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γ-Hydroxybutyric Acid

Neurobiology and Toxicology of a Recreational Drug

C. Guin Ting Wong,[1,2] *Katherine F.Y. Chan*,[1,3] *K. Michael Gibson*[4] and *O. Carter Snead III*[1,2,3,5,6]

1 Institute of Medical Sciences, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada
2 Brain and Behavior Research Program, The Hospital for Sick Children, Toronto, Ontario, Canada
3 Department of Pharmacology, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada
4 Department of Molecular and Medical Genetics, Oregon Health & Science University, Portland, Oregon, USA
5 Department of Pediatrics, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada
6 Division of Neurology, The Hospital for Sick Children, Toronto, Ontario, Canada

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Abstract

γ-Hydroxybutyric acid (GHB) is a short-chain fatty acid that occurs naturally in mammalian brain where it is derived metabolically from γ-aminobutyric acid (GABA), the primary inhibitory neurotransmitter in the brain. GHB was synthesised over 40 years ago and its presence in the brain and a number of aspects of its biological, pharmacological and toxicological properties have been elucidated over the last 20–30 years. However, widespread interest in this compound has arisen only in the past 5–10 years, primarily as a result of the emergence of GHB as a major recreational drug and public health problem in the US. There is considerable evidence that GHB may be a neuromodulator in the brain. GHB has multiple neuronal mechanisms including activation of both the γ-aminobutyric acid type B ($GABA_B$) receptor, and a separate GHB-specific receptor. This complex GHB-$GABA_B$ receptor interaction is probably responsible for the protean pharmacological, electroencephalographic, behavioural and toxicological effects of GHB, as well as the perturbations of learning and memory associated with supra-physiological concentrations of GHB in the brain that result from the exogenous administration of this drug in the clinical context of GHB abuse, addiction and withdrawal. Investigation of the inborn error of metabolism succinic semialdehyde deficiency (SSADH) and the murine model of this disorder (SSADH knockout mice), in which GHB plays a major role, may help dissect out GHB- and $GABA_B$

receptor-mediated mechanisms. In particular, the mechanisms that are operative in the molecular pathogenesis of GHB addiction and withdrawal as well as the absence seizures observed in the GHB-treated animals.

1. History of γ-Hydroxybutyric Acid (GHB)

γ-hydroxybutyric acid (GHB) is a naturally occurring short-chain fatty acid[1,2] that possesses ubiquitous neuropharmacological and neurophysiological properties. In 1947, γ-butyrolactone (GBL), a related compound, was shown to have central nervous system depressant properties.[3] However, interest in GBL lay dormant until 1960 when GHB was synthesised in an attempt to create an analogue of γ-amino butyric acid (GABA), a ubiquitous inhibitory neurotransmitter in the brain, that would cross the blood-brain barrier.[4] GHB was found to have 'sedative' properties similar to those of GBL[4] and for a time there was uncertainty as to whether GBL and GHB had behavioural and pharmacological activity independent of one another. However, GHB has been shown to be the active metabolite of GBL, which is biologically inactive.[5-7] An active lactonase that rapidly converts GBL to GHB is present in serum and liver, but not brain or cerebrospinal fluid.[8,9]

Soon after it was synthesised in the early 1960s GHB found initial use as an anaesthetic; however, it was essentially abandoned as a useful agent for this purpose because of seizures and lack of analgesia.[10-13] In the 1970s, GHB was found to be beneficial in the treatment of the sleep disorder narcolepsy.[14] This finding has been borne out in a double-blind, crossover clinical trial that tested the therapeutic efficacy of GHB in narcolepsy patients.[15] GHB has recently been approved by the US FDA for treatment of that disorder.[16,17]

GHB was manufactured in the US during the late 1980s, and in the beginning of the 1990s began to be marketed and used as a dietary supplement.[18] Around the same time GHB and its prodrug, GBL, also began to be used and abused, by body builders[19] because of the ability of these compounds to stimulate growth hormone production.[20-22] With increased illicit use of GHB and GBL, addictive properties have emerged for both of these compounds.[23] By the late 1990s, GHB had became a popular club drug and gained significant notoriety both as a major recreational drug of abuse[24-26] and as a date-rape drug.[27] The FDA banned the sale of nonprescription GHB in 1990 and on March 13, 2000, GHB was classified as a schedule I drug.[28]

2. GHB Metabolism

At least two pathways for the production of GHB exist in mammals (figure 1). The first, and quantitatively most important, entails conversion of GABA to succinic semialdehyde (SSA). This reaction is catalysed by mitochondrial GABA-transaminase and is followed by reduction of SSA to GHB, catalysed by cytosolic succinic semialdehyde reductase[29,30] Mitochondrial succinic semialdehyde dehydrogenase (SSADH), converting SSA to succinate, couples neurotransmitter metabolism to mitochondrial energy production. Therefore, unless aldo-keto reductase(s) exist within the mitochondria capable of catalysing the reduction of SSA to GHB, the production of cytosolic GHB requires transport of SSA from the mitochondria.[30,31] A minor pathway for GHB production involves partial oxidation of 1,4-butanediol (1,4-BD).[32] Potential endogenous sources of the small amounts of 1,4-BD detected in mammalian brain include the polyamines ornithine, spermine, spermidine and putrescine.[33]

There are at least four pathways for GHB degradation in mammals. The first, and quantitatively most significant, is the $NAD(P)^+$-linked oxidation of GHB to SSA, the latter undergoing further metabolism to either GABA or succinate catalysed by SSADH. Kinetically, the latter is likely of primary metabolic significance, based upon the low Michaelis-Menten constant (Km) of SSADH for SSA (approximately 1–4 μmol/L).[34] Additional pathways for GHB catabolism have not been as extensively investigated. Kaufman and colleagues identified a mitochondrial $NAD(P)^+$-independent transhydrogenase in rat kidney, liver and brain capable of metabolising GHB to SSA with the stoichiometric production of *D*-2-hydroxyglutaric acid from 2-oxoglutarate.[35] The transhydrogenase has been partially purified, but no kinetic parameters have been reported.[36,37] Walkenstein and colleagues[38] have presented evidence for β-oxidation of GHB. Similarly, urine obtained from patients with inherited SSADH deficiency contain intermediates consistent with the β-oxidation of GHB, including 3-oxo-4-hydroxybutyric, 3,4-dihydroxybutyric and glycolic acids.[39] However, other investigators have been unable to demonstrate significant metabolism of GHB via a β-oxidation scheme.[40]

3. GHB: A Biologically Active Neuromodulator or Incidental Metabolite of γ-Aminobutyric Acid (GABA)?

GHB has many properties suggesting that it may play a role in the brain as a neurotransmitter or neuromodulator.[19,29,41,42] These characteristics include a discrete, subcellular anatomical distribution for GHB and its synthesising enzyme in neuronal presynaptic terminals. Succinic semialdehyde reductase is present in GABAergic neurons suggesting that GHB and GABA may colocalise in

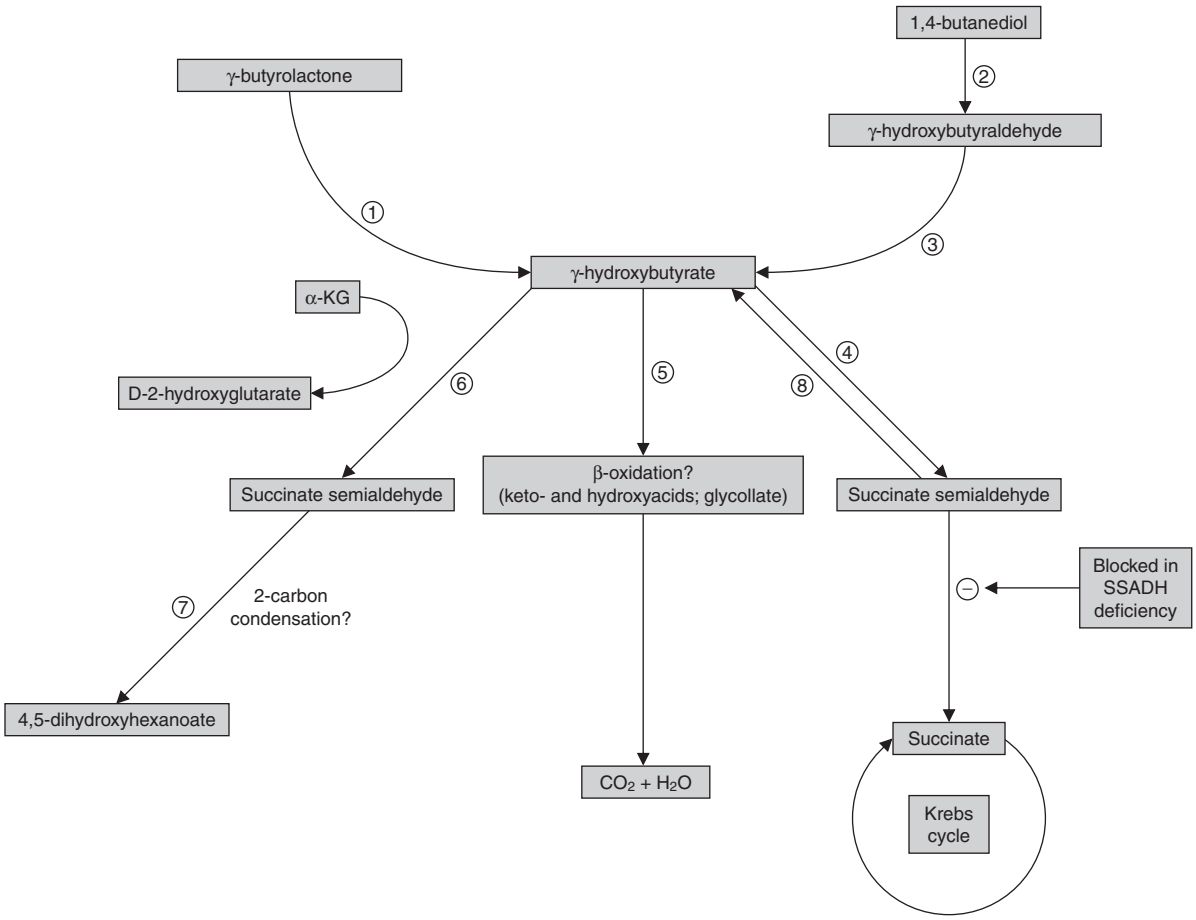

Fig. 1. Putative metabolic interrelationships of GHB, γ-butyrolactone and 1,4-butanediol in mammals. Numbered enzymes/reactions include: (1) serum lactonase; (2) alcohol dehydrogenase; (3) aldehyde dehydrogenase; (4) GHB dehydrogenase; (5) fatty-acid β-oxidation spiral; (6) D-2-hydroxyglutarate transhydrogenase; (7) an uncharacterised reaction forming 4,5-dihydroxyhexanoic acid from SSA; (8) SSA reductase. The site of the block in murine and human SSADH deficiency is depicted on the right. SSA also derives from GABA, primarily in the CNS, in a reversible reaction catalysed by GABA-transaminase (not shown), and SSA may be metabolised to GABA as well. **GABA** = γ-aminobutyric acid; **GHB** = γ-hydroxybutyric acid; **SSA** = succinic semialdehyde; **SSADH** = succinic semialdehyde dehydrogenase; **α-KG** = α-ketoglutarate.

certain inhibitory nerve terminals,[43] raising the possibility of a GHB-derived pool of GABA. GHB is released by neuronal depolarisation in a calcium-dependent fashion.[44] A sodium-dependent GHB uptake system has been demonstrated in the brain[45] and an active vesicular uptake system has been reported that is most likely driven by the vesicular inhibitory amino acid transporter – the same transporter involved in GABA and glycine uptake.[46]

Although GHB has been proposed as a biologically active neuromodulator,[30] the precise function of endogenous GHB in brain is unknown. In fact, there remains an ongoing controversy about whether GHB is a neuromodulator in its own right or whether it is an incidental metabolite of GABA. The difficulty in resolving this question lies in the sorting out of which of the physiological, pharmacological behavioural, and toxicological effects of GHB are directly GHB-mediated, which are related to an action of GHB at the $GABA_B$ receptor ($GABA_BR$), and which are dependent on both GHB- and $GABA_BR$-mediated mechanisms. In order to understand these issues, a discussion of the GHB-specific receptor (GHBR), the $GABA_BR$ and their relationship is necessary. The $GABA_BR$ is the focus of the discussion on GHB-GABA interaction in the brain because with few exceptions[47,48] there is little evidence to support the hypothesis that GHB interacts with the ionotropic $GABA_A$ receptor.[49-51]

4. The GHB Receptor

A GHBR is suggested by specific, high-affinity [^{3}H]GHB binding sites that occur in rat and human brain.[52-54] In all of these studies, [^{3}H]GHB binding was saturable, reversible and displayed both a high affinity binding site (30–580 nmol/L) and a low affinity binding site (1.5–16 μmol/L). The highest density of GHB binding sites is observed in the hippocampus, followed by the cortex, then thalamus and amygdala.[55-57] Brain regions associated

with dopaminergic transmission (such as caudate putamen, substantia nigra and ventral tegmental area) also exhibit GHB binding. No GHB binding is observed in the cerebellum. The anatomical distribution of [^{3}H]GHB binding correlates with GHB turnover and displays a distinct ontogeny. GHB binding is a postnatal event, appearing in the third postnatal week of life.[58]

The GHBR appears to be coupled to G proteins because the addition of GTP and pertussis toxin inhibits GHB binding in rat brain.[59,60] Physiologically relevant concentrations of GHB have been shown to inhibit forskolin-stimulated increases in cyclic adenosine monophosphate (cAMP) levels in the cortex and hippocampus, an effect likely due to activation of a G protein-coupled receptor since GHB stimulates [^{35}S]GTPγS binding and low-K_m GTPase activity. The effect of GHB on cAMP levels are detected in presynaptic synaptosome fractions but not synaptosoneurosome fractions, which contain primarily postsynaptic proteins. Furthermore, these effects do not occur until after postnatal day (P) 21, a developmental timepoint when specific [^{3}H]GHB binding is emerging.[61] Since presynaptic cAMP is known to be functionally coupled to neurotransmitter release, GHB may activate a specific presynaptic GHBR to inhibit neurotransmitter release. *In vivo* microdialysis studies indicate that GHB induces a robust decrease in the basal and potassium-evoked release of GABA.[62,63]

In the rat, doses of GHB below 500 mg/kg have been reported to increase hippocampal levels of cGMP.[64] However, doses at or above 500 mg/kg did not change the overall concentrations of cGMP. It should be noted that administration of doses >100 mg/kg GHB to rat result in levels of GHB in the brain that are far in excess of physiological concentrations of this compound.[7] These supra-physiological concentrations of GHB have also been shown to inhibit mitogen-activated protein kinase phosphorylation *in vitro*.[65] This inhibition was mimicked by application of baclofen and blocked by the $GABA_BR$ antagonist CGP56999A.

More recently, data have been reported that suggest that activation of the GHBR may not be G protein-coupled. Two GHB analogues that are specific GHBR agonists, NCS-435 and *trans*-γ-hydroxycrotonic acid, failed to stimulate [^{35}S]GTPγS binding in rat cortical homogenates and slices. GHB stimulated [^{35}S]GTPγS binding in the same studies, but this effect appeared to be due to activation of the $GABA_BR$ because [^{35}S]GTPγS binding was blocked by the $GABA_BR$ antagonist CGP 35348, but not by the GHBR antagonist (2E)-(5-hydroxy-5,7,8,9-tetrahydro-6H-benzo[α][7]annulen-6-ylidene)ethanoic acid (NCS-382).[66]

Recently Andriamampandry and colleagues have cloned a putative GHB receptor that is activated by GHB and G protein-coupled.[67] However, this newly cloned receptor displays no affinity for the specific GHB antagonist, NCS 382, suggesting an NCS 382-insensitive subtype of the GHB receptor.

4.1 The $GABA_B$ Receptor ($GABA_BR$)

The $GABA_BR$ is a heterodimer in which $GABA_BR$ 1 is co-expressed with $GABA_BR$ 2 to form a functional $GABA_BR$.[68] The $GABA_BR$ couples to various effector systems, through the signal transducing G protein. Presynaptically, activation of $GABA_BR$ autoreceptors (located on GABAergic neurons) and heteroreceptors (located on other neurotransmitter-releasing neurons) have been reported to inhibit neurotransmitter release through inhibition of calcium channels. Postsynaptically, $GABA_BR$ activation produces slow inhibitory postsynaptic potentials via G protein-coupled inward rectifying potassium channels.[68]

4.1.1 Effect of Exogenously Administered GHB on the $GABA_BR$

Many, if not most, of the pharmacological, clinical, behavioural and toxicological effects of exogenously administered GHB, are most likely mediated via the $GABA_BR$.[42] GHB has been proposed to be a weak $GABA_BR$ agonist,[69] with an affinity for the $GABA_BR$ in the mmol/L range,[70] which is well above the 1–4 μmol/L physiological concentrations of GHB in the brain.[2,29] Therefore, the high concentrations of GHB in brain that would be predicted to accrue from exogenous administration of this drug[7] may exert their protean pharmacological, toxicological and behavioural effects through $GABA_BR$-mediated mechanisms.

From a physiological perspective, GHB can activate $GABA_BR$ heterodimers co-expressed with Kir3 channels in *Xenopus* oocytes, but only with a concentration which produces a 50% effective response (EC_{50}) of approximately 5 mmol/L,[70] 1000 times the physiological concentration of GHB in brain. Similarly, millimolar concentrations of GHB are required to mimic the postsynaptic electrophysiological effects of baclofen on the $GABA_BR$. This electrophysiological effect of GHB is blocked by a specific $GABA_BR$, but not the GHB antagonist, NCS-382.[71-74] Indeed, aside from the recent studies by Godbout et al.[75] and Kemmel and colleagues[76] there are few electrophysiological data obtained from *in vitro* investigations to support a neuronal GHB receptor-mediated electrophysiological response.[77] However, there are two broad problems with the literature on the electrophysiological effects of GHB. First, the threshold concentration of GHB for $GABA_BR$-mediated effects in *in vitro* electrophysiological experiments appears to be around 1 mmol/L.[78] Secondly, most slice recordings are done in brains taken from rats at P12–22.[77] While $GABA_BR$ binding is demonstrable in the brains of this age animal, binding to the GHBR does not appear until P15–18 in rat and does not reach adult levels until the fourth and fifth weeks of postnatal age in that species.[58] Hence, the absence of a GHB-specific electrophysiological response in a brain slice

Table I. Comparison of the $GABA_B$ receptor to the GHB receptor

	$GABA_B$ receptor	GHB receptor
GHB	Low affinity	High affinity
GHB antagonist (NCS-382)	No affinity	High affinity, no affinity for at least one subtype
Baclofen	High affinity	No affinity
G-protein coupled	Yes	Inconclusive data
Ontogeny	Present at birth	Appears postnatal week 3
Cerebellum	High density	No binding
Hippocampus	Moderate density	High density
Cortex	Layer IV–VI	Layer I–III
Thalamus	High density	Moderate density
Effects on forskolin-stimulated cAMP	Inhibits	Inhibits

cAMP = cyclic adenosine monophosphate; **$GABA_B$** = γ-aminobutyric acid type B; **GHB** = γ-hydroxybutyric acid.

from a rat younger than 15–18 days is to be expected and does not exclude the presence of a functional GHBR in older animals.

Alternatively, GHB could activate the $GABA_B$ receptor indirectly via its conversion to GABA[29] (figure 1). This hypothesis could explain the inordinately high concentration of GHB required to produce $GABA_B$ receptor-mediated effects since high μmol/L to low mmol/L concentration of GHB are required to produce enough GHB-derived GABA to activate $GABA_B$ receptors.[79] The conversion of GHB to GABA can be inhibited by ethosuximide and valproate. When these two drugs are included in binding assays, GHB no longer competes with [^{3}H]GABA for binding at $GABA_B$ receptor.[79] Although some studies using concentrations of GHB below 100 μmol/L have failed to provide support for the GHB-derived GABA hypothesis,[80] the possibility of GHB-derived GABA playing a role when the concentration of GHB is inordinately high (figure 1), as would be the case in GHB abuse and toxicity, has not been fully addressed experimentally.

5. The GHB Receptor and $GABA_BR$ are Separate and Distinct

There are several lines of evidence to support the hypothesis that the GHBR is a distinct receptor that is separate from the $GABA_BR$ (table I). $GABA_BR$ agonists do not displace [^{3}H]GHB binding[81,82] and the ability of GHB to displace [^{3}H]baclofen binding is quite weak.[42] GHB and its antagonist, NCS 382,[29] do not compete for [^{3}H]-GABA binding in autoradiographic binding assays on rat brain sections.[81] Neither [^{3}H]GHB nor the GHB receptor antagonist [^{3}H]NCS 382 have any affinity for $GABA_BR1$, $GABA_BR2$, or the $GABA_BR$ heterodimer expressed in recombinant human embryonic kidney cells.[83] Furthermore, the ontogeny of the GHBR[58] and GHB itself[84] is decidedly different from that of GABA and the $GABA_BR$. The GHBR and $GABA_BR$ have distinct developmental profiles. $GABA_BR$ binding is detectable at birth, while GHB binding is not detected until P18.[58] Moreover, there are major differences in the regional distribution of GHB and $GABA_BR$ binding. GHB binding is most prevalent in superficial layers of cerebral cortex and the CA1 of the hippocampus while $GABA_BR$ binding is most intense in deeper cortical laminae, the thalamus, and the cerebellum, a region notable for the absence of GHB binding sites.[30,51,55,57,59-61,85]

The recent development of mutant mice in which the gene encoding the $GABA_BR1$ subunit has been deleted has helped to delineate GHBR-specific and $GABA_BR$-specific effects of GHB. In these mutant mice $GABA_BR$ binding and function is completely lost while GHBR binding is fully retained. Hence, these mutant animals provide a promising model to examine GHBR-specific effects of GHB. A recent study examining the effects of baclofen and GHB on core body temperature in $GABA_B$ knockout animals demonstrates the utility of this approach.[86] Administration of baclofen caused a large decrease in body temperature in wild-type control mice and $GABA_BR^{+/-}$ mice, but the hypothermic effects of baclofen were abolished in $GABA_BR^{-/-}$. GHB administration also is usually associated with a robust hypothermia;[87,88] however, in the $GABA_BR^{-/-}$ animals, GHB administration failed to induce hypothermia suggesting that the ability of GHB to reduce core body temperature is $GABA_BR$ mediated. Recently, Kaupmann and colleagues, in a more extensive studies in $GABA_BR^{-/-}$ mice have confirmed the absence of GHB-induced hypothermia in these mutant animals and also have shown that additional pharmacological effects of GHB are $GABA_BR$ mediated. These include GHB-induced hypolocomotion, stimulation of GTPγ[^{35}S], increased striatal dopamine synthesis and slowing on the EEG, all of which were absent in the $GABA_BR^{-/-}$ mice.[89] However, despite the fact that most of the pharmacological effects of exogenously administered GHB were absent in the $GABA_BR^{-/-}$ mice, Kaupmann et al. also demonstrated that there was a similar spatial distribution of

[^{3}H]GHB and [^{3}H]GHB binding sites in the brains of $GABA_BR^{-/-}$ and $GABA_BR^{+/+}$ mice. Hence, the GHBR and the $GABA_BR$ are indeed separate and distinct from one another, but those authors conclude that virtually all of the pharmacological, behavioural and toxicological effects of GHB are mediated by the $GABA_BR$; however, again, the high doses of GHB used in the mutant mouse experiments (1 g/kg) would select out for $GABA_BR$-mediated effects because GHB is a weak partial agonist at the $GABA_BR$.

5.1 GHB Modulation of Trafficking of the $GABA_BR$

Recently, a third potential mechanism of action by which GHB may modulate $GABA_BR$ function has emerged. Recent experiments have examined the effect of GHB on the intracellular trafficking of the $GABA_BR$, and demonstrated two distinct trafficking mechanisms of action for GHB at the $GABA_BR$. These data provide support for the hypothesis that GHB is converted to GABA and suggest that GHB-derived GABA activates the $GABA_BR$ and, in so doing, induces an agonist-induced internalisation of $GABA_BR$ resulting in a decrease of the number of $GABA_BR$s present on the cell surface. However, the situation is made more complex by the finding that GHB itself binds to the $GABA_BR$ and *increases* the number of $GABA_BR$s present on the cell surface of cortical neurons, a diametrically opposite effect from the $GABA_BR$ agonist effect. Hence, by inhibiting the endocytosis of $GABA_BR$ GHB increases the overall number of cell surface $GABA_BR$ and increases $GABA_BR$-mediated function. These data suggest that the end result of GHB action on the $GABA_BR$ is that GHB-derived GABA immediately activates the $GABA_BR$ and induces endocytosis of $GABA_BR$, while GHB itself opposes this process and, retains $GABA_BR$ on the cell surface, and thus prolongs the functionality of the receptor.[90] Although baclofen and GHB both activate the $GABA_BR$, they have opposite effects on trafficking, which may potentially explain certain differences in the physiological effects between these two $GABA_BR$ agonists.

While many studies have demonstrated that supra-physiological concentrations of GHB bind to and increase $GABA_BR$ function, one must also consider the fact that high concentrations of GHB also will simultaneously saturate the high-affinity sites on GHBRs. Thus, the pharmacological effects of supra-physiological concentrations of GHB may be due to activation of $GABA_BR$s, saturation of GHBRs and/or a combination of the two events.

6. GHB Modulation of Dopaminergic Function

Although GHB has protean effects on serotonergic, cholinergic and opiatergic neurotransmitter systems,[29] the bulk of the literature on its effects on other neurotransmitter systems in the brain concerns the attenuation of dopamine-mediated neuronal activity.[91] GHB has long been known to increase the brain levels of dopamine,[92-94] decrease dopamine turnover,[95] and decrease neuronal cell firing of dopaminergic neurons in the striatum.[74,96] However, low 'sub-anaesthetic' doses of GHB have been reported to increase cell firing in striatal dopaminergic neurons in unanaesthetised rats.[97] The initial reports of an increase in brain dopamine concentrations following GHB administration were later followed by studies which indicated a decrease of dopamine levels in animals treated with GHB occurs in response to an inhibition of dopamine release,[98] an effect that can be reversed by treatment with the opiate antagonist naloxone.[99,100]

Chronic treatment with GHB results in upregulation of D_1 and D_2 mRNA expression in brain regions rich in GHB receptors[101] and a tolerance to the effect of GHB on brain dopamine levels. Also, chronic treatment with the dopamine D_2 receptor antagonist sulpiride increase GHB receptor maximum specific binding (B_{max}) values.[102]

The attenuation of dopamine neurotransmission that follows GHB administration may underlie the loss of locomotor activity that can occur in humans and experimental animals. Rodent studies have shown that GHB given in various supra-physiological doses, ranging from 200 to 800 mg/kg, can lead to sedation and a dose-dependent decrease in motor activity.[103-105] These effects were shown to be effectively antagonised by pretreatment with various $GABA_B$ receptor antagonists, CGP 35348, CGP 46381 and SCH 50911.[106] Carai and colleagues have reported similar findings and demonstrated that the GHB-induced loss of motor activity was associated with a highly significant decrease in striatal 3-methoxytyramine (3-MT) concentrations.[105] Since dopamine is inactivated in the synaptic cleft by the action of catechol-*O*-methyltransferase resulting in 3-MT formation, these observations provide further evidence that GHB may act to inhibit dopamine release. Pre-treatment with $GABA_B$ antagonists, CGP 35348 and CGP 36742 not only antagonised the motor effects but also the loss of striatal 3-MT.[107] Collectively, the above data strongly support an acute pharmacological action of GHB to stimulate $GABA_B$ receptors, which results in inhibition of dopamine release. The effects of chronic high doses of GHB on dopaminergic neurotransmission have yet to be studied.

7. EEG-Behavioural Effects of GHB

When administered systemically, both GHB and its prodrug GBL produce a predictable sequence of dose-dependent EEG changes associated with progressive obtundation in both animals and humans.[12,88,108-115] Parenteral administration of doses of 100–200 mg/kg of GHB or GBL to rats result in EEG and

behavioural perturbations that closely resemble generalised absence seizures seen in humans.[116-118] The EEG of a rat given 100–200 mg/kg GHB or GBL is characterised by bilaterally synchronous spike-and-wave discharges (SWD) that are associated with immobility, starring, facial myoclonus and vibrissal twitching. When doses of 200–300 mg/kg of GHB or GBL are given to rats the bilaterally synchronous discharge first becomes continuous and then evolves into generalised slowing. When the dose is increased to >300 mg/kg burst suppression appears with the isoelectric period between bursts increasing as the dose increases. There is deepening coma associated with a prolonged loss of the righting reflex that appears at doses of 200 mg/kg and higher.[119] Developmentally, this progression of EEG and behavioural changes in response to systemic GHB or GBL occurs in reverse. For the first 10–12 days of life, GHB or GBL administration in the rat results in burst suppression on the EEG associated with coma. From 12–16 days of age, EEG slowing appears in response to GHB/GBL administration. The bilaterally synchronous SWD that is the hallmark of experimental absence seizures in the GHB model of this disorder in the rat appear at postnatal day 16–18.[120] The GHB/GBL-induced SWD in rat is blocked by both anti-absence anticonvulsant drugs[118,121] and $GABA_BR$ antagonists.[122] Neither the EEG slowing nor the EEG burst suppression induced by GHB or GBL in adult or developing rats are blocked by anti-absence drugs; however, these EEG changes in response to higher doses of GHB or GBL are aborted by $GABA_BR$ antagonists.[58]

Generalised absence seizures may be defined as a paroxysmal loss of consciousness of abrupt and sudden onset and offset which is associated with bursts of bilaterally synchronous 3 cycles/sec SWD recorded on the EEG. There is no aura or postictal state. This particular type of seizure usually occurs in children between the ages of 4 years and adolescence, although they may occur at either ends of that age spectrum.[123-125] In order to be a valid investigative tool, an animal model of generalised absence seizures should reflect the clinical and pharmacological characteristics of this disorder[121] (table II). The animal model should manifest bilaterally synchronous SWD that can be recorded on EEG only from thalamocortical and not hippocampal circuitry.[126-132] During the SWD the animal should have behavioural immobility, the onset and offset of which comport precisely with the onset and offset of the SWD.[132] The experimental absence seizures should be blocked by anti-absence drugs and exacerbated by phenytoin and carbamazepine.[115,118,121] As with any animal model of seizures, the animal model of absence seizures should be reproducible, predictable and quantifiable.[121] Animal models of absence seizures should reflect the fact that both clinical and experimental absence seizures are exacerbated by both direct and indirect GABA agonists[122,133-136] and abolished by $GABA_BR$ antagonists.[58,122] Finally, animal models of absence seizures should reflect the fact that this is a disorder of children and have a unique developmental profile.

Table II. Characteristics of γ-hydroxybutyric acid-induced absence seizures

Bilaterally synchronous SWD associated with behavioural immobility
Blocked by ethosuximide, trimethadione, valproic acid and benzodiazepines
Exacerbated by carbamazepine and phenytoin
Developmental onset that comports with development of thalamocortical circuitry
Hippocampal circuitry is silent during SWD
Involvement of thalamocortical mechanisms
Exacerbated by $GABA_AR$ and $GABA_BR$ agonists
Blocked by $GABA_BR$ antagonists
Reproducible and predictable
Quantifiable

$GABA_AR$ = γ-aminobutyric acid type A receptor; **$GABA_BR$** = γ-aminobutyric acid type B receptor; **SWD** = spike-and-wave discharges.

The GHB model of absence seizures in the rat fulfils all of the criteria for an experimental model of absence seizures[118,121] (table II), and is used routinely as such. A standard dose of 100mg GBL in 0.09 mL/kg given intraperitoneally (IP) reliably produces onset of bilaterally synchronous 7–9 cycles/sec SWD within 2–5 minutes. Associated with these hypersynchronous electrographic changes are behavioural arrest, facial myoclonus and vibrissal twitching. This model is therefore predictable, reproducible and replicates the electrographic and behavioural events that occur in the human condition.[118] An additional advantage of the GHB model is that it affords control of pharmacokinetic variables in any pharmacological study since the concentration of GBL and GHB can be determined in the brain and the kinetics are known.[7]

The rapid onset of SWD induced by GBL in the rat correlates with an almost immediate appearance of GHB in the brain. In contrast, animals that receive IP GHB do not manifest EEG changes until about 20 minutes after administration, when its levels in the brain are peaking. The threshold brain concentration of GHB for EEG changes in both GHB and GBL-treated rats is 240 μmol/L. GBL concentration in brain peaks 1 minute after GBL administration and falls rapidly to undetectable levels within 5 minutes. Bilateral microinjection of GHB into thalamus resulted in brief bursts of SWD, while GBL administered into the thalamus has no effect. These data confirm the hypothesis that GBL is biologically inactive in the brain and support the validity of the use of GBL as a pro-drug for GHB in this model of absence seizures.[7]

The GHB model of absence seizures is quantitated in a matter similar to other EEG models of absence seizures.[137] GHB-induced SWD may be quantitated in terms of cumulative duration (seconds) per 20-minute epoch of time, or as a percentage of control SWD duration. In this way, the GHB model of absence may be compared with any other rodent model of generalised absence seizures using the same pharmacological paradigm.[137]

8. Putative Mechanisms of GHB-Induced Absence Seizures

Although there is some evidence of involvement of glutamatergic[66,138-142] and $GABA_AR$-mediated[143-146] mechanisms in the pathogenesis of GHB-induced absence seizures, most of the data on this subject point to either $GABA_BR$ and/or GHBR-mediated activity as being critical to the phenomenon of GHB-induced absence seizures. The discovery that the late, long lasting, potassium-dependent inhibitory postsynaptic potential (IPSP) was mediated by the $GABA_BR$ and that $GABA_BR$-mediated IPSPs in thalamus activated low-threshold calcium potentials which led to burst firing and oscillatory behaviour in thalamic neurons gave rise to the hypothesis that $GABA_BR$-mediated mechanisms might be operative in the pathogenesis of generalised absence seizures.[147] In support of this hypothesis, a specific $GABA_BR$ antagonist, such as CGP 35348, has been shown either to attenuate or block SWD in the GHB and pentylenetetrazole absence seizure models in the rat,[122,148] a genetic rat model of absence seizures,[149] and the *lh/lh* absence seizure model in the mouse[148,150,151] all in a dose-dependent fashion.

The absence seizure animal model experiments, taken in conjunction with *in vitro* data that demonstrate the role of $GABA_BR$-mediated IPSPs in regulating thalamocortical oscillatory behaviour via low-threshold calcium currents,[147,152-154] strongly suggest that $GABA_BR$-mediated mechanisms might be involved in the pathogenesis of absence seizures. Indeed, the animal model data suggest that the $GABA_BR$ is the final common pathway in absence seizures.

More recent data in favour of a $GABA_BR$-mediated mechanism in GHB-induced absence seizures may be found in the recent report that a mutant mouse, in which there was a null mutation of the α (1G) subunit of T-type Ca^{2+} channels, was resistant to GHB-induced absence seizures.[155] In addition, concentrations of GHB that are threshold in the brain for absence seizures (i.e. 250 µmol/L) have been shown to reversibly decrease the amplitude of electrically evoked excitatory postsynaptic currents and $GABA_AR$-mediated inhibitory postsynaptic currents (IPSCs) via activation of $GABA_BR$; however, approximately 60% of the IPSCs were insensitive to this concentration of GHB. The GHBR antagonist NSC 382 applied alone had no action on, or further increased, the GHB-elicited effects on synaptic currents. 250 µmol/L GHB also was effective in increasing absence-like intrathalamic oscillations evoked by cortical afferent stimulation.[156]

Although the data described above provide compelling evidence in support of the hypothesis that a $GABA_BR$-mediated mechanism is operative in GHB/GBL-induced absence seizures, it should be noted that GHB-induced absence seizures also are blocked by the GHB antagonist NCS 382,[148] raising the possibility that there is a GHBR-specific mechanism also involved in the molecular pathogenesis of GHB-induced absence seizures. As discussed in sections 9 and 11.1, all of the experimental data aimed at elucidating the biochemical and electrophysiological mechanisms of GHB- and GBL-induced absence seizures are referable to the other clinical effects of GHB on learning and memory and may be useful in explaining the addictive properties of this compound.

9. Effect of GHB on Learning and Memory

The precise effect of GHB on learning and memory is poorly defined and there is a distinct dichotomy between the clinical and preclinical data on this issue. Most of the clinical information concerning the effect of GHB on memory has been gleaned from case reports of GHB abuse and overdose; however, two clinical studies have been reported in which the cognitive effect of acute GHB exposure in healthy individuals was examined. In 1971, Grove-White and Kelman observed that subjects who were administered GHB 10 mg/kg intravenously exhibited a significant short-term memory deficit in digit recall test at a prolonged delay interval of 20 seconds, but not at the shorter interval of 4 seconds. These authors postulated that the GHB accelerated the decay of memory trace and disrupted the consolidation process during memory formation.[157] In contrast, in 1999 Ferrara et al. reported no change in short-term memory with an acute oral dose of GHB 12.5 or 25 mg/kg.[158] There were no correlative measures of serum concentrations of GHB in these studies and the choice of reaction time as a measure of short-term memory[159] did not allow for the assessment of learning and retention ability at varying delay intervals. Rather, this paradigm evaluated short-term memory on the basis of response latency and error frequency.

The two studies also differed greatly in the pharmacokinetic parameters utilised. The impaired performance reported by Grove-White and Kelman was observed 5 minutes following, but not at later times (15, 30 and 90 minutes), after intravenous (IV) GHB administration, suggesting that the effect of parenteral GHB is rapid in onset and short in duration, characteristics of GHB that have been observed in experimental animals.[7,112,160] Ferrara et al.

tested the effect of orally administered GHB initially at 15 minutes, but made no repeated measures until 60, 120 and 180 minutes following GHB administration. Ferrara and colleagues also noted that GHB was absorbed easily after an oral dose with a time to maximum concentration (t_{max}) of 20–45 minutes for a dose of 25 mg/kg.[161-163] The majority of subjects in the Ferrara study experienced dizziness or dullness that occurred 20 minutes following GHB administration. These symptoms disappeared within 30–60 minutes of receiving the oral dose of GHB. The absence of testing during the 15–60 minute interval following the oral administration could explain the fact that no change in performance was observed following an oral dose of GHB. The findings of Grove-White and Kelman comport with reports of memory impairment associated with GHB abuse and overdose. GHB intoxication is commonly reported to be associated with mental confusion[164-166] and/or amnesia.[165]

Endogenous serum concentrations of GHB in healthy individuals is <2.5 mg/L;[167] however, patients who are abusing GHB in the absence of other psychoactive substances have been reported to have blood levels of GHB that range from 90 to 127 mg/L,[165] which is equivalent to an oral dose of >75 mg/kg.[168] Significant short- and long-term memory impairment have been reported following daily consumption of GHB at 395–477 mg/kg/day for 2.5 years.[169] A strict clinical dose-response effect of GHB in this regard is difficult to ascertain because most of the patients who were subjects of these reports had purchased the drug illegally for recreational or body-building purposes. Hence, the purity and size of the described 'capful' or 'teaspoon' dosages vary widely and there is no accurate method of assessment of the actual dose. In addition, the confound of other drugs of abuse may be a problem in interpretation of the contribution of GHB to memory deficits since GHB often is not the only recreational drug ingested.

GHB has been shown to affect long-term potentiation in the hippocampus,[170,171] a well-accepted neuronal substrate for learning and memory. The ability of GHB to induce amnesia may be mediated through the $GABA_BR$ since $GABA_BR$ agonists (e.g. baclofen) also have been reported to impair learning and memory retention[172-177] in both spatial maze and fear-motivated avoidance paradigms. Conversely, $GABA_BR$ antagonists (e.g. CGP 36742 and its analogues) have been shown to enhance cognitive function in the rat.[178-183]

In summary, the clinical data suggest that both short- and long-term use of GHB, particularly at doses >75 mg/kg, have the potential to impair human memory. The result is that there may be 'no memory of event' that transpires during GHB intoxication.[166]

10. Neuroprotective Effect of GHB

In contrast to the human experience, similar or higher doses of GHB administered to rodents have been shown to be anti-amnesic in the presence of a variety of brain insults known to impair memory. For example, prenatal challenges during mid-gestation with either sodium nitrite-induced ischaemia[184] or an exposure to a 2-hour hypobaric[185] hypoxic insult both can lead to a disturbed memory in rats born of mothers subjected to these insults as demonstrated by passive and active avoidance paradigms. However, GHB, when administered daily at a dosage of 150 mg/kg/day to mothers from embryonic day 12 to 19, abolished the perturbations in cognition observed in control offspring in the sodium nitrite model.[186] GHB had the same effect when given in a daily dosage of 50 mg/kg/day from P8–20 in the hypobaric hypoxia model.

Intrastriatal administration of endothelin-1 and the four-vessel occlusion model of carotid arteries result in focal[187] and global[188] cerebral ischaemia, respectively. Both of these insults are associated with significant spatial learning and memory deficit with a prolonged escape latency in the Morris water maze task. GHB affords neuroprotection against ischaemia-induced impairment of cognition and greatly attenuates the perturbed spatial memory observed in this model when given in an IP dose of 300 mg/kg followed by 100 mg/kg twice daily for 10 days after these ischaemic insults, but lower doses are ineffective. The improved Morris water maze performance was accompanied by a substantial reduction in CA1 hippocampal neuronal loss and improvement in sensorimotor orientation and coordinated limb use.

Sodium or lithium salts of GHB given in a dose of 50–100 mg/kg for 2 weeks prevented impaired learning in response to stress associated with alcohol intoxication induced pre- or postnatally.[189-192] GHB administration throughout the second week of life also antagonises disturbances in conditioned avoidance, extrapolation, habituation, motor hyperactivity and movement coordination induced by the suppression of protein synthesis by acute cycloheximide injection on P7.[193,194] GHB may owe its 'anti-amnestic' effect in these models to a more global neuroprotective action occasioned by the ability of GHB to decrease energy utilisation,[195,196] lower oxygen demand and consumption[197-199] dampen neuronal firing rates,[75,200] and/or diminish the release of excitatory amino acids.[62,201,202]

The effect of GHB on animal models subjected to prior neurological insult may have limited relevance to the effect of GHB on human memory. However, the salt forms of GHB, lithium and sodium gammahydroxybutyrate have been shown to exacerbate retrograde amnesia caused by electroshock in the passive avoidance task in the rat at doses of 50 mg/kg; however, a higher dose of

200 mg/kg of GHB had the opposite effect and abolished electroshock-induced amnesia.[203] The reason for the biphasic action of GHB in these experiments is not clear. The authors postulated that the anti-amnestic effect of the higher dose of GHB might be related to the promotion of leucine incorporation during protein formation.[204,205] Alternatively, the sedation produced by a dose of GHB 200 mg/kg[103,206-209] could confound the fear-motivated task of the passive avoidance parodyne and mask any improved memory that might result from a lower, non-sedating dose of GHB.

11. Toxicology of GHB

The pharmacokinetics of GHB are species-dependent. GHB has a half-life of about 1 hour in the rat,[8] but a longer half-life in the cat,[210] dog,[211,212] mouse,[212] non-human primate[213] and human.[162,214] It crosses rapidly and passively across the blood-brain barrier following systemic administration.[7,210,211,213,215] GHB elimination kinetics are non-linear in animals and humans.[162,211,212,216,217] Oral administration of increasing doses of GHB does not proportionately result in a decrease in peak plasma concentrations and an increase in the median time-to-peak concentration suggesting a capacity-limited absorption.[162] Food is reported to significantly alter the bioavailability of GHB by decreasing mean peak plasma concentration, increasing median time-to-peak concentration and decreasing the area under the plasma concentration-time curve.[218] Following a dose of 1 or 2g of GHB, the drug first appears in the urine less than 10 hours post-dosing.[219] Urinary levels under these conditions are in the low mg/L range. The pharmacokinetics of GHB are not affected by sex[218] or hepatic dysfunction.[162]

GHB, GBL, and 1,4-butanediol are all potentially toxic compounds.[24,28,220,221] GBL owes all of its pharmacological and toxicological effects to its conversion to GHB, since GBL itself is biologically inactive.[6-9] Similarly, most of the toxic effects of 1,4-butanediol result from its metabolism to GHB[32,222-224] via an alcohol dehydrogenase;[105,224,225] however, the diol itself also carries an inherent toxicity[225] which is enhanced in the presence of ethanol, probably due to a competition of the two compounds for alcohol dehydrogenase.[226]

Clinically, intoxication with GHB and its analogues is characterised by aggressive behaviour, ataxia, amnesia, vomiting, somnolence, bradycardia, respiratory depression and apnoea, seizures and coma.[227-232] Well over 70 deaths have been reported with overdose of either GHB and/or its congeners.[220] Many patients who present with GHB intoxication have abused other drugs as well[233,234] or are on medications with which there may be a life-threatening interaction with GHB.[235]

The management of GHB intoxication in a spontaneously breathing patient is primarily supportive with intravenous access, oxygen supplementation, and atropine for any persistent bradycardia.[233,236,237] Because of the relatively short half-life of GHB in humans (within hours), if the patient has recovered within 6 hours they can be sent home; otherwise, they should be admitted to the hospital. Several groups have reported a beneficial effect of physostigmine in reversal of the clinical signs of GHB intoxication;[238-241] however, a recent analysis of these clinical data concluded that there is insufficient evidence to support the routine use of physostigmine in the treatment of GHB toxicity.[242]

Clinically, the effects of GHB are dose-dependent. Low doses (10 mg/kg) induce short-term anterograde amnesia, increased libido, euphoria, suggestibility and passivity, all of which contribute to the utility of GHB in sexual assaults.[19,243,244] Doses of 20–30 mg/kg result in drowsiness and sleep, while doses of 50 mg/kg lead to general anaesthesia, with no analgesia. Doses higher than 50 mg/kg can result in coma, cardiorespiratory depression, seizures and death.[232,233]

In non-human experimental models, dose-dependent effects also are observed for GHB. Doses of 10–50 mg/kg in rats causes memory deficits, while doses ranging from 75–200 mg/kg induce absence seizures.[148] Administration of 200–300 mg/kg to rat results in stupor and dependence, while doses higher than 300 mg/kg causes coma that can be blocked by $GABA_BR$ antagonists but not the GHBR antagonist.[245] The median lethal dose (LD_{50}) of GHB in the rat is 1.7 g/kg.[246]

11.1 GHB Abuse, Addiction and Withdrawal

Street names for GHB include G, liquid ecstasy, grievous bodily harm, Georgia home boy, gib, liquid X, soap, easy lay, salty water, scoop and nitro.[28] GHB abuse has arisen from the euphoria, disinhibition and heightened sexual awareness said to be associated with its use.[27] The recent surge in the use of GHB as a major recreational drug has led to reports of addictive properties of this compound in humans.[27] In addition, GHB withdrawal symptoms similar to those observed in ethanol withdrawal have been reported in humans.[25,169] In the majority of these cases, tolerance to GHB occurred.

The data from studies in rodents are in concordance with the clinical data. GHB is self-administered by rats and mice, and rats undergoing chronic GHB administration exhibit tolerance and withdrawal.[247-249] Animals that receive GHB chronically also develop conditioned place preference suggesting a rewarding effect of GHB.[250,251] The mechanism(s) of action underlying the addictive properties of GHB are not clear. There is some evidence to suggest that the addictive properties may be mediated by the

GHBR because the GHB antagonist, NCS-382 is reported to block GHB self-administration.[252] Alternatively, there are data that indicate that the addictive properties of GHB are mediated through the $GABA_B$ receptor. GHB self-administration is blocked by administration of the $GABA_B$ receptor agonist baclofen.[253]

Although there is some disagreement about the quality of the evidence,[254] GHB appears to be effective in the treatment of both alcoholism[255] and alcohol withdrawal syndrome.[256,257] However, in alcoholic patients treated with GHB, relapse rates are high. Moreover, the use of GHB in the treatment of alcohol withdrawal has the potential to lead to GHB addiction or withdrawal.[258-260]

In order to utilise basic experimental data to formulate testable and credible hypotheses concerning the molecular mechanisms that underlie GHB abuse, addiction and withdrawal, one must consider the dose response of GHB observed in both *in vitro* and *in vivo* experiments and correlate those data with brain levels of GHB achieved in the clinical situation under discussion. Theoretically, low concentrations of GHB in the brain (i.e. <100 μmol/L) would be predicted to activate the GHBR while higher concentrations in the brain, such as would result from GHB, GBL, or 1,4-butanediol intoxication, abuse or addiction, would activate the $GABA_BR$ both by a direct effect of GHB and by an effect of GHB-derived GABA. In addition, these high concentrations of GHB also would saturate brain GHBR. Therefore, the clinical signs and symptoms that result from the doses of GHB seen in intoxication, abuse, addiction and withdrawal represent a cumulative effect of maximal stimulation of GHBR and $GABA_BR$ in the brain by GHB.

12. Succinic Semialdehyde Dehydrogenase Deficiency

SSADH deficiency is a rare autosomal recessive defect in the catabolic pathway of GABA (figure 1) that was first reported in 1981.[39] The clinical phenotype of SSADH deficiency is rather nonspecific with most patients presenting a picture of global developmental delay, hypotonia, ataxia and poorly developed to absent speech. Approximately 50% of SSADH-deficient patients manifest overt seizure activity. Seizure subtypes include absence, myoclonic, generalised tonic-clonic and status epilepticus. Patients with SSADH deficiency accumulate both GHB and GABA in body fluids including plasma, urine and cerebrospinal fluids. Accumulation of GHB establishes the diagnosis of SSADH deficiency.[261,262] In addition, both human and murine SSADH deficiency demonstrate accumulation of other intermediates, including GABA, homocarnosine (the GABA-histidine dipeptide), and 4,5-dihydroxyhexanoic acid, a postulated derivative of SSA with an activated, but uncharacterised, two-carbon species, Patients with SSADH deficiency also manifest significantly decreased levels of glutamine.[263-266]

In order to delineate pathological mechanisms in human SSADH deficiency and develop a model system of chronic GHB accumulation in the brain, a murine knockout model of SSADH deficiency has been developed.[267] These mice are born at the expected Mendelian frequency for an autosomal recessive disorder[268] and accumulate very high levels of GHB and GABA in tissues and physiological fluids – both in the periphery and in the central nervous system.[267,269] $SSADH^{-/-}$ mice manifest early absence seizures from about P14 that evolve into generalised convulsions with an explosive onset of status epilepticus at 3–4 postnatal weeks of age. The status epilepticus in the mutant mice is uniformly fatal.[267,269,270] The transition from absence seizures to generalised convulsive seizures in the $SSADH^{-/-}$ mice appears similar to the transition from absence seizures to generalised convulsive seizures that is observed clinically in about half of children with absence epilepsy.[271] Hence, the $SSADH^{-/-}$ mouse may represent an animal model for this clinical phenomenon. A potential explanation for the transition from absence to generalised convulsive seizures in the $SSADH^{-/-}$ mice may derive from the fact that the accumulated GHB and GABA in the brains of these mutant animals leads to a desensitisation, and consequent down-regulation, of $GABA_BR$ with a resultant decrease in inhibitory neurotransmission.[272]

The murine model of SSADH deficiency may be used to develop novel therapies for this inborn error of metabolism. Therapeutic intervention for human SSADH deficiency historically has been limited to vigabatrin (VGB). Theoretically, VGB should be highly effective since its pharmacological action of GABA-transaminase (GABA-T) inhibition should lead to lowered succinic semialdehyde, and therefore GHB, levels. Unfortunately, the clinical efficacy of VGB in the treatment of SSADH deficiency has been variable at best.[273,274] Moreover, if the hypothesis that increased GABA in the brains of children with SSADH deficiency contributes to a desensitisation of the $GABA_BR$, increasing brain GABA levels further by blocking GABA-T with VGB may not be desirable. Other antiepileptic drugs (i.e. carbamazepine, lamotrigine) have been employed with variable success,[262,263] and behavioural problems have been treated symptomatically.

Survival of $SSADH^{-/-}$ mice was compared following administration of high-dose VGB, CGP 35348 (a $GABA_BR$ antagonist) or the non-protein amino acid taurine. The latter was employed because of deterioration in suckling mice at weaning, and the high concentration of taurine in murine breast milk. In addition, the efficacy of NCS-382, a specific GHB receptor antagonist, in prolonging survival of $SSADH^{-/-}$ mice was assessed. All interventions led to significant lifespan extension (22–61%), with

NCS-382 being most effective.[266] These data indicate that both GHBR and $GABA_BR$ are involved in the pathophysiology of SSADH deficiency. Based on the hypothesis put forth earlier in this review, these data support the concept that the clinical signs and symptoms of SSADH deficiency result from a cumulative effect of maximal stimulation of GHBR and $GABA_BR$ in the brain by the markedly elevated brain levels of GHB. These high levels of GHB activate the $GABA_BR$ by both a direct effect of GHB as well as by an effect of GHB-derived GABA. This is further exacerbated by the GABA that accrues from the SSADH deficiency. Furthermore, the data suggest that taurine and NCS-382 may hold therapeutic promise in the treatment of human SSADH deficiency as they are most effective in prolonging the survival of the $SSADH^{-/-}$ mice.

An example of the utility of the mutant $SSADH^{-/-}$ mouse in testing hypotheses of the pathogenesis of certain clinical manifestations of SSADH deficiency may be seen in experiments testing the hypothesis that monoamines are involved in the pathogenesis of behavioural disturbances in patients with SSADH deficiency. Catecholamine metabolites were determined in $SSADH^{-/-}$mouse brain extracts in which the GHB concentration is approximately 200–250 μmol/L. Only homovanillic acid (HVA), the end-product of dopamine metabolism, was significantly increased in mutant mice. There was a significant linear correlation between brain GHB concentration and both HVA and 5-hydroxyindoleacetic acid (5-HIAA, the end-product of serotonin metabolism). There was no alteration in serotonin turnover.[275]

Based on these data from the $SSADH^{-/-}$ mouse, the hypothesis that behavioural disturbances in SSADH-deficient patients, including hallucinations, anxiety, aggression and altered sleep patterns, would correlate with altered catecholamine metabolism was formulated. Catecholamine patterns were thus determined in cerebrospinal fluid (CSF) derived from patients. The CSF GHB concentration ranged from 200–800 μmol/L, comparable to levels in $SSADH^{-/-}$ mouse brain. Similar to the findings in the mutant mouse, in the patients studied there was a linear correlation between GHB levels and HVA/5-HIAA concentrations, but no significant difference in HVA and 5-HIAA levels between controls and patients.[265] Since serotonin is critically important for maintenance of mood, sleep patterns and anxiety levels, altered serotinergic neurotransmission in SSADH deficient patients may underlie a significant portion of the behavioural abnormalities seen in these children.

13. Conclusion

Although an unfortunate social happenstance, the emergence of GHB addiction and abuse as a public health problem has instigated a significant amount of research into the fundamental properties of this interesting compound. As a result, there has been significantly increased understanding of the neurobiology, pharmacology and toxicology of GHB. Also, much clinical experience has been gained in the diagnosis and treatment of GHB abuse, addiction and withdrawal as well as the with the therapeutic use of GHB in drug and alcohol addiction and withdrawal. However, a number of important questions about GHB remain to be answered. Prime among these is what is the elusive neurobiological function of GHB? The answer to this question will be facilitated by the molecular cloning of the GHBR that is specifically antagonised by NCS 382 and has the regional distribution and binding kinetics reported for the classical GHBR, and subsequent engineering of GHBR null mutant mice. This molecular development also should lead to a more precise elucidation of the relationship, if any, between GHBR and $GABA_BR$. Such $GHBR^{-/-}$ mice could be used to tease out the relative contribution of the GHBR, the $GABA_BR$, and GHB-derived GABA in GHB toxicity, abuse, addiction and withdrawal as well to formulate and test hypotheses of the fundamental mechanisms that underlie GHB-induced absence seizures.

Finally, a number of practical clinical questions have arisen from all of the clinical and experimental data accrued to date on GHB. Would $GABA_BR$ or GHBR antagonists, be useful therapeutically in the treatment of GHB overdose, addiction, tolerance and withdrawal? Would these drugs be useful in the treatment of addiction to and/or withdrawal from opiates, cocaine or other drugs of abuse? Would $GABA_BR$ or GHBR antagonists be useful therapeutically in SSADH deficiency? If so, when should treatment be started? What would be the developmental consequences of such treatment in young children?

The experimental data reviewed in this paper provide compelling support for the hypothesis that $GABAB_BR$ antagonists would be effective for GHB toxicity, abuse and withdrawal and that both $GABA_BR$ and GHBR antagonists hold therapeutic promise in the treatment of children with SSADH deficiency. However, valid clinical proof of efficacy for these compounds will have to await the availability of $GABA_BR$ and GHBR antagonists that can be used in clinical trials specifically designed to address these important questions.

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Correspondence and offprints: Dr *O. Carter Snead*, III, Division of Neurology, Hospital for Sick Children, 555 University Avenue, Toronto, ON, Canada M5G 1X8.