999).

GHB is considered to act on specific binding sites present in the brain, densely localized especially in the hippocampus (Hechler et al., 1989). However, at high concentrations, GHB also acts as a weak agonist for $GABA_B$ receptors (Lingenboehl et al., 1999). Indeed, GHB and the prototype $GABA_B$ receptor agonist baclofen share several pharmacological actions including cognitive deficits (McNamara and Skelton, 1996; Stackman and Walsh, 1994). Moreover, drug discrimination studies have shown that baclofen elicits discriminative stimulus effects similar to those produced by GHB (Colombo et al., 1998).

Since acetylcholine is one of the most important neurotransmitters involved in the control of the cognitive processes in the hippocampus (Vizi and Kiss, 1998), we studied whether GHB may affect hippocampal acetylcholine release and whether this effect is mimicked by baclofen and antagonized by the $GABA_B$ receptor antagonist (2*S*)(+)-5,5-Dimethyl-2-morpholineacetic acid (SCH 50911).

2. Materials and methods

Male Sprague–Dawley rats (225–250 g, Charles River, Calco, Lecco, Italy) were housed in groups of three per

☆ Experiments were carried out in accordance with the recommendations from the declaration of Helsinki and the internationally accepted principles in the care and use of experimental animals.

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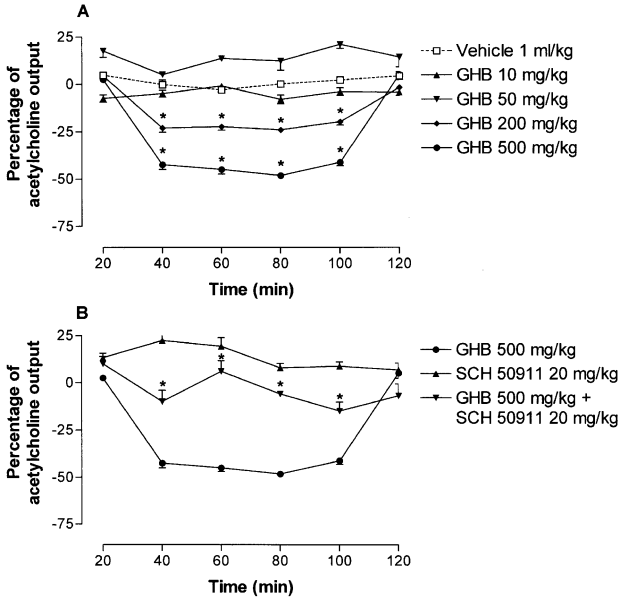

Fig. 1. Reduction of hippocampal extracellular acetylcholine concentrations induced by γ-hydroxybutyric acid (GHB) (A) and antagonism by SCH 50911 (B). $^{*}P < 0.05$ vs. controls (Student–Newman–Keuls test). Data are expressed as percentage (mean ± S.E.M.; $n = 5$) of the baseline concentrations. Basal values of extracellular acetylcholine concentrations, prior to drug administration, were: 1.35 ± 0.23 fmol/μl for control group, 1.25 ± 0.34, 1.45 ± 0.18, 1.18 ± 0.22 and 1.33 ± 0.23 fmol/μl for the groups treated with GHB at the doses of 10, 50, 200 and 500 mg/kg, respectively, and 1.42 ± 0.21 and 1.35 ± 0.16 fmol/μl for the groups treated with SCH 50911 and GHB + SCH 50911, respectively. SCH 50911 was given 20 min before GHB treatment.

cage for at least 10 days before use. Food and water were freely available and animals were maintained under an artificial 12/12-h light/dark cycle (lights were on from 07:00 a.m. to 07:00 p.m.). Experiments were carried out between 08:00 a.m. and 05:00 p.m. Rats were anaesthetised with Equithesin (4 mg/kg, i.p.) and dialysis tubes (AN 69-HF, with a wet fiber outer diameter of 320 μm; Hospal-Dasco, Bologna, Italy) were implanted transversally at the level of the hippocampi ($A = -3.2$ from the bregma and $V = -3.6$ from the skull), according to the atlas by Paxinos and Watson (1986). The localization of the dialysis probe was verified hystologically at the end of the experiments. All biochemical determination started 24 h after surgery. The Ringer solution containing 3 mM KCl, 125 mM NaCl, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, 23 mM $NaHCO_3$, 1.5 mM potassium phosphate buffer (pH 7.3), and 0.1 mM neostigmine bromide was pumped through the dialysis probe at a constant rate of 2 μl/min. Samples were collected every 20 min, corresponding to a volume of 40 μl, and were injected in a high-performance liquid chromatography (HPLC) with electrochemical detection according to the techniques described by Damsma and Westerink (1991). The detection limit for acetylcholine was 0.05 fmol/1 μl of sample. Samples were collected every 20 min (40 μl) and the average concentration of acetylcholine in the last three pre-drug samples, obtained after 1 h of perfusion, was taken as 100%, and all subsequent post-treatment values were expressed as mean (± S.E.M.) percent variation of basal values. The basal extracellular concentration of acetylcholine in the hippocampus, at 24 h after surgery, was 1.45 ± 0.23 fmol/μl ($n = 5$).

GHB, the GHB receptor antagonist 6,7,8,9-Tetrahydro-5-hydroxy-5*H*-benzocyclohept-6-ylideneacetic acid (NCS 382), the $GABA_B$ receptor agonist baclofen and its antagonist SCH 50911 were purchased from RBI, Italy. All drugs were dissolved in a saline solution (NaCl 0.9%) and administered in a volume of 1 ml/kg. Control rats were treated with the vehicle used to dissolve the active ingredient.

Statistical analyses were assessed by a two-way analysis of variance (ANOVA) for repeated measures, factors being treatment and time points. Post-hoc analyses were performed using the Student–Newman–Keuls test.

3. Results

As shown in Fig. 1, the i.p. administration of GHB at the dose of 200 and 500 mg/kg reduced extracellular hippocampal acetylcholine levels by about 25% and 50%, respectively (main effect of treatment $F(2,1) = 295.8$, $P < 0.001$; main effect of repeated measures $F(5,1) = 160.7$,

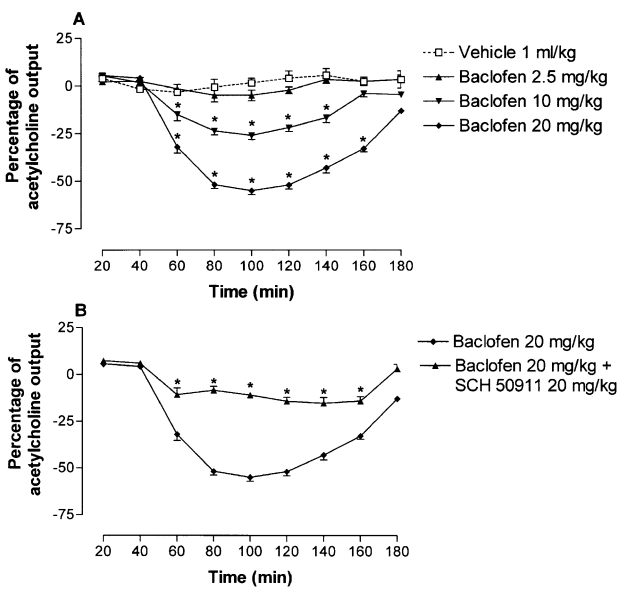

Fig. 2. Reduction of hippocampal extracellular acetylcholine concentrations induced by baclofen (A) and antagonism by SCH 50911 (B). $^{*}P < 0.05$ vs. controls (Student–Newman–Keuls test). Data are expressed as percentage (mean ± S.E.M.; $n = 5$) of the baseline concentrations. Basal values of extracellular acetylcholine concentrations, prior to drug administration, were: 1.25 ± 0.18 fmol/μl for control group, 1.32 ± 0.28, 1.13 ± 0.14 and 1.23 ± 0.12 fmol/μl for the groups treated with baclofen at the doses of 2.5, 10 and 20 mg/kg, respectively, and 1.32 ± 0.11 and 1.51 ± 0.23 fmol/μl for the groups treated with SCH 50911 and baclofen + SCH 50911, respectively. SCH 50911 was given 20 min before baclofen treatment.

$P < 0.001$; time × group interaction $F(5,1) = 37.7$, $P < 0.001$). Maximal inhibition of acetylcholine concentrations occurred within 40 min after treatment and disappeared at 120 min. The doses of 10 and 50 mg/kg did not modify acetylcholine levels. The inhibitory effect induced by the highest GHB dose was prevented by a pre-treatment with the $GABA_B$ receptor antagonist SCH 50911 (main effect of treatment $F(2,6) = 219.1$, $P < 0.001$; main effect of repeated measures $F(5,1) = 27.2$, $P < 0.001$; time × group interaction $F(5,1) = 22.2$, $P < 0.001$), given i.p. at the dose of 20 mg/kg, which per se did not modify acetylcholine concentrations (Fig. 1).

The putative GHB receptor antagonist NCS 382 was ineffective in blocking the reduction of extracellular hippocampal acetylcholine release induced by GHB (data not shown).

Similar to GHB, the $GABA_B$ receptor agonist baclofen at the doses of 10 and 20 mg/kg i.p. reduced hippocampal acetylcholine levels by about 25% and 60%, respectively (main effect of treatment $F(2,1) = 236.1$, $P < 0.001$; main effect of repeated measures $F(8,1) = 126.4$, $P < 0.001$; time × group interaction $F(8,1) = 45.1$, $P < 0.001$) (Fig. 2). A significant reduction was observed at 60 min from treatment, and acetylcholine concentrations returned to baseline at 180 min. The dose of 2.5 mg/kg was ineffective. The reduction of acetylcholine levels induced by the highest dose of baclofen was prevented by the $GABA_B$ receptor antagonist SCH 50911 (main effect of treatment $F(1,8) = 202$, $P < 0.001$; main effect of repeated measures $F(8,8) = 142.8$, $P < 0.001$; time × group interaction $F(8,8) = 40.7$, $P < 0.001$), given i.p. at the dose of 20 mg/kg (Fig. 2).

4. Discussion

This study shows that both GHB and baclofen reduce extracellular acetylcholine levels in the hippocampus. These effects are antagonized by the $GABA_B$ receptor antagonist SCH 50911 indicating that they are mediated by $GABA_B$ receptors. These data are in accordance with previous evidence indicating that both GHB (Sethy et al., 1976) and its precusor γ-butyro-lactone (GBL) (Ladinsky et al., 1983), increase acetylcholine content in several rat brain regions including hippocampus, suggesting that they reduce cholinergic neurotransmission. Our results support a possible involvement of $GABA_B$ receptors in the control of hippocampal acetylcholine release and cognitive processes. Moreover, they offer a possible mechanism to explain the cognitive deficits induced by both GHB and baclofen.

Finally, this study confirms that several of the effects elicited by GHB are $GABA_B$ receptor-mediated and suggests a potential therapeutic use of SCH 50911 in the treatment of cognitive deficits.

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