Multi-Faceted Aspects of Gamma-Hydroxybutyric Acid: A Neurotransmitter, Therapeutic Agent and Drug of Abuse

M. Paola Castelli[1,2,*]

[1] *"B. B. Brodie" Department of Neuroscience and* [2] *Center of Excellence 'Neurobiology of Addiction', University of Cagliari, Cagliari, Italy*

Abstract: Gamma hydroxybutyric acid (GHB), an endogenous constituent of the mammalian brain, acts as i) a neurotransmitter or neuromodulator, ii) a medicine used for the treatment of narcolepsy and alcoholism, and iii) a drug illicitly used for its psychotropic effects. GHB is thought to act as a specific GHB receptor agonist as well as a weak gamma-aminobutyric acid type B ($GABA_B$) receptor agonist. Here, I review the *in vivo* and *in vitro* pharmacological properties of GHB and its interaction with GHB and $GABA_B$ receptors. When exogenously administered, GHB is rapidly absorbed, crosses the blood-brain barrier, penetrates into the brain and exerts a number of pharmacological effects including anxiolysis, sedation/hypnosis and anesthesia. Due to its effects on the central nervous system, GHB has been used for the treatment of narcolepsy and as an anesthetic adjuvant. More recently, a role for GHB in the pharmacotherapy of alcohol dependence has been described. In this review, I also focus on the abuse liability and reinforcing properties of GHB in humans and laboratory animals.

Key Words: GHB, GHB receptor, $GABA_B$ receptor, NCS-382.

INTRODUCTION

Gamma-hydroxybutyric acid (GHB), a short-chain fatty acid, was first synthesized in the late 1950s by Henri Laborit [1] in an attempt to produce a gamma-aminobutyric acid (GABA) analog capable of crossing the blood-brain barrier with ease. The chemical structure of GHB is shown in Fig (**1**). Subsequent studies revealed that GHB occurs naturally in the mammalian brain, where it is currently believed to function as a neurotransmitter or neuromodulator [2, 3]. When exogenously administered, GHB is rapidly absorbed, crosses the blood-brain barrier with ease, penetrates into the brain and produces a number of pharmacological effects including (as the dose is increased) anxiolysis [4], sedation/hypnosis and anesthesia [3]. GHB has been used as an anesthetic in the laboratory [5, 6] and clinic [1] since the 1970s. Recently, GHB has been marketed for treatment of narcolepsy (Xyrem®) in the USA and alcoholism (Alcover®) in Italy. In addition to its therapeutic use, by the late 1990s GHB was also used recreationally as a "club drug" and "date rape drug" in the USA [7, 8] and Europe [9]. The Food and Drug Administration banned the sale of nonprescription GHB in 1990, and in 2000 the agency classified GHB as a Schedule I substance in the USA (United States Federal Register, 2000). Despite the obvious biological and pharmacological importance of GHB, its exact mechanism of action remains elusive.

GHB has affinity for two distinct receptor binding sites in the brain, the GHB receptor and at higher concentrations the $GABA_B$ receptor [10-12]. In this article, I review the neurobiology of endogenous GHB, its *in vivo* and *in vitro* pharmacological properties, and its interaction with GHB and $GABA_B$ receptors [for recent reviews see 13-15]. In addition, I focus on the therapeutic and addictive properties of this drug.

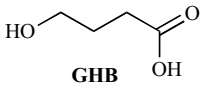

Fig. (1). Chemical structure of GHB.

1. GHB AS A NEUROTRANSMITTER

GHB is present at micromolar concentrations in both the brain and peripheral tissue [2]. However, its function outside the nervous system remains unknown. In the rat brain, GHB is unevenly distributed with the highest concentrations (42-46 pmol/mg protein) present in the substantia nigra and hypothalamus, and the lowest concentrations (4-8 pmol/mg protein) present in the frontal cortex and cerebellum [16]. The primary precursor of GHB in the brain is GABA. GHB is derived from the GABA-transaminase-mediated transformation of GABA into succinic semialdehyde (SSA), followed by the reduction of SSA in the presence of NADPH into GHB and and succinic semialdehyde reductase (SSR) in the neural cytosol [2]. Although this source of GHB is the most documented, other sources have been identified including polyamines, lipids, 1,4 butanediol (1,4BD) and γ-butyrolactone (GBL) [for review see 17]. The transformation of 1,4BD into GHB in the brain is catalyzed by alcohol deydrogenase and aldehyde deydrogenase [18]. Systemically administered GBL is converted into GHB by a circulating lactonase that is not present in the brain tissue [2].

*Address correspondence to this author at the "B. B. Brodie" Department of Neuroscience and [2]Center of Excellence 'Neurobiology of Addiction', University of Cagliari, Cagliari, Italy; Tel: +39-070-6754065; Fax: +39-070-6754320; E-mail: castelli@unica.it

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It has been hypothesized that GHB may act as a neurotransmitter/neuromodulator in the central nervous system (CNS), since GHB reportedly localizes in neurons at the synaptic level, exists in the brain at specific binding sites (high- and low-affinity) as well as with presynaptically located synthesizing enzymes, has its own vesicular uptake system [19], and its depolarization-evoked release may be Ca^{2+}-dependent [2, 3]. GHB has been postulated to act *via* the $GABA_B$ receptor, where it acts indirectly or directly as a weak agonist, and *via* independent GHB specific receptor sites in the brain [13-15].

1.1. Sites of Actions: GHB and $GABA_B$ Receptors

1.1.1. GHB Receptor

In Vitro Receptor Binding Studies

High-affinity binding sites for [^{3}H]GHB were first described in membranes isolated from the rat cerebral cortex nearly 25 years ago [10]. Since then, several studies on rat and human brains have revealed both high- (Kd_1= 300-500 nM; Bmax= 0.5-1.8 pmol/mg protein) and low-affinity (Kd_2= 1-11 μM; Bmax= 8-46 pmol/mg protein) [2, 3] GHB binding sites. These Kd values are in the range of the endogenous concentration of GHB found in the brain. The GHB binding sites are expressed only in neuronal cells, at the synaptic level in neurons in the brain and in neuronal cell lines [2, 20]. GHB binding has not been observed in glia or in cells of glial origin such as astrocytes in primary culture or C6 lines [2]. Bourguignon *et al*. examined the effects of several structural analogs of GHB on [^{3}H]GHB binding to rat membranes [21]. The binding of GHB and GHB analogs are dependent on the protein concentration, pH of the incubation medium and are stereoselective [22]. Although inhibitors of [^{3}H]GHB binding have been identified, to date the only compound reported to be a purported antagonist of the GHB receptor sites is 6,7,8,9-tetrahydro-5-hydroxy-5H-benzocycloheptene-6-ylideneacetic acid (NCS-382), a semirigid compound structurally related to GHB [23]. We recently demonstrated that NCS-382 is a stereoselective ligand for both the high- and low-affinity GHB binding sites [24]. Although NCS-382 did not display affinity for $GABA_A$, $GABA_B$ or any other known receptors, inverse and partial agonist actions of NCS-382 have been previously reported in biochemical and electrophysiological studies. Thus, NCS-382 is a good ligand, but not a selective antagonist, for GHB receptors [25].

Quantitative autoradiographic studies have established that GHB binding sites are heterogeneously distributed in the rat brain [26, 27]. Previously, we have shown using autoradiography that the distribution of the GHB binding sites in squirrel monkey and human brains is similar to that observed in the rat, including the absence of receptors in the hindbrain and cerebellum [12]. As illustrated in Fig. (**2**), incubation of monkey brain sections with 20 nM [^{3}H]GHB revealed a heterogeneous density of binding sites. In both human and squirrel monkey brains, the highest densities of GHB receptors were found in the hippocampus, particularly in the CA1 and CA2 fields (Figs. **2-3**). High to moderate densities of GHB receptors were found in the cortical areas (frontal, temporal, insular, cingulate and entorhinal), whereas low densities were observed in the nucleus accumbens (NAc), putamen and nucleus caudate (Table **1**). Interestingly, a simi-

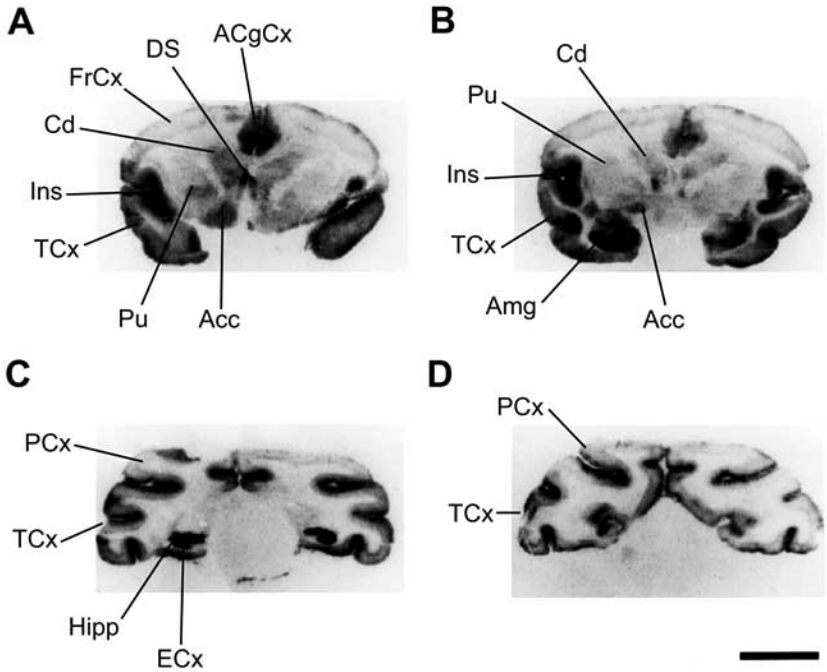

Fig. (2). Rostrocaudal (A-D) distribution of [^{3}H]GHB binding sites through the squirrel monkey brain. [^{3}H]GHB autoradiography was carried out as described in Castelli *et al*. [12]. Note the absence of signal in the caudal midbrain (C) and in the pons and the cerebellum (D). Abbreviations: Hipp, hippocampus; DS, lateral septal nuclei (dorsal part); FrCX, frontal cortex; PCx, parietal cortex; TCx, temporal cortex; Ins, insular cortex; ECx, enthorinal cortex; ACgCx, anterior cingulate cortex; Acc, nucleus accumbens; Cd, caudate; Pu, putamen; Amg, amygdala. Scale bar, 1 cm.

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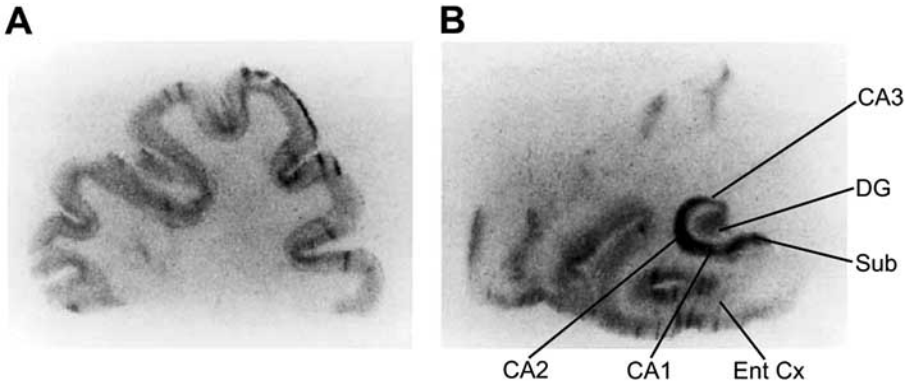

Fig. (3). Autoradiograms of [^{3}H]GHB binding sites in human brain tissue. [^{3}H]GHB binding sites in coronal (20 μm) sections detected by quantitative autoradiography using 20 nM [^{3}H]GHB (60 Ci/mmol). Autoradiograms were generated, quantified and analysed as described in Castelli *et al*. [12]. (A) Left hemisphere, frontal cortex. (B) Left hemisphere, hippocampal formation: CA1, field CA1 of Ammon's horn; CA2, field CA2 of Ammon's horn; CA3, field of CA3 of Ammon's horn; DG, dentate gyrus; Ent Cx, entorhinal cortex; Sub; subiculum. Source: Reprinted from Castelli *et al*. [12] with permission from Elsevier B.V./ECNP.

lar distribution of GHB binding sites was observed using [^{3}H]NCS-382 as the radioligand instead of [^{3}H]GHB [28]. The use of [^{3}H]NCS-382 appears to offer some advantage over [^{3}H]GHB for radioligand binding in both homogenate and autoradiography studies due to the fact that, unlike GHB, [^{3}H]NCS-382 does not interact with $GABA_A$ or $GABA_B$ receptors.

Table 1. Distribution and Relative Densities (ROD) of High-Affinity [3H] GHB Binding Sites in the Monkey and Human Brain[a]

Neuroanatomical Structure	Monkey	Human
Frontal cortex	30±3	22±2
Cingulate cortex	37±4	n.d.[b]
Temporal cortex	50±4	18±2
Insular cortex	69±6	n.d.
Entorhinal cortex	36±9	22±2
Caudate nucleus	11±2	9±2
Putamen	9±2	8±1
Accurnbens	11±1	n.d.
Globus pallidus	0 b	0
Lat. septum nucleus (dorsal part)	44±5	n.d.
Amygdala	76±8	n.d.
Hippocampal formation:		
Dentate gyrus	65±5	28±2
CA1 field	129±8	46±4
CA2 field	135±7	51±3
CA3 field	79±4	29±2
Subiculum	n.d.	28±4
Cerebellum	0	0

[a]Binding densities were determined in autoradiograms from three human and one monkey brains. Data are mean± S.E.M. values of five density readings, expressed in fmol mg tissue equivalent, as described in Castelli *et al.* [12].

[b]n.d. not determined; O, no detectable binding. Source: Reprinted from Castelli *et al*. [12] with permission from Elsevier B.V./ECNP.

Novel Ligands for the GHB Receptor

In order to examine the role of GHB receptors in the behavioral profile of GHB, novel GHB ligands that lack affinity for $GABA_A$ and $GABA_B$ receptors and are not metabolized to GABA have been developed [29]. Wu *et al*. [30] reported the synthesis of tertiary alcohol analogs of GHB and its homolog, 5-hydroxypentanoic acid (UMB58) such as 4-hydroxy-4-methylpentanoic acid (UMB68) and 5-hydroxy-5-methylhexanoic acid (UMB75). Binding studies against [^{3}H]NCS382 showed that the tertiary alcohol analog of GHB (UMB68) has similar affinity to GHB, with the longer chain analogs possessing lower affinity. Against [^{3}H]GABA, UMB68 showed no affinity (IC_{50} >100 μM)at $GABA_A$ or $GABA_B$ receptors. Later, Carter *et al*. [31] introduced aromatic substituents on the GHB molecule to prevent metabolism to compounds with affinity for GABA receptors. Radioligand assays identified UMB86 (4-hydroxy-4-napthylbutanoic acid, sodium salt), UMB72 ([4-(3-phenylpropyloxy) butyric acid, sodium salt), 3-HPA (3-hydroxyphenylacetic acid), and 4-hydroxy-4-phenylbutyric acid as compounds that displace [^{3}H]NCS-382 from GHB receptors at concentrations that do not affect [^{3}H]GABA binding to $GABA_B$ receptors. These compounds are unable to mimic discriminative stimulus effects, catalepsy and, in part, the loss of righting reflex elicited by GHB, effects which are blocked by GABAB receptor antagonists.

Although these compounds have been shown to be useful in *in vivo* studies, at best they are equipotent with GHB.

Wellendorph *et al*. have synthesized conformationally restricted GHB analogs and assayed for [^{3}H]NCS-382 binding studies in rat homogenate [32]. The chemical structures of these compounds are shown in Fig. (**4**). The cyclohexene and cyclopentene analogs RS-3-hydroxycyclohex-1-enecarboxylic acid (RS-HOCHCA) and RS-3-hydroxycyclopent-1-enecarboxylic acid (RS-HOCPCA) were found to be high affinity GHB ligands, with IC_{50} values in the nanomolar range, and had respectively 9 and 27 times higher affinity than GHB for GHB receptors. As shown in Fig. (**5B**), the stereoselectively synthesized R, R isomer of trans-2-(hydroxymethyl)cyclopropanecarboxylic acid (HOCPrCA) (Ki=1.1

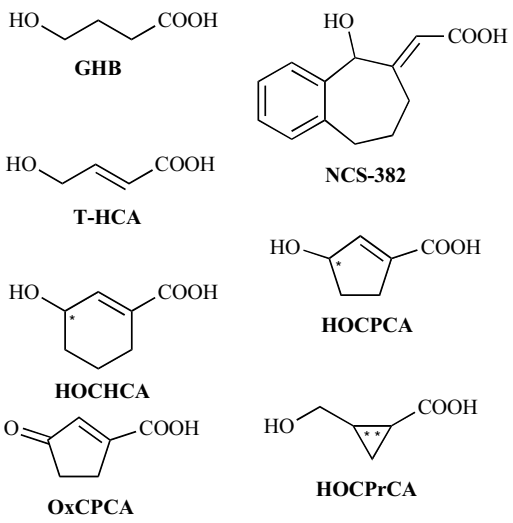

Fig. (4). Chemical structure of GHB, the reference compounds t-HCA and NCS-382, and the novel GHB analogs HOCHCA, HOCPCA, OxCPCA, and HOCPrCA. Chiral carbon atoms are marked by asterisk. Reprinted from Wellendorph *et al.* [32] with permission from ASPET.

μM) was found to have 10-fold higher affinity than its enantiomer. Likewise the R-enantiomers of HOCHCA and HOCPCA selectively inhibited [^{3}H]NCS-382 binding Figs. (**5C-D**): the latter being the most potent ligand identified to date for the native GHB binding site (39-fold higher affinity than GHB). These compounds, devoid of affinity for GABA receptors, are a good tool for studying and understanding the physiological role of endogenous GHB.

Second Messenger Studies

Contradictory data have been reported on the generation of second messengers in response to activation of native GHB receptors as well as the intracellular events that occur after second messenger activation. Snead demonstrated that GHB decreased forskolin-stimulated cyclic AMP levels in rat cortical and hippocampal membranes with concurrent stimulatory effects on high affinity GTPase activity and guanosine 5'-O-(3-[35S]GTPγS) binding in these brain regions [33]. The GHB-induced increase in [^{35}S]GTPγS binding and GTPase activity were blocked by NCS-382 but not by a specific $GABA_B$ antagonist, suggesting that these effects are mediated by the GHB receptor. Subsequent studies failed to demonstrate the GHB-mediated activation of both high affinity GTPase activity and GTPγS binding in these brain regions [34-36].

Results from our laboratory [35] confirmed Snead's initial observation [33] that GHB shares the ability to stimulate G protein activity with the $GABA_B$ receptor agonist baclofen. However, as shown in Fig. (**6B**), doses of 0.5 mM and 1 mM GHB led to a maximal stimulation of approximately 40% and 30%, respectively, of GTPγS binding. Neither γ-(p-methoxybenzyl)-γ-hydroxybutyric acid (NCS-435) nor trans-4-γ-hydroxycrotonic acid (t-HCA), selective ligands for GHB binding sites, stimulated GTPγS binding when administered at concentrations up to 1 mM (Fig. **6B**). Moreover, the $GABA_B$ receptor antagonist CGP 35348 completely abolished both the GHB- and baclofen-GTPγS-induced effect, while the GHB receptor antagonist NCS-382 failed to modify GHB-induced-GTPγS stimulation (Fig. **6C**), indicating that the GHB-induced G protein activation is mediated

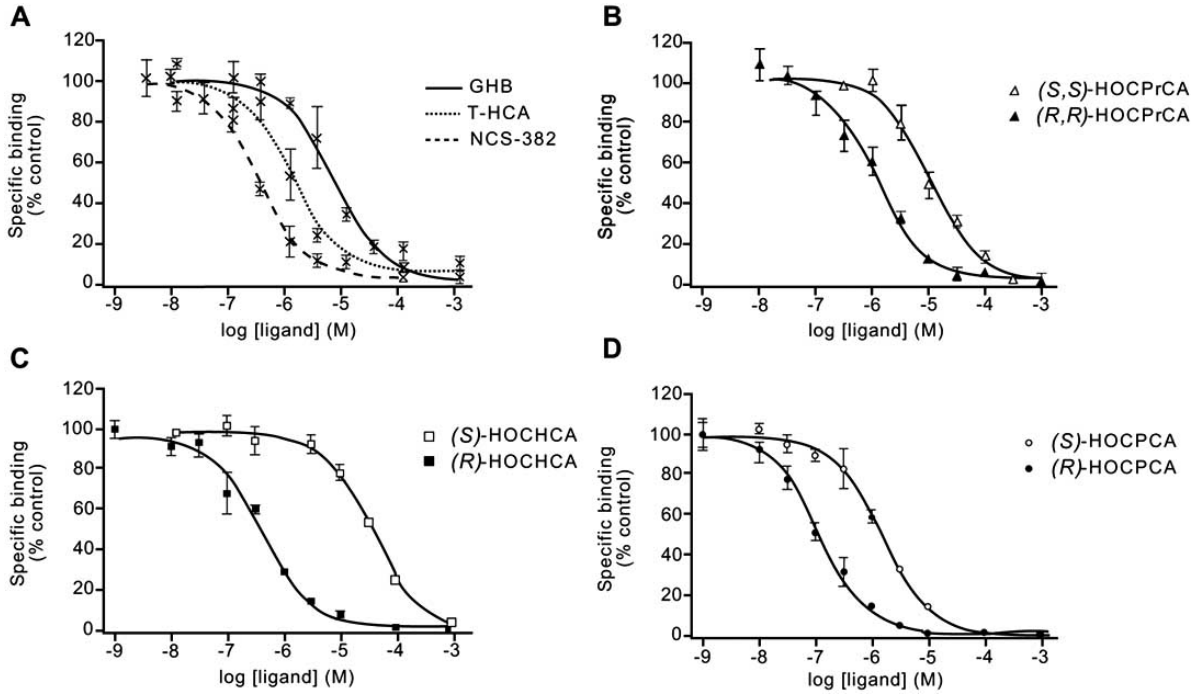

Fig. (5). Concentration-dependent inhibition of [^{3}H]NCS-382 binding to rat synaptic membranes by the established compounds GHB, t-HCA, and NCS-382 (A) and by the novel cyclic analogs (S,S)- and (R,R)- HOCPrCA (B), (S)- and (R)- HOCHCA (C), and (S)- and (R)- HOCPCA (D). Data shown are mean±S.D. of a single representative experiment performed in triplicate. Reprinted from Wellendorph *et al.* [32] with permission from ASPET.

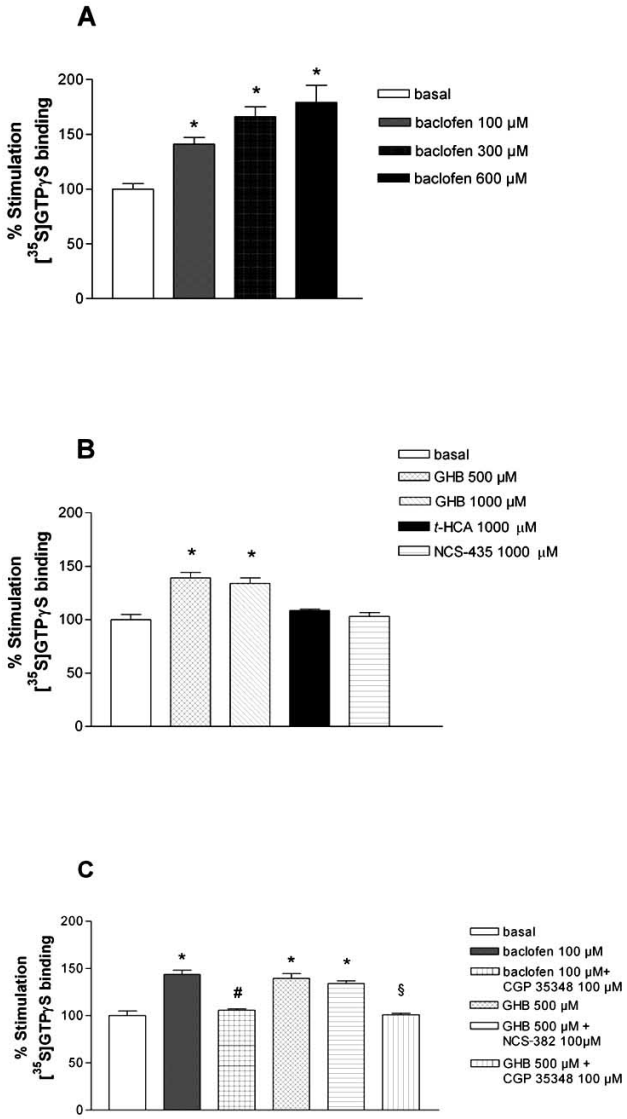

Fig. (6). Effect of baclofen (A) and GHB, *t*-HCA, NCS-435 (B) on [^{35}S]GTPγS binding in rat cerebral cortex. Data are the means ± S.E.M. of six determinations and expressed as percent over basal values. Results: Baclofen experiments ANOVA: F(3,25)= 9.959, P<0.0002. *P<0.01 with respect to basal values (Dunnett test). GHB experiments: ANOVA: F(4,23)= 17, P<0.0001 ; *P<0.01 with respect to basal values (Dunnett test).

Effect of baclofen and GHB (C) on [^{35}S]GTPγS binding either alone or with $GABA_B$ antagonist CGP 35348 or GHB antagonist NCS-382 in rat cerebral cortex. Data are the means ± S.E.M. of six determinations and expressed as percent over basal values. ANOVA: F(5,20)= 38.78, P<0.0001; *P<0.001 with respect to basal values, #P<0.001 with respect to baclofen 100 μM; §P<0.001 with respect to GHB 500 μM (Newman-Keuls test). Reprinted from Castelli *et al*. [35] with permission from Wiley-Blackwell.

via activation of the $GABA_B$ receptor and not the GHB receptor. It was also shown that although GHB (≥ 1 mM) stimulated GTPγS binding in wild type mice, this effect was completely abolished in $GABA_{B1}$ knockout mice [36]. We have no explanation for the discrepancy between these latter findings and the data reported by Snead [33].

Cloning of Rat and Human GHB Receptors

The cloning of both rat and human putative GHB receptors has been recently reported [37, 38]. The cloned rat GHB receptor has a molecular mass of 56 kDa and shows a predicted secondary structure consisting of seven-transmembrane (7TM)-spanning regions (similar to G-protein-coupled receptors [GPCRs]) [37]. However, while the peptide sequence has no significant homology with any G-protein linked receptor including the $GABA_B$ receptor, it does share 65% homology with the tetraspanin protein family. The mRNA expression pattern of the GHB receptor in the brain was similar to that of the native GHB binding sites, except for its expression in the cerebellum. In addition, the pharmacological profile of the cloned receptor expressed in Chinese hamster ovarian (CHO) cells was similar to that of the native GHB receptor, although differences were observed including the presence of only one component of the GHB binding site as well as the lack of NCS-382 binding. Moreover, t-HCA and its derivatives, which are good ligands for the native GHB binding sites, were more potent than GHB itself at binding to the cloned receptor. Taken together, these data suggest that several subtypes of GHB receptors exist.

The cloning and functional characterization of a GHB receptor identified in the human brain was reported four years later [38]. Two clones, termed C12K32 and GHBh1, were isolated from a human frontal cortex cDNA library. These clones are identical except in their C terminal, which differs due to a cytosine deletion in the C12K32 nucleotide sequence. The structures of C12K32 and GHBh1 do not correspond to the typical 7TM model, since both clones were predicted to have ten potential TM domains by SOSUI (TM prediction algorithm). Using the SMART program, a structure consisting of six TM domains and a seventh PFAM (protein family database) domain (called DUF1011) of unknown function was described. A number of eukaryotic proteins share this conserved domain including human putative GPCRs.

Adriamampandry *et al*. [38] claimed that both human GHB clones belong to a "clan", a term used to describe these GPCRs, that constitute a group of different families with a wide range of functions and no significant sequence similarity between them. Both GHB clones have no significant homology with any known GPCRs including the $GABA_B$ receptor. Moreover, their cDNA sequences showed only 50-60% similarity with the rat brain GHB receptor. Examination of the pharmacological properties of the GHB receptor in C12K32-expressing CHO cells revealed a Kd of 114 nM for GHB binding with no affinity for GABA and glutamate. In contrast to the cloned rat GHB receptor, the cloned human GHB receptor had affinity for NCS-382 and 4-phenyl-butyrate, a selective GHB analog, but not for t-HCA. [^{35}S]GTPγS binding studies demonstrated that GHB stimulates GTPγS binding in a dose-dependent manner [38]. Furthermore, this effect was not reproduced by GABA, and was inhibited by pertussis toxin and antagonized by NCS-382 [38]. These data support the concept that cloned human GHB belongs functionally to the GPCR family and that a Gi or Go protein is probably involved in the coupling of the receptor. Patch clamp studies in CHO-transfected cells with human GHB clones revealed that GHB induced a dose-dependent inward

current that was inhibited by NCS-382 [38]. GABA did not induce this effect when used at concentrations up to 100 μM. The two human GHB clones differed markedly in the reproducibility of this response, with strong and rapid desensitization of GHBh1 occurring after the first GHB application. Similar to the cloned rat GHB receptor, activation of the human GHB receptor expressed in CHO cells resulted in cationic channel opening [38]. However, CHO cells expressing cloned human GHB showed more depolarized Er values than cloned rat GHB, indicating a higher selectivity for Na^+ ions than K^+ ions, and suggesting that the human and rat GHB receptors are coupled to different signal transduction pathways. Together these data revealed a significant discrepancy in the molecular structures, pharmacological and functional profiles, and receptor distribution of cloned human, rat and native GHB receptors.

1.1.2. $GABA_B$ Receptors

$GABA_B$ receptors, members of the GPCR family have a molecular structure characterized by 7TM domains and a large extracellular N-terminal ligand-binding domain [39]. Among GPCRs, $GABA_B$ receptors are unique in that they require distinct subunits for their function. The $GABA_B$ receptor exists as a heterodimer, comprised of two 7TM-spanning units, named R1 and R2, that are linked by their C-termini [39]. In neurons, $GABA_B$ receptors are coupled *via* a G protein to adenylyl cyclase, and potassium and calcium channels at the membrane. $GABA_B$ receptor activation decreases adenylyl cyclase activity and calcium conductance, and increases potassium conductance, leading to inhibition of neuronal excitability and neurotransmitter release [39].

While studies on native $GABA_B$ receptor subtypes have predicted pharmacologically distinct $GABA_B$ receptor subtypes, molecular studies have failed to identify the expected receptor subtypes. Pharmacological stimulation of the $GABA_B$ receptor produces a variety of effects including muscle relaxation, anxiolysis and anti-addiction. Due to its ability to induce muscle relaxation, the prototypic $GABA_B$ receptor agonist, baclofen, has been used for more than 30 years to treat muscle rigidity associated with multiple sclerosis. The molecular structure, function and pharmacological profile of the $GABA_B$ receptor is described elsewhere [40-42].

GHB has been characterized as a weak agonist of $GABA_B$ receptors [11, 43-46]. The reported Ki values of GHB in rats are similar to those obtained in three $GABA_B$ agonist radioligand assays, such as [^{3}H]CGP 27492 and [^{3}H]baclofen (~80-100 μM), and are much lower than the Ki value (~3,000 μM) measured with $GABA_B$ receptor antagonists such as [^{3}H]CGP 54626 [11]. The Ki values of GHB observed with three agonist radioligands and the higher Ki values obtained in a $GABA_B$ antagonist assay are similar in the cortex, hippocampus and cerebellum [11]. The large discrepancy in the Ki values between agonist and antagonist radioligand binding assays is a characteristic of all $GABA_B$ receptor agonists [47]. In contrast, $GABA_B$ receptor antagonists have similar Ki values in the antagonist radioligand assay as those observed in the agonist radioligand assay [47]. The Ki values determined using $GABA_B$ agonist and antagonist radioligands indicate that GHB acts as an agonist at $GABA_B$ receptors [11]. In addition, Gpp(NH)p produces a significant decrease in GHB affinity for $GABA_B$ receptors in antagonist binding assays, confirming that GHB shares the same binding characteristics as GABA, baclofen and other $GABA_B$ receptor agonists.

1.1.3. GHB and $GABA_B$ Receptors: Distinctive Entities

Several studies demonstrate that the GHB receptor is not the same as the $GABA_B$ receptor, since the binding of GHB in brain tissues does not overlap with $GABA_B$ receptor distribution, and has a differential developmental profile [12, 48, 49]. Selective GHB ligands such as t-HCA, NCS-356, NCS-435, H-OCPCA and NCS-382 do not possess any affinity for either $GABA_A$ or $GABA_B$ receptors [32, 35]. GHB and its putative antagonist NCS-382 do not compete for [^{3}H]GABA binding in autoradiographic binding assays on rat brain sections [48]. Furthermore, neither [^{3}H]GHB nor [^{3}H]NCS-382 were found to have any affinity (up to 100 nM) for recombinant $GABA_{B1b}$, $GABA_{B2}$ and $GABA_{B1b+2}$ receptors expressed in HEK-293 cells [50]. In addition, [^{3}H]GHB and [^{3}H]NCS-382 binding was still observed in two lines of $GABA_B$ receptor knockout mice generated from C57B16/j and BALB/c backgrounds [36, 50]. All these data strongly indicate that GHB and $GABA_B$ receptors are separate molecular entities.

1.2. Pharmacological Effect of GHB on GHB Receptors

Electrophysiological and Microdialysis Studies

To date only a few *in vitro* studies have reported full antagonism of the electrophysiological effects of GHB by the GHB receptor antagonist NCS-382. In the presence of the $GABA_B$ receptor antagonists CGP 35348 (500 μM) or CGP 55485 (1 μM), application of GHB (600 μM) decreased NMDA- and AMPA/kainite-mediated EPSCs or $GABA_A$ IPSPs, respectively, in CA1 neurons *in vitro*: this effect was abolished by NCS-382 [51, 52]. Using a similar experimental model, GHB receptor-mediated action was not detected on thalamic EPSCs and IPSCs [53]. However, the lack of action on thalamic EPSCs and IPSCs by NCS-356 and 4-HCN (two high-affinity GHB receptor agonists in cell lines) does not support the existence of an electrophysiological GHB receptor-mediated response [54]. It is not clear if the differences between these studies reflect regional differences (thalamus versus hippocampus) or imply that in the hippocampal studies GHB is still acting on presynaptic $GABA_B$ receptors, since 1 mM GHB is required to fully block presynaptic $GABA_B$ receptors in this area [55]. Other studies show that GHB (300-600 μM), again in the presence of CGP 55485 (1 μM), depressed both the frequency and amplitude of IPSCs and EPSCs on DAergic neurons recorded *in vitro* in the substantia nigra [56, 57]. All of these actions were $GABA_B$-independent and were blocked by NCS-382. These findings are in contrast to the observations of Pistis *et al*. [58] described in the paragraph 1.4. Diverse experimental conditions (i.e. *in vitro* versus *in vivo*) may explain the discrepancies between these studies.

Together these studies demonstrated that i) submicromolar to low micromolar GHB concentrations are needed to evoke GHB-mediated responses at the GHB receptor, and ii) a prior block at the $GABA_B$ receptor is essential to disclose the electrophysiological GHB-induced effects mediated at

the GHB receptor. The only exception is the GHB (<100 μM)-mediated modulation of Ca^{2+} currents in NCB-20 cell lines, which were found to be NCS-382 sensitive [20, 54]. Thus, *in vitro* studies do not support the notion that there is a neuronal GHB-receptor-mediated electrophysiological response [59]. With respect to *in vivo* studies, opposite effects on the neuronal firing rate were observed in the prefrontal cortex with high and low doses of GHB: only the increased firing rate of neurons in response to low doses of GHB was blocked by NCS-382 [60].

The paucity of GHB receptor agonists and lack of selective antagonists, devoid of any inverse and/or partial agonistic action, have made it difficult to clarify the exact role of GHB receptors on the electrophysiological responses induced by endogenously and/or exogenously administered GHB.

In vivo microdialysis studies in rats indicate that GHB modifies the basal and K^+-evoked release of GABA [61, 62] and glutamate [63, 35]. Administration of doses of GHB < 2.0 mmol/kg (which induces a maximal concentration of GHB in the brain below 400-500 μM) led to inhibition of GABA release in the thalamus and frontal cortex [61, 62]. Treatment with GHB plus NCS-382 completely blocked the GHB-induced decrease in extracellular GABA levels. This GHB-induced inhibitory control of GABA release might represent the physiological effect of endogenous GHB that is mediated *via* presynaptic GHB receptors. At higher doses (>4.0 mmol/kg; approximately 800-1000 μM in the brain) GHB or the GHB-selective agonist NCS-356 led to a large increase in GABA levels in the cortex, which was blocked by administration of a high dose (1 mM) of NCS-382 [62].

In the hippocampus, nanomolar concentrations of GHB (100-500 nM) induce a large increase in extracellular levels of glutamate and the *in vitro* K^+-evoked release of glutamate from rat hippocampal synaptosomes [35, 63]. Since these effects were blocked by NCS-382, and not by the $GABA_B$ receptor antagonist CGP 35348, they must be mediated *via* the GHB receptor. Furthermore, while a high concentration of GHB (1 mM) reduced extracellular levels of glutamate *via* $GABA_B$ receptors, two GHB analogs (t-HCA and NCS-435), consistent with their lack of affinity for the $GABA_B$ receptor, only exhibited a stimulatory effect on extracellular glutamate levels, even at millimolar concentrations [35]. These effects were abolished by NCS-382, but not by CGP 35348, suggesting that this was a GHB receptor-mediated effect. Alone, neither NCS-382 nor CGP 35348 modified extracellular glutamate levels or the basal and K^+-evoked efflux from rat hippocampal synaptosomes *in vitro*.

1.3. Behavioral Studies: GHB Acting at the GHB Receptor

Drug Discrimination Studies

Administration of a combination of NCS-382 (25-50 mg/kg i.p.) and GHB resulted in the complete blockade of the DS effects induced by both 300 and 700 mg/kg GHB (i.g.) in rats trained to discriminate 300 and 700 mg/kg GHB from water in a T-maze food reinforced paradigm [64]. These findings suggest that the activation of GHB receptors constitutes a salient component of the GHB cue. However, subsequent work by the same author demonstrated that NCS-382 dramatically reduces alcohol absorption from the gastrointestinal system [65]. Therefore, further studies are required to verify whether the blockade of the DS of GHB is due to a reducing effect of NCS-382 on GHB absorption from the gastrointestinal tract.

Rats were trained to discriminate GHB from compounds that share pharmacological mechanisms with GHB using a two-lever response under a food-reinforced FR schedule [66]. Rats were trained to discriminate a) GHB *vs* saline (group 1), b) GHB *vs* either saline or baclofen (group 2) and c) GHB *vs* saline, baclofen and the positive allosteric $GABA_A$ modulator diazepam (group 3). The study revealed that rats could discriminate GHB from baclofen supporting the hypothesis that the effects of GHB and baclofen are not identical. Thus, the DS effects that GHB does not share with baclofen may be mediated *via* GHB receptors or differential interaction with $GABA_B$ receptors. In addition, it was shown that selective GHB ligands, which cannot be metabolized to GABA-active compounds, did not mimic or attenuate the DS effects of GHB. [30, 31]. 4-Hydroxy-4-napthyl-butanoic acid (UMB86), one selective GHB ligand, did not mimic the DS effects of GHB in any of the three groups, but tended to attenuate the DS effects of GHB more in rats trained to discriminate GHB from baclofen and diazepam than in the other groups. These findings suggest that UMB86 may possess antagonistic properties at the GHB receptor and that the GHB receptor may be prominently involved in the GHB versus baclofen or diazepam discrimination. Finally, the $GABA_B$ receptor antagonist CGP 35348 was found to differentially attenuate the DS effects of GHB and $GABA_B$ receptor agonists [67]. Dose effect curves were determined for GHB and two receptor agonists, baclofen and SKF 97541, alone and together with CGP 35348, a $GABA_B$ receptor antagonist. CGP 35348 significantly antagonized the baclofen and SKF 97541 dose effect (i.e. shifted the curves to the right). Although CGP 35348 attenuated the DS effects of GHB (320 mg/kg GHB), the magnitude of antagonism (i.e. curve shift to the right) of GHB was not as large as that observed for baclofen or SKF 97541. In addition, the GHB and SKF 97541 dose effect curves were determined alone and together with baclofen. Although baclofen enhanced the DS and rate-decreasing effects of SKF 97541, a similar response was not observed for GHB. These data suggest that GHB receptors as well as $GABA_B$ receptors may contribute to the DS and rate-decreasing effects of GHB.

The Role of the GHB-Receptor Mediated Component on the Reinforcing Effect of GHB

To date, only Martellotta *et al*. [68] have demonstrated that the reinforcing effects induced by GHB in drug naïve mice were completely abolished by the putative GHB receptor antagonist NCS-382. In this study, animals self-injected GHB acutely by nose-poking in single 30 min sessions. Under this conditions, every nose-poke resulted in a tail vein injection of doses of GHB ranging from 0.01 to 0.5 mg/kg. As commonly observed in studies testing the intravenous self-administration of drug abuse [69], GHB was acutely self-administered by mice according to a concentration-dependent bell-shaped curve. Treatment with NCS-382, at a dose of 12.5 mg that did not affect the spontaneous motor

activity in these animals, completely inhibited the self-administration of GHB (0.1 mg/kg).

1.4. Pharmacological Effect of GHB on $GABA_B$ Receptors

Increasing evidence suggests that the majority, if not all, of the behavioral and neurochemical actions elicited by GHB are mediated through $GABA_B$ receptors.

Electrophysiological and Microdialysis Studies

Electrophysiological studies examining the effect of GHB both *in vivo* and *in vitro* highlight $GABA_B$ receptor-mediated responses [59]. In rat and cat thalamocortical (TC) neurons, $GABA_B$ receptor antagonists were found to block postsynaptic GHB hyperpolarization [70] that, depending on the concentration, produces either an increase or decrease in excitability [59]. $GABA_B$ receptor antagonists also blocked the decrease in sensory excitatory postsynaptic potentials (EPSPs), cortical EPSCs and intrathalamic inhibitory postsynaptic potentials (IPSPs) in rat and cat TC neurons. *In vitro* electrophysiological studies have shown that all GHB actions are mediated by $GABA_B$ receptors in all brain regions examined to date including the substantia nigra [71], hippocampus [72, 73], ventral tegmental area (VTA) [74], and cortex [75]. Suppression of monosynaptic and polysynaptic IPSCs by GHB has recently been confirmed in rat neocortical slices [76]. GHB has also been shown to significantly suppress NMDA and AMPA mediated EPSCs. All of these GHB-mediated effects were reversed by the specific $GABA_B$ receptor antagonist CGP 62349, but not by the putative GHB receptor antagonist NCS-382 [76]. It has been suggested that some of the actions of GHB might be mediated by conversion of GHB to GABA [77], a process that is blocked by sodium valproate. Application of sodium valproate, at a concentration expected to block this conversion, had no detectable effect on the suppression of polysynaptic IPSCs induced by GHB [76]. Furthermore, GHB has been shown to inhibit mitogen-activated protein kinase (MAPK) phosphorylation *via* $GABA_B$ receptors in the presence of sodium valproate [78].

Despite the increasing problems associated with GHB abuse and its potential anti-craving properties in the alcohol withdrawal syndrome, only a few electrophysiological *in vivo* studies have examined the actions of GHB on the mesolimbic dopamine (DA) system. Although low doses of GHB (<200 mg/kg i.v.) induce a small increase in the firing rate of DAergic neurons in the pars compacta of the substantia nigra in anaesthetized rats, higher doses (200-400 mg/kg i.v.) lead to an overall decrease in firing rate. The $GABA_B$ receptor agonist baclofen (8-16 mg/kg i.v.) mimics every action of GHB, whereas the $GABA_B$ receptor antagonist CGP 35348 blocks all the actions of GHB and baclofen [79].

A dual action of intravenously injected GHB has recently been described on mesolimbic DAergic neurons in the VTA and their target cells in the NAc in urethane anaesthetized rats [58]. GHB, in common with the majority of drugs of abuse, inhibited the excitability of the NAc. This effect was mimicked by the $GABA_B$ receptor agonist baclofen, but not by the selective GHB receptor agonist NCS-435 [35], and was blocked by the $GABA_B$ receptor antagonist SCH 50911. As shown in Fig. (**7C**), GHB heterogeneously affects DAergic neurons in the VTA. Two populations of neurons were identified, which were either inhibited or stimulated by GHB. Both the excitatory and inhibitory actions of GHB were blocked by SCH50911, a $GABA_B$ receptor antagonist Fig. (**7A,B**). The actions of GHB were dependent on the baseline firing rate of the cells, since cells with a low firing rate were predominantly excited, whereas fast firing cells were slightly inhibited Fig (**7D**). This may be due to the fact that GHB biphasically affects the activity of the mesolimbic system [80]. GHB exerts preferential inhibition of GABAergic neurons over DAergic neurons in the VTA, due to a lower EC_{50} for $GABA_B$-evoked G protein-activated inwardly rectifying K+ (GIRK) currents in the GABAergic population. Consequently, GHB may indirectly excite slow firing DAergic neurons by preferentially inhibiting fast firing GABAergic neurons. Conversely, the direct inhibitory effects of GHB may prevail against the indirect effect on fast firing DAergic neurons, since in these neurons the GABAergic tone is presumably lower. Accordingly, GHB concentrations less than 1 mM enhanced release of DA [81], due to inhibition of GABAergic interneurons through the low affinity-GHB binding site for the $GABA_B$ receptor (100 μM), thereby disinhibiting the DAergic cells. At higher concentrations (>1 mM), GHB inhibited DAergic neurons by direct hyperpolarization of these neurons, leading to decreased DA release [74, 82]. Previous studies have also indicated that activation of the $GABA_B$ receptor by GHB is responsible for its action on DAergic neurons [83]. Taken together these results substantiate that in the mesolimbic system the effects of GHB are mostly, if not all, due to its affinity for $GABA_B$ receptors and do not necessarily involve the high affinity GHB receptor binding site.

In vivo microdialysis studies indicate that GHB induces a decrease in hippocampal extracellular acetylcoline levels *via* the $GABA_B$ receptor, since this action was blocked by the $GABA_B$ receptor antagonist SCH 50911, but not by the GHB receptor antagonist NCS-382 [84]. Exogenous administration of GHB (>300 mg/kg i.p.) increased allopregnanolone (AP) and allo-tetrahydrodeoxycorticosterone (THDOC) levels in the rat cerebral cortex. In addition, elevated levels of their precursors progesterone and pregnenolone were reported. Furthermore, the GHB-induced increase in progesterone levels was mimicked by baclofen. All GHB-mediated effects were blocked using $GABA_B$ receptor antagonists while NCS-382, the GHB receptor antagonist, had no effect [85].

1.5. Behavioral Studies: GHB Acting at the $GABA_B$ Receptor

Drug Discrimination Studies

The drug discrimination assay is a suitable and sensitive tool for determining the receptor systems involved in the mediation of the discriminative stimulus (DS) effects of a drug [86]. This assay is based on the findings that i) drugs acting in a similar manner at a specific class of receptors possess similar DS, and ii) antagonists acting at a specific receptor block the DS effect of the receptor agonist. Although both GHB and $GABA_B$ receptors have been implicated in mediating the DS effects of GHB, their relative importance is still unclear.

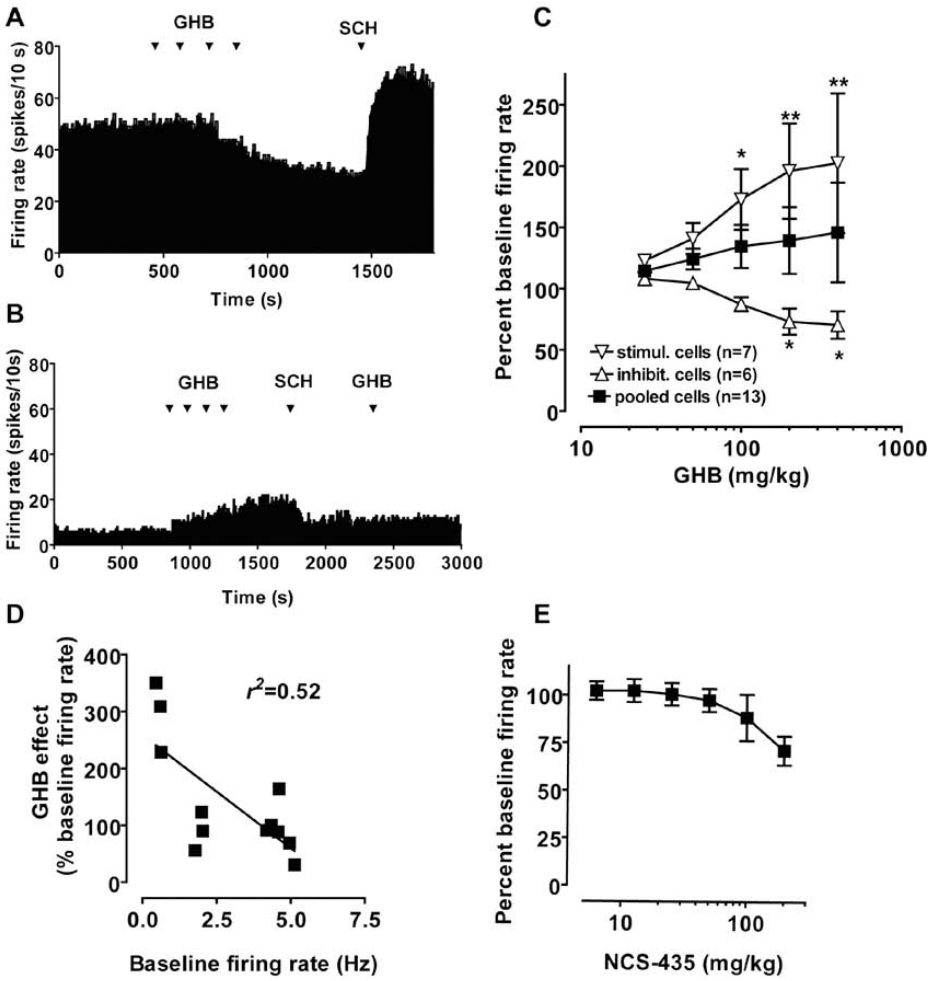

Fig. (7). GHB heterogeneously affects mesoaccumbens DA neurons. Illustrative firing rate histograms of antidromically identified DA neurons that shows both inhibitory (A) and excitatory (B) effects on firing rate of GHB (25-500 mg/kg, i.v.). In both examples the effects of GHB are reversed by the $GABA_B$ receptor antagonist SCH50911 (SCH, 32 mg/kg, i.v.). Arrows indicate time of administration. (C) Curves displaying the effects of systemically administered cumulative doses of GHB (logarithimic scale, abscissa) on the firing rate of DA neurons. In this graph, pooled cells (filled squares), stimulated cells (down triangles) and inhibited cells (up triangles) are plotted for comparison. (D) Baseline firing rate predicts the effect of GHB on DA neurons. This graph displays the significant correlation (r^2=0.52; $p<0.01$) between the baseline firing rate of the cells and the maximal effects of GHB. (E) The GHB analog NCS-435 (6.25-200 mg/kg, i.v.) did not dignificantly changed DA neuron firing rate. Reprinted from Pistis *et al*. [58] with permission from Elsevier B.V./ECNP.

The prototypical $GABA_B$ receptor agonist baclofen substituted for low (200-300 mg/kg i.g.) and high (700 mg/kg i.g.) doses of GHB in rats trained to discriminate 300 and 700 mg/kg GHB from water in a T-maze food-reinforced drug discrimination paradigm [64]. However, the potency of baclofen in substituting for GHB was higher in the high training dose than in the low training dose. Consistent with these findings, the $GABA_B$ receptor antagonist CGP 35348 was more potent and effective in blocking the DS effects of 700 mg/kg than 300 mg/kg GHB [64]. Later studies using two-lever or two-key food-reinforced drug discrimination procedure in rats and pigeons, respectively, confirmed the role of $GABA_B$ receptors in mediating the DS effects of GHB [87, 88]. Furthermore, in pigeons NCS-382 failed to attenuate the GHB-induced DS effects and when administered alone produced a substantial (70%) GHB-appropriate response [88]. The GHB-like effects of NCS-382 were antagonized by CGP 35348, suggesting that $GABA_B$ receptors might be involved in this GHB-mediated increase in DS. As the $GABA_B$-like activity of GHB might result from both its direct binding to $GABA_B$ receptors and through the conversion of GHB to GABA, GHB analogs that cannot be metabolized to GABA and are devoid of affinity for $GABA_B$ receptors have been developed [30]. These selective analogs, UMB86, UMB72 and 3-HPA, did not induce GHB-like DS effects in rat and pigeons or catalepsy in mice [31]. Together these findings indicate that the $GABA_B$-mediated cue is a prominent component of the GHB-induced DS effect.

The Role of the $GABA_B$-Mediated Component on the Depressant Behavioral Effects of GHB

Exogenously administered GHB can lead to decreased locomotor activity [89], ataxia [90, 91], decreased operant responding [90, 91], loss of righting reflex [92], and catalepsy [93]. $GABA_B$ receptor antagonists inhibit these GHB-induced effects suggesting that these actions are all due to

the activation of $GABA_B$ receptors. Consistent with this hypothesis, all of these GHB-mediated actions are reproduced by the prototypical $GABA_B$ receptor agonist baclofen [31, 87]. Moreover, transgenic mice that do not express functional $GABA_B$ receptors do not exhibit hypolocomotion or hypothermia after administration of baclofen, GHB or GBL [36, 94]. Finally, the GHB-selective analogs that have affinity for [^{3}H]NCS-382-labeled GHB receptors and (unlike GHB) not for $GABA_B$ receptors did not produce catalepsy [31]. The absence of catalepsy following the administration of these selective GHB analogs, and the attenuation of catalepsy induced by baclofen, SKF 97541 (a $GABA_B$ receptor agonist) or GHB by the $GABA_B$ receptor antagonist CGP 35348 provide strong evidence that $GABA_B$ receptor activation is necessary for GHB-induced catalepsy in mice [31].

2. GHB AS A THERAPEUTIC AGENT

Since the early sixties GHB has been tested for different therapeutic uses and in the treatment of a number of pathologies. Some of its medicinal uses include anesthesia, and treatment of narcolepsy, schizophrenia, depression, alcohol dependence and alcohol withdrawal syndrome [95]. The present review focuses only on the therapeutic effects of GHB on narcolepsy and alcoholism.

GHB and Narcolepsy

Narcolepsy is a chronic neurological disorder characterized by a tetrad of symptoms: excessive daytime sleepiness (EDS), catalepsy (brief loss of muscle tone in response to emotional arousal), hypnagogic hallucinations (visual or auditory dream-like hallucinations at sleep onset) and sleep paralysis [for reviews see 96, 97].

In healthy subjects, normal sleep is characterized by progression through a series of stages: slow-wave sleep (stages III and IV) followed by periodic rapid eye movement (REM) sleep and dreaming. In contrast, in narcoleptic patients sleep is often fragmented, and more time is spent awake or in stage I sleep than in stage III or IV sleep. These patients enter REM sleep more rapidly than usual, sometimes after following sleep [98]

The effects of GHB on sleep have been well documented [99, 100]. A double-blind study demonstrated that GHB significantly increased the time spent in stages III and IV (deep sleep), decreased the time spent in stage I (light sleep) and did not affect the time spent in REM sleep [101]. Several clinical studies examining the effects of GHB in patients with narcolepsy have shown that this compound is capable of reducing the signs and symptoms of catalepsy without suppression of REM sleep [95].

Two small clinical trials have shown that sodium oxybate, the sodium salt of GHB, may be potentially useful in the treatment of narcolepsy [101, 102]. In addition, two large, randomized, double-blind, parallel-group, placebo-controlled, multicentre USA (US Xyrem® Multicenter Study group, 2002; [103] and international trials [104] have examined the efficacy of oral administration of sodium oxybate on the treatment of this neurological disorder. In these trials, administration of sodium oxybate at doses of 4.5-9g/night significantly reduced the frequency of cataleptic attacks in patients with narcolepsy by 57-87% in a dose-dependent manner. In addition, sodium oxybate was found to reduce EDS (in 4- and 8-week trials), the median frequency of daytime sleep attacks and the median Epworth Sleepiness Scale scores [102].

Some minor side effects are associated with sodium oxybate including gastrointestinal adverse effects, dizziness, weight loss and urinary urgency. Moreover, its withdrawal is not associated with REM rebound. The major clinical disadvantage of sodium oxybate therapy appears to be its short duration of action, due to its short half-life. Other pharmacotherapies such as tricyclic antidepressants (TCAs, e.g. clomipramine, imipramine) and selective serotonine reuptake inhibitors (SSRIs, e.g. fluoxetine, paroxetine) can induce serious side effects, and lose efficacy on continuous treatment (development of tolerance). In conclusion, sodium oxybate is well tolerated and effective in the treatment of narcoleptic symptoms. Although its short half-life necessitates twice-nightly administration, it is highly effective in reducing the frequency of catalepsy, improving sleep architecture and reducing EDS in patients with narcolepsy. Recently published European Federation of Neurological Societies guidelines suggest that sodium oxybate is now the first-line treatment for catalepsy in patients with narcolepsy [105]. Sodium oxybate is approved in Europe for the treatment of narcolepsy with catalepsy and in the USA for the treatment of catalepsy and EDS in patients with narcolepsy.

GHB was banned by the FDA in 1990 and listed as a Schedule I drug in the USA in 2000 after developing notoriety as a substance of abuse reported to induce euphoria, disinhibition and sexual arousal. As a result, the potential for abuse/misuse of sodium oxybate has led to the development of strict risk-management strategies. Thus, in the USA, sodium oxybate is subjected to prescription restriction and is controlled under Schedule III, through the Xyrem® Success program, a restricted-drug distribution system [106]. Through this system, sodium oxybate is distributed from a single central pharmacy by courier and requires physician registration and verification of eligibility to prescribe, and registration and required reading of educational materials by patients. In Europe, a risk-management system as strict as that in the USA has not been planned.

GHB and Alcoholism: Preclinical and Clinical Studies

Several studies have demonstrated the effectiveness of GHB in animal models of alcoholism [for review see 107]. GHB reduces the severity of ethanol withdrawal signs in rats rendered physically dependent on ethanol [108] as well as reducing the voluntary ethanol intake in Sardinian alcohol-preferring rats (sP) [109] and P rats [110]. In both sP and P rats the acute administration of a non-sedative dose of GHB (200-300 mg/kg) resulted in a significant reduction (60-70%) in voluntary alcohol intake during the first 15-30 min, with intake returning to control group levels thereafter [111]. The short duration of the GHB-reducing effect on ethanol-intake in both sP and P rats is a consequence of the short half-life of the drug [112]. Interestingly, in clinical practice the best treatment outcome is consistently achieved by administration of GHB six times a day [113]. GHB and alcohol possess similar DS effects and cross-tolerance to the motor impairing

effects of GHB and ethanol has been reported [114, 115]. Recently, it was shown that administration of relatively low doses of GHB reduces the breakpoint for alcohol under a progressive ratio (PR) of reinforcement and extinction responding for alcohol in sP rats [116]. As breakpoint and extinction responding procedures are validated indices of the appetitive strength of alcohol in laboratory animals [117], these results indicate that GHB specifically reduced the motivational properties of alcohol (the possible animal correlate of human craving for alcohol). On the basis of these findings, it has been suggested that GHB may exert its effects on alcohol dependence by mimicking the actions of alcohol in the CNS, and that similarities to alcohol may constitute the mechanism of action by which GHB reduces the severity of alcohol withdrawal symptomatology, alcohol craving and alcohol consumption [107].

In humans, the efficacy of non-hypnotic doses of GHB administered orally to suppress alcohol withdrawal was first reported by Gallimberti *et al*. [118]. This randomized double-blind study recruited 23 patients meeting the DMS III-R criteria for alcohol withdrawal syndrome. Treatment with GHB led to a dramatic decrease in alcohol withdrawal severity over a seven-hour observation period, and its only side effect was a slight and transient dizziness. A subsequent randomized single-blind design study compared the effectiveness of GHB and diazepam, administered orally for 6-10 days to 30 patients, on the alcohol withdrawal syndrome [119]. The major disadvantage against the clinical use of GHB is the short duration of its effects. Thus, a total dose of 0.5-0.75 mg/kg/day of diazepam and 50 mg/kg/day of GHB were divided into six and three daily administrations, respectively. The protection from alcohol withdrawal syndrome elicited by three daily administrations of GHB was similar to that exerted by a double fractioning of the daily dose of the reference compound, diazepam. Subsequent studies (double-blind and open-label) demonstrated the efficacy of GHB in reducing the intensity of alcohol withdrawal symptoms and signs using doses of GHB ranging from 50 to 150 mg/kg/day in four or three administrations, respectively [120, 121]. None of the above investigations reported symptoms of withdrawal from GHB, craving for GHB or somnolence following GHB administration.

The efficacy of GHB on alcohol craving as well as for the daily consumption of alcoholic beverages has been assessed. In alcoholic patients treated with GHB at a dose of 50 mg/kg (divided into three daily doses for three months), the number of daily drinks was reduced by approximately 60% and the overall number of days of abstinence was increased [122]. GHB treatment also led to a significant reduction in alcohol craving. These results were confirmed by two subsequent studies [123, 124]. GHB was shown to be effective in reducing alcohol craving and increasing the abstinence rate, measured as a decrease in relapse monitored at six months and one year after discontinuation. Transitory side effects associated with GHB including vertigo, increased sleepiness and fatigue were observed in approximately 30% of patients and were resolved within 2-3 weeks of GHB intake. However, an increase in GHB consumption and some GHB craving was reported in 10% of patients during chronic treatment [123]. Furthermore, in the majority of studies, approximately 30-40% of alcoholics treated with GHB were "non-responders" [125]. The rapid metabolism of GHB in human alcoholics [126] and its short-lasting effect on alcohol intake in animal studies may be one of the reasons why this drug fails to maintain total abstinence from alcohol. Recent investigations demonstrated that fractioning to six administrations of the same daily dose of GHB (50 mg/kg) compared to the standard dosage regimen (three administrations/day) resulted in abstinence from alcohol in a larger percentage of "non-responders".

3. GHB AS A DRUG OF ABUSE

Preclinical and Clinical Studies

In the last 15 years, GHB has emerged as a recreational drug of abuse in Anglo-saxon countries [14, 113, 127]. GHB has been reported to have positive reinforcing properties and abuse potential at both preclinical and clinical levels. Several animal models, using different experimental procedures such as self-administration, conditioned place preference and drug-discrimination to elicit DS effects similar to those produced by abused drugs, have been shown to possess high predictive validity for abuse and dependence potential of drugs in humans. Rodent studies demonstrated that GHB produced conditioned place preference [128], is easily self-administered both orally and intravenously [68, 129] and decreases intravenous cocaine self-administration in rats [130]. Drug discrimination studies using rats trained to discriminate GHB from vehicle demonstrated that 1.0 g/kg alcohol completely substituted for 300 mg/kg GHB [114], whereas the benzodiazepines, chlordiazepoxide [131] and diazepam [86] substituted only partially (eliciting 60-70% of GHB-appropriate responding) for low to moderate doses of GHB. In contrast, d-amphetamine, cocaine, phencyclidine [131] or the cannabinoid receptor agonist WIN 55212-2 [86] did not elicit GHB-like internal cues. Although GHB substituted for alcohol [114], it failed to substitute for heroin, phencyclidine and cocaine [132]. In summary, administration of GHB produces unique DS effects with some characteristics most similar to those produced by non-sedative doses of alcohol and benzodiazepines, suggesting that the alcohol and benzodiazepine-like effects of GHB may contribute to its abuse potential.

The few studies that tested the positive reinforcing properties and abuse potential of GHB in primates generated more ambiguous results. Two studies examined the self-administration of GHB in monkeys trained to self-administer phencyclidine and methohexital, respectively [132, 133]. In the first study, the number of intravenous infusions of GHB exceeded control levels (i.e. mean infusions of saline) in only one monkey (four rhesus monkeys were used) and this increase occurred solely at the dose of 3 mg/kg/injection GHB [132]. In the latter study, the number of GHB injections/session was significantly higher than that of saline injection in two monkeys (three monkey were used) at concentrations of 3.2 mg/kg injection and 10 mg/kg injection, respectively [133]. Based on these findings, it was concluded that GHB is only a weak positive reinforcer and has little potential for recreational use and abuse. However, it has recently been demonstrated that chronic administration of GHB and its precursor GBL produce physical dependence in

baboons [134, 135]. Chronic GHB and GBL decreased food-maintained behavior, disrupted performance on fine-motor tasks and produced ataxia, muscle relaxation, tremors and jerks in a dose-dependent manner. Administration of the $GABA_B$ receptor antagonist SGS742 (formerly CGP 36742), during either GHB or GBL administration, precipitated a withdrawal syndrome characterized by self-directed behaviors, vomiting, tremors and/or jerks and increased aggression. Signs of physical dependence were also observed after discontinuation of chronic GHB or GBL treatment. In summary, chronic GHB and GBL administration produced physical dependence in baboons that was probably mediated *via* $GABA_B$ receptors.

GHB has been used clinically in Europe for decades without reports of severe side effects and incidence of abuse. However, GHB and its analogs 1,4BD and GBL have recently gained notoriety for their popularity as drugs of abuse, initially among bodybuilders and subsequently among participants of "rave" dance parties and poly-drug abusers [136]. Despite the ban on sales in 1990 by The Food and Drug Administration, illegally produced GHB, GBL and 1,4 BD continued to be abused. Users of GHB and its analogs claimed to experience an alcohol-like euphoria, disinhibition and sexual arousal without unpleasant hangover effects. However, the increase in GHB use was associated with an increasing number of GHB users experiencing overdoses serious enough to require hospital emergency care [137, 138]. The intensity of these GHB-induced effects depended on the dose taken and were significantly affected by the different types and quantities of coingestants. Instances of withdrawal syndrome (characterized by anxiety, insomnia, muscle cramps, tremors, delirium) after GHB discontinuation revealed the development of physical dependence upon GHB [139, 140]. GHB withdrawal is unlikely in once-daily users, but can be expected in the subpopulation that uses GHB more frequently (e.g. every 3-4 hours) [138]. Most cases of severe withdrawal have been reported in bodybuilders and others who use exceedingly high doses, several times daily, for a prolonged period of time [138]. Clinically, withdrawal from GHB and its analogs is almost identical to the alcohol or sedative-hypnotic withdrawal syndrome (e.g. benzodiazepines).

The possibility that craving for GHB may develop during its low-dose use as a therapy for alcoholism was first described by Addolorato *et al*., who reported that a number of subjects abused the drug by six to seven times the recommended dose [123]. GHB abuse in treated alcoholics was subsequently confirmed by other studies [113, 141].

Conclusions

In conclusion, GHB, a naturally occurring endogenous substance in the mammalian brain, is a neurotransmitter or neuromodulator with widespread effects on $GABA_B$ and GHB receptors. Several studies strongly suggest that GHB and $GABA_B$ receptors are separate molecular entities. However, while the pharmacological effects of GHB mediated by $GABA_B$ receptors are well known, the exact role of the GHB receptor remains elusive. The development of novel potent, selective GHB analogs that are insensitive to metabolic degradation, together with well-validated functional assays for the GHB receptor will help elucidate the functional relationship between GHB and $GABA_B$ receptors. Furthermore, full characterization of the cloned GHB receptor is required to clarify the discrepancies between the properties of cloned and native GHB receptors as well as to understand the physiological role of the GHB receptor.

GHB is also a therapeutic agent used for treatment of narcolepsy and alcoholism. This drug constitutes a replacement therapy for alcoholism similar to methadone in heroin addiction. Since the capability of GHB to induce salient feelings of euphoria, disinhibition and anxiolysis, as well as the increasing number of illicit and dangerous episodes of self-administration, the development of a GHB dependence syndrome has been proposed. However, the analysis of data on GHB abuse should distinguish between the illicit use of GHB for recreational purposes and episodes occurring in narcoleptic and alcoholic patients under medical conditions. While the recreational abuse of GHB clearly constitutes a medical and social problem, data from clinical trials and post-marketing surveillance have thus far not demonstrated any clear evidence of abuse in patients receiving sodium oxybate. Moreover, based on the data currently available self-directed intake of GHB among alcoholics undergoing therapy with GHB is limited (10-15% of patients) and should not undermine its medical use. Thus, given the encouraging preclinical and clinical evidence of the efficacy of GHB in alcoholism, further double-blind investigations, examining larger samples of alcoholic patients, are required to further assess the effectiveness of GHB in the treatment of alcoholic dependence.

ABBREVIATIONS:

1,4BD	=	1,4 butanediol
7TM	=	Seven-transmembrane-spanning regions
CHO	=	Chinese hamster ovarian
CNS	=	Central nervous system
DA	=	Dopamine
DS	=	Discriminative stimulus
EPSPs	=	Excitatory postsynaptic potentials
FR	=	Fixed ratio
GABA	=	Gamma-aminobutyric acid
GBL	=	γ-butyrolactone
GHB	=	Gamma hydroxybutyric acid
GIRK,	=	G protein-activated inwardly rectifying K+
GPCRs	=	G-protein-coupled receptors;
HOCPrCA	=	Trans-2-(hydroxymethyl)cyclopropanecarboxylic acid
IPSPs	=	Inhibitory postsynaptic potentials
NAc	=	Nucleus accumbens
NCS-382	=	6,7,8,9-tetrahydro-5-hydroxy-5H-benzocyclohept-6-ylideneacetic acid

NCS-435	=	γ-(p-methoxybenzyl)-γ-hydroxybutyric acid
PFAM	=	Protein family database
RS-HOCHCA	=	RS-3-hydroxycyclohex-1-enecarboxylic acid
RS-HOCPCA	=	RS-3-hydroxycyclopent-1-enecarboxylic acid
REM	=	Rapid eye movement
SSA	=	Succinic semialdehyde
SSR	=	Succinic semialdehyde reductase
t-HCA	=	Trans-4-γ-hydroxycrotonic acid
TH	=	Thalamocortical
UMB86	=	4-hydroxy-4-napthyl-butanoic acid
VTA	=	Ventral tegmental area

REFERENCES

[1] Laborit, H. *Int. J. Neuropharmacol*., **1964**, *3*, 433.
[2] Maitre, M. *Prog. Neurobiol*., **1997**, *51*, 337.
[3] Bernasconi, R.; Mathivet, P.; Bischoff, S.; Marescaux, C. *Trends Pharmacol. Sci*., **1999**, *20*, 135.
[4] Schmidt-Mutter, C.; Pain, L.; Sandner, G.; Gobaille, S.; Maitre, M. *Eur. J. Pharmacol*., **1998**, *342*, 21.
[5] Vickers, M.D. *Int. Anesthesiol. Clin.,* **1969**, *1,* 75.
[6] Kleinschmidt, S.; Grundmann, U.; Janneck, U.; Kreienmeyer, J.; Kulosa, R.; Larsen, R. *Eur. J. Anaesthesiol.,* **1997**, *14*, 590.
[7] Li, J.; Stokes, S.A.; Woeckener, A. *Ann. Emerg. Med.* **1998**, *31*, 723.
[8] Romanelli, F.; Smith, K.M.; Pomeroy, C. *Top. HIV Med*., **2003**, *11*, 25.
[9] Liechti, M.E.; Kunz, I.; Greminger, P.; Speich, R.; Kupferschmidt, H. *Drug Alcohol Depend.,* **2006**, *81*, 323.
[10] Benavides, J.; Rumigny, J. F.; Bourguignon, J.J.; Cash, C.; Wermuth, C. G.; Mandel, P.; Vincendon, G.; Maitre, M. *Life Sci.,* **1982**, *30*, 953.
[11] Mathivet, P.; Bernasconi, R.; De Barry, J.; Marescaux, C.; Bittiger, H. *Eur. J. Pharmacol*., **1997**, *321*, 67.
[12] Castelli, M. P.; Mocci, I.; Langlois, X.; Gommerendagger, W.; Luyten, W. H.; Leysen, J. E.; Gessa, G. L. *Brain Res. Mol. Brain Res.,* **2000**, *78*, 91.
[13] Wong, C. G.; Gibson, K. M.; Snead, O.C. 3rd. *Trends Pharmacol. Sci*., **2004**, *25*, 29.
[14] Snead, O. C.; Gibson, K.M. *N Engl. J. Med.,* **2005**, *352*, 2721.
[15] Crunelli, V.; Emri, Z.; Leresche, N. *Curr. Opin. Pharmacol*., **2006**, *6*, 44.
[16] Vayer, P.; Maitre, M. *Neurosci. Lett.,* **1988**, *87*, 99.
[17] Kafman E. E. In *Gamma-Hydroxybutyrate. Molecular, Functional and Clinical Aspects*; Tunnicliff, Cash, Ed.; Taylor and Francis, London and New York, **2002**, pp. 17-27
[18] Vayer, P.; Mandel, P.; Maitre, M. *Life Sci*., **1987**, *41*, 1547.
[19] Muller, C.; Viry, S.; Miehe, M.; Andriamampandry, C.; Aunis, D.; Maitre, M. *J. Neurochem*., **2002**, *80,* 899.
[20] Kemmel, V.; Taleb, O.; Perard, A.; Andriamampandry, C.; Siffert, J. C.; Mark, J.; Maitre, M. *Neuroscience,* **1998**, 86, 989.
[21] Bourguignon, J. J.; Schmitt, M.; Didier, B. *Alcohol,* **2000**; *20*, 227.
[22] Cash, C. D.; Tunnicliff, G. In *Gamma-Hydroxybutyrate. Molecular, Functional and Clinical Aspects*; Tunnicliff, Cash, Ed.; Taylor and Francis, London and New York, **2002**; pp. 17-27.
[23] Maitre, M.; Hechler, V.; Vayer, P.; Gobaille, S.; Cash, C. D.; Schmitt, M.; Bourguignon, J. J. *J. Pharmacol. Exp. Ther.,* **1990**, *255*, 657.
[24] Castelli, M. P.; Mocci, I.; Pistis, M.; Peis, M.; Berta, D.; Gelain, A.; Gessa, G. L.; Cignarella, G. *Eur. J. Pharmacol.,* **2002**, *445*, 1.
[25] Castelli, M. P; Pibiri, F.; Carboni, G.; Piras, A. P. *CNS Drug Rev*., **2004**, *10*, 243.
[26] Hechler, V.; Weissmann, D.; Mach, E.; Pujol, J. F.; Maitre, M. *J. Neurochem.,* **1987**, *49*, 1025.
[27] Hechler, V.; Gobaille, S.; Maitre, M. *Brain Res*., **1992**, *572*, 345.
[28] Mehta, A. K.; Muschaweck, N. M.; Maeda, D. Y.; Coop, A.; Ticku, M. J. *J. Pharmacol. Exp. Ther.,* **2001**, *299*, 1148.
[29] Murphy, T. C.; Poppe, C.; Porter, J. E.; Montine, T. J.; Picklo, M. J. *J. Neurochem.,* **2004**, *89*, 1462.
[30] Wu, H.; Zink, N.; Carter, L. P.; Mehta, A. K.; Hernandez, R. J.; Ticku, M. K.; Lamb, R.; France, C. P.; Coop, A. *J. Pharmacol. Exp. Ther.,* **2003**, *305*, 675.
[31] Carter, L.P.; Wu, H.; Chen, W.; Matthews, M. M.; Mehta, A. K.; Hernandez, R. J.; Thomson, J. A.; Ticku, M. K.; Coop, A.; Koek, W.; France, C. P. *J. Pharmacol. Exp. Ther*., **2005**, *313*, 1314
[32] 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**, 346.
[33] Snead; O. C III. *J. Neurochem.,* **2000**, *75*, 1986.
[34] Odagaki, Y.; Yamauchi, T. *Basic Clin. Pharmacol. Toxicol.,* **2004**, *94*, 89.
[35] Castelli, M. P.; Ferraro, L.; Mocci, I.; Carta, F.; Carai, M. A.; Antonelli, T.; Manganelli, S.; Cignarella, G.; Gessa, G. L. *J. Neurochem.,* **2003**, *87*, 722.
[36] Kaupmann, K.; Cryan, J. F.; Wellendorph, P.; Mombereau, C.; Sansig, G.; Klebs, K.; Schmutz, M.; Froestl, W.; van der Putten, H.; Mosbacher, J.; Bräuner-Osborne, H.; Waldmeier, P.; Bettler, B. *Eur. J. Neurosci.,* **2003**, *18*, 2722.
[37] Andriamampandry, C.; Taleb, O.; Viry, S.; Muller, C.; Humbert, J. P.; Gobaille, S.; Aunis, D.; Maitre, M. *J. FASEB,* **2003**, *17*, 1691.
[38] Andriamampandry, C.; Taleb, O.; Kemmel, V.; Humbert, J. P.; Aunis, D.; Maitre, M. *J. FASEB,* **2007**, *21*, 885.
[39] Bowery, N. G.; Bettler, B.; Froestl, W.; Gallagher, J. P.; Marshall, F.; Raiteri, M.; Bonner, T. I.; Enna; S. J. *Pharmacol. Rev*., **2002**, *54*, 247.
[40] Bettler, B.; Kaupmann, K.; Mosbacher, J.; Gassmann, M. *Physiol. Rev.,* **2004**, *84*, 835.
[41] Bettler, B.; Tiao, J. Y. *Pharmacol. Ther.,* **2006**, *110*, 533.
[42] Bowery, N. G. *Curr. Opin. Pharmacol.,* **2006**, *6,* 37.
[43] Bernasconi, R.; Lauber, J.; Marescaux, C.; Vergnes, M.; Martin, P.; Rubio, V.; Leonhardt, T.; Reymann, N.; Bittiger, H. *J. Neural Transm. Suppl.,* **1992**, *35*, 155.
[44] Ishige, K.; Aizawa, M.; Ito, Y.; Fukuda, H. *Neuropharmacology,* **1996**, *35*, 45.
[45] Ito, Y.; Ishige, K.; Zaitsu, E.; Anzai, K.; Fukuda, H. *J. Neurochem.,* **1995**, *65*, 75.
[46] Lingenhoehl, K.; Brom, R.; Heid, J.; Beck, P.; Froestl, W.; Kaupmann, K.; Bettler, B.; Mosbacher, J. *Neuropharmacology*, **1999**, *38*, 1667.
[47] Bittiger, H.; Reymann, N.; Froestl W. *Pharmacol. Commun*., **1992**, *2*, 23.
[48] Snead, O. C. 3rd. *Biochem. Pharmacol*., **1996**, *52*, 1235.
[49] Snead, O. C. 3rd. *Brain Res*., **1994**, *659*, 147.
[50] Wu, Y.; Ali, S.; Ahmadian, G.; Liu, C. C.; Wang, Y. T.; Gibson, K. M.; Calver, A. R.; Francis, J.; Pangalos, M. N.; Carter Snead, O. 3rd. *Neuropharmacology,* **2004**, *47*, 1146.
[51] Berton, F.; Brancucci, A.; Beghè, F.; Cammalleri, M.; Depuro, A.; Francesconi, W.; Gessa, G. L. *Eur. J. Pharmacol.,* **1999**, *380*, 109.
[52] Cammalleri, M.; Brancucci, A.; Berton, F.; Loche, A.; Gessa, G. L.; Francescani, W. *Neuropsychopharmacology,* **2002**, *27*, 960.
[53] Gervasi, N.; Monnier, Z.; Vincent, P.; Paupardin-Tritsch, D.; Hughes, S. W.; Crunelli, V.; Leresche, N. *J. Neurosci.,* **2003**, *23*, 11469.
[54] Kemmel, V.; Taleb, O.; Andriamampandry, C.; Aunis, D.; Maitre, M. *Neuroscience,* **2003**, *116*, 1021.
[55] Davies, C. H.; Starkeys, S. H.; Pozza, M. F.; Collingridge, G. L. *Nature,* **1991**, *349*, 611.
[56] Brancucci, A.; Berretta, N.; Mercuri, N. B.; Francesconi, W. *Neuropsychopharmacology*, **2004**, *29,* 537.
[57] Brancucci, A.; Berretta, N.; Mercuri, N. B.; Francesconi, W. *Brain Res*., **2004**, *30*, 62.
[58] Pistis, M.; Muntoni, A. L.; Pillola, G.; Perra, S.; Cignarella, G.; Melis, M.; Gessa, G. L. *Neuroscienze,* **2005**, *131*, 465.
[59] Crunelli, V.; Leresche, N. In *Gamma-Hydroxybutyrate. Molecular, Functional and Clinical Aspects*; Tunnicliff, Cash, Ed.; Taylor and Francis, London and New York, **2002**; pp. 75-110.

[60] Godbout, R.; Jelenic, P.; Labrie, C.; Schmitt, M.; Bouruignon, J. J. *Brain Res.,* **1995**, *673*, 157.

[61] Banerjee, P. K.; Snead, O. C. *J. Pharmacol. Exp. Ther.,* **1995**, *273*,1534.

[62] Gobaille, S.; Hechler, V.; Andriamampandry, C.; Kemmel, V.; Maitre, M. *J. Pharmacol. Exp. Ther.,* **1999**, *290*, 303.

[63] Ferraro, L.; Tanganelli, S.; O'Connor, W. T.; Francesconi, W.; Loche, A.; Gessa, G.L. Antonelli T. *J. Neurochem.,* **2001**, *78*, 929.

[64] Colombo, G.; Agabio, R.; Lobina, C.; Reali, R.; Gessa, G. L. *Physiol. Behav.,* **1998**, *64*, 293.

[65] Colombo, G.; Agabio, R.; Bourguignon, J. J.; Lobina, C.; Loche, A.; Maitre, M.; Reali, R.; Gessa, G. L. *Alcohol.,* **1999**, *17*, 93.

[66] Koek, W.; Carter, L. P.; Lamb, R. J.; Chen, W.; Wu, H.; Coop, A.; France, C. P. *J. Pharmacol. Exp. Ther*., **2005**, *314*, 170.

[67] Carter, L. P.; Chen, W.; Coop, A.; Koek, W. ; France, C. P. *Eur. J. Pharmacol.,* **2006**, *538*, 85.

[68] Martellotta, M. C.; Cossu, G.; Fattore, L.; Gessa, G. L.; Fratta, W. *Eur. Neuropsychopharmacol* .,**1998***, 8,* 293.

[69] Katz, J. L. In *The Neuropharmacological Basis of Reward* Liebman, J. M.; Cooper, S. J. Ed. Oxford University Press, Oxoford, **1989**; pp. 164-213.

[70] Williams, S. R.; Turner, J.P.; Crunelli, V. *Neuroscience,* **1995**, *66*, 133.

[71] Harris, N. C.; Webb, C.; Greenfield, S. A. *Neuroscience* **1989**, *31*, 363.

[72] Xie, X.; Smart, T. G. *Eur. J. Pharmacol.,* **1992**, *223*, 193.

[73] King, M. A.; Thinschmidt, J.; Walker, D. W. *J. Neural. Transm.,* **1997**, *104*, 1177.

[74] Madden, T. E.; Johnson, S. W. *J. Pharmacol. Exp. Ther.,* **1998**, *287*, 261.

[75] Jensen, K.; Mody, I. *Cereb. Cortex*., **2001**, *11*, 424.

[76] Li, Q.; Kuhn, C. M.; Wilson, W. A.; Lewis, D. V. *Neuroscience,* **2007**, *30*, 82.

[77] Hechler, V.; Ratomponirina, C.; Maitre, M. J. *Pharmacol. Exp. Ther.,* **1997**, *281*, 753.

[78] Ren, X.; Mody, I. *J. Biol. Chem.,* **2003**, *278*, 42006.

[79] Engberg, G.; Nissbrandt, H. *Naunyn-Schmiedberg's Arch. Pharmacol.,* **1993**, *348*, 491.

[80] Cruz, H. G.; Ivanova, T.; Lunn, M. L.; Stoffel, M.; Slesinger, P.A.; Lüscher, C. *Nat. Neurosci.,* **2004**, *7*, 153.

[81] Feigenbaum, J. J.; Howard, S. G. *Intern. J. Neurosci.,* **1996**, *88*, 53.

[82] Erhardt, S.; Andersson, B.; Nissbrandt, H.; Engberg, G. *Naunyn-Schmiedberg's Arch. Pharmacol.,* **1998**, *357*, 611.

[83] Howard, S. G.; Banerjee, P. K. In *Gamma-Hydroxybutyrate. Molecular, Functional and Clinical Aspects*; Tunnicliff, Cash, Ed.; Taylor and Francis, London and New York, **2002**; pp. 111-119.

[84] Nava, F.; Carta, G.; Bortolato, M.; Gessa, G. L. *Eur. J. Pharmacol.*, **2001**, *403*, 261.

[85] Barbaccia, M. L.; Colombo, G.; Affricano, D.; Carai, M. A.; Vacca, G.; Melis, S.; Purdy, R, H.; Gessa, G. L. *Neuropharmacology*, **2002**, *42,* 782.

[86] Goudie, A. J.; Leatley, M.J. In: *Behavioural Neuroscience: A pratical approach.* Sahgal A, Ed., Oxford: IRL Press, **1993**; pp. 145-167.

[87] Carter, L. P.; Flores, L. R.; Wu, H.; Chen, W.; Unzeitig, A. W. ; Coop, A.; France, C. P. *J. Pharmacol. Exp. Ther.,* **2003**, *305*, 668.

[88] Koek, W.; Flores, L. R.; Carter, L. P.; Lamb, R. J.; Chen, W.; Wu, H.; Coop, A.; France, C. P. *J. Pharmacol. Exp. Ther.,* **2004**, *308*, 903.

[89] Nissbrandt, H.; Engberg, G. *J. Neur. Trasm.* **1996**, *103*, 1255.

[90] Cook, C. D.; Aceto, M. D.; Coop, A., Beardsley, M.P. *Psycopharmacology,* **2002**, *160*, 99.

[91] Goodwin, A. K.; Froestl, W.; Weerts, E. M. *Psychopharmacology*, **2005**, *180*, 342.

[92] Carai, M.A.; Colombo, G.; Brunetti, G.; Melis, S.; Serra, S.; Vacca, G.; Mastinu, S.; Pistuddi, A. M.; Solinas, C.; Cignarella, G.; Minardi, G.; Gessa G. L. *Eur. J. Pharmacol.,* **2001**, *428*, 315.

[93] Itzhak, Y.; Ali, S. F. *Ann. N. Y. Acad. Sci.,* **2002**, *965*, 451.

[94] Queva, C.; Brenner-Danielsen, M.; Edlund, A.; Ekstrand, A. J.; Elg, S.; Erickson, S.; Johansson, T.; Lehmann, A.; Mattsson, J. P. *Br. J. Pharmacol*., **2003**, *140*, 315.

[95] Agabio, R.; Gessa, G. L. In *Gamma-Hydroxybutyrate. Molecular, Functional and Clinical Aspects*; Tunnicliff, Cash, Ed.; Taylor and Francis, London and New York, **2002**; pp. 169-187.

[96] Zeman, A.; Britton, T.; Douglas, N.; Hansen, A.; Hicks, J.; Howard, R.; Meredith, A.; Smith, I.; Stores, G.; Wilson, S.; Zaiwalla, Z. *BMJ,* **2004**, *329*, 724.

[97] Scammel, T. E. *Ann. Neurol.,* **2003**, *53*, 154.

[98] Roger, A.; Aldrich, M. S.; Caruso, C. C. *Sleep,* **1994**, *17*, 590.

[99] Mamelak, M.; Scharf, M.B.; Woods, M. *Sleep,* **1986**, *9*, 285.

[100] Metcalf, D. R.; Emde, R. N.; Stripe, J. T. *Electroenceph. Clin. Neurophysiol.,* **1966**, 20, 506.

[101] Lapierre, O.; Montplaisir, J.; Lamarre, M.; Bedard, M. A. *Sleep*, **1990**, *13*, 24.

[102] Littner, M.; Johnson, S. F.; McCall, W. V.; Anderson, W. M.; Davila, D.; Hartse, S. K.; Kushida, C. A.; Wise, M. S.; Hirshkowitz, M.; Woodson, B. T.; Standards of Practice Committee. *Sleep,* **2001**, *24*, 451.

[103] US Xyrem® Multicenter Study group. *Sleep,* **2002**, *25*, 42.

[104] US Xyrem® International Study group. *J. Clin. Sleep Med.,* **2005***, 1,* 391.

[105] Billiard, M.; Bassetti, C.; Dauvilliers, Y.; Dolenc-Groselj, L.; Lammers, G. J.; Mayer, G.; Pollmächer, T.; Reading, P.; Sonka, K.; EFNS Task Force. *Eur. J. Neurol.,* **2006**, *13*, 1035

[106] Fuller, D. E.; Hornfeldt, C. S.; Kelloway, J. S.; Stahl, P. J.; Anderson, T. F. *Drug Saf.,* **2004**, *27*, 293.

[107] Gessa, G. L.; Agabio, R.; Carai, M. A. M.; Lobina, C.; Pani. M.; Reali, R.; Colombo, G. *Alcohol,* **2000**, *20*, 271.

[108] Fadda, F.; Colombo, G.; Mosca, E.; Gessa, G. L. *Alcohol Alcohol,* **1989**, *24*, 447.

[109] Agabio, R.; Colombo, G.; Loche, A.; Lobina, C.; Pani, M. L.; Reali, R.; Gessa, G. L. *Alcohol Alcohol*., **1998**, *33*, 465.

[110] June, H. L.; Williams, J. A.; Cason, C. R.; Devaraju, S.; Lin M.; Murphy, J. M.; Lewis, M. J.; Lumeng, L.; Li, T. K. *Alcoholism: Clin. Exp. Res.,* **1995**, *19*, 14.

[111] Colombo, G. *Alcohol Alcohol,* **1997**, *32*, 443.

[112] Lettieri, J. T.; Fung, H. L. *J. Pharmacol. Exp. Ther*., **1979**, *208*, 7.

[113] Addolorato, G.; Caputo, F.; Capristo, E.; Stefanini, G. F.; Gasbarrini, G. *Alcohol*, **2000**, *20*, 217.

[114] Colombo, G.; Agabio, R.; Lobina, C.; Reali, R.; Fadda, F.; Gessa, G. L. *Physiol. Behav.,* **1995**, *57*, 105.

[115] Colombo, G.; Agabio, R.; Bourguignon, J. J.; Fadda, F.; Lobina, C.; Maitre, M.; Reali, R.; Schmitt, M.; Gessa, G. L. *Physiol. Behav.,* **1995**, *58*, 587.

[116] Maccioni, P.; Pes, D.; Fantini, N.; Carai, M. A. M.; Gessa, G. L.; Colombo, G. *Alcohol,* **2008**, *1*, 7.

[117] Markou, A.; Weiss, F.; Gold, L. H.; Caine, S. B.; Schulteis, G.; Koob, G. F. *Psychopharmacology,* **1993**, *112*, 163.

[118] Gallimberti, L.; Canton, G.; Gentile, N.; Ferri, M.; Cibin, M.; Ferrara, S. D.; Fadda, F.; Gessa, G. L. *Lancet,* **1989**, *2*, 787.

[119] Addolorato, G.; Balducci, G.; Capristo, F.; Attilia, M. L.; Taggi G.; Gasbarrini, G.; Ceccanti, M. *Alcohol. Clin. Exp. Res*., **1999**, *23*, 1596.

[120] Moncini, M.; Masini, E.; Gambassi, F.; Mannaioni, P. F. *Alcohol,* **2000**, *20*, 285.

[121] Nimmerrichter, A. A.; Walter, H.; Gutierrez-Lobos, K. E.; Lesch, O. M. *Alcohol Alcohol*, **2002**, *37*, 67.

[122] Gallimberti, L.; Ferri, M.; Ferrara, S. D.; Fadda, F.; Gessa, G. L. *Alcohol Clin. Exp. Res*., **1992**, *16*, 673.

[123] Addolorato, G.; Castelli, E.; Stefanini, G. F.; Casella, G.; Caputo, F.; Marsigli, L.; Bernardi, M.; Gasbarrini, G. *Alcohol Alcohol*, **1996**, *31*, 341.

[124] Addolorato, G.; Stefanini, G. F.; Casella, G.; Marsigli, L.; Caputo, F.; Gasbarrini, G. *Alcologia Eur. J. Alcohol. Study,* **1995***, 7,* 233.

[125] Addolorato, G.; Cibin, M.; Capristo, E.; Beghé, F.; Gessa, G. L.; Stefanini, G. F.; Gasbarrini, G. *Lancet,* **1999**, *351*, 38.

[126] Ferrara, S. D.; Zotti, S.; Tedeschi, L.; Frison, G.; Castagna, F.; Gallimberti, L.; Gessa, G. L.; Palatini, P. *Br. J. Clin. Pharmacol*., **1992**, *34*, 231.

[127] Drasbek, K. R.; Christensen, J.; Jensen, K. *Acta Neurol. Scand*., **2006**, *114*, 145.

[128] Martellotta, M. C.; Fattore, L.; Cossu, G.; Fratta, W. *Psychopharmacology*, **1997**, *132*, 1.

[129] Colombo, G.; Agabio, R.; Lobina, C.; Reali, R.; Fadda, F.; Gessa, G. L. *Eur. J. Pharmacol*., **1995**, *273*, 235

[130] Martellotta, M. C.; Balducci, C.; Fattore, L.; Cossu, G.; Gessa, G. L.; Pulvirenti, L.; Fratta, W. *Pharmacol. Biochem. Behav.,* **1998**, *59*, 697.

[131] Winter, J. C. *Psychopharmacology,* **1981**, *73*, 371.

[132] Beardsley, P. M.; Balster, R. L.; Harris, L.S. *Psychopharmacology,* **1996**, *127*, 315.
[133] Woolverton, W. L.; Rowlett, J. K.; Winger, G.; Woods, J. H.; Gerak, L. R.; France, C. P. *Drug Alcohol Depend*., **1999**, *54,* 137.
[134] Weerts, E. M.; Goodwin, A. K.; Griffiths, R. R.; Brown, P. R.; Froestl, W.; Jakobs, C.; Gibson, K. M. *Psychopharmacology,* **2005**, *179*, 678.
[135] Goodwin, A. K.; Griffiths, R. R.; Brown, P. R.; Froestl, W.; Jakobs, C.; Gibson, K. M.; Weerts, E. M. *Psychopharmacology,* **2006**, *189*, 71.
[136] Nicholson, K. L.; Balster, R. L. *Drug Alcohol Depend.* **2001**, *63*, 1.
[137] McDonough, M.; Kennedy, N.; Glasper, A.; Bearn, J. *Drug Alcohol Depend*., **2004**, 75, 3.
[138] Tarabar, A. F.; Nelson, L. S. *Toxicol. Rev*., **2004**, *23*, 45.
[139] Galloway, G. P.; Frederick, S. L.; Staggers, F. E. Jr.; Gonzales, M.; Stalcup, S. A.; Smith, D. E. *Addiction*, **1997**, *92*, 89.
[140] Hernandez, M.; McDaniel, C. H.; Costanza, C. D.; Hernandez, O. J. *Am. J. Drug Alcohol Abuse*., **1998**, *24*, 179.
[141] Gallimberti, L.; Spella, M. R.; Soncini, C. A.; Gessa G. L. *Alcohol* **2000**, *20*, 257.

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