

INTRODUCTION

Gamma-hydroxybutyrate (GHB) is both a naturally occurring compound in the human brain and a synthetic drug. Couper and Marinetti (2002) recently summarized the history of the clinical and recreational uses of this drug, which is one of the products of the gamma-amino butyric acid (GABA) metabolic pathway (see Figure 1). When administered externally, GABA does not readily cross the blood-brain barrier and, as a result, has little therapeutic value. On the other hand, GHB crosses the blood-brain barrier quite easily. It was originally synthesized in 1960 in the hope that it would succeed where GABA failed as an experimental treatment for seizures. Later, it found use as an anesthetic due to its properties as a central nervous system (CNS) depressant. GHB did not produce analgesia, however, so it lost much of its popularity as an anesthetic agent (Tunnicliff, 1997). In fact, GHB is not currently approved for any medical use in the United States. However, researchers are looking into possible uses for GHB in the treatment of sleep disorders such as narcolepsy, alcohol and opiate withdrawal symptoms, schizophrenia, and fibromyalgia. GHB has also gained in popularity in recent years as a drug of abuse. It produces effects similar to those of ethanol, including euphoria, mood enhancement, and relaxation at low doses and sleep, decreased cognitive function, and coma at higher doses. Those who abuse the drug over long periods of time can experience addiction, tolerance, and withdrawal symptoms (Bernasconi, et al., 1999).

In spite of all the current knowledge about GHB's physiological effects, however, not as much is known about how GHB produces those effects. A starting point for many researchers has been to investigate the endogenous GHB system in the human brain, including its receptors and its function as a neurotransmitter. The next step is to examine what happens in this endogenous system when a dose of GHB is administered externally. How does exogenous GHB affect the function of other neurotransmitters, such as dopamine and serotonin? How does GHB

interact with opioid mechanisms? Finally, and most importantly, researchers have tried to make connections between GHB's mechanism of action and its clinical effects. Although the conclusions drawn have been a point of contention within the scientific community at times, the overall goal is clear: "A better understanding of its mechanism of action might help uncover new therapeutic uses and/or foster a greater appreciation of potential adverse effects" (Madden and Johnson, 1998). A current picture of the mechanism of action of GHB reveals many clues to further this understanding as well as many unanswered questions.

ENDOGENOUS GHB SYSTEM

GHB was first demonstrated to be an endogenous substance in mammals in 1970 (Roth and Giarmann). It occurs naturally in all regions of the brain as well as in several other body tissues, including the lung, liver, heart, muscle, and kidney (Vayer, et al., 1987). In the brain, the highest concentrations of GHB are found in the hippocampus, striatum, and substantia nigra (Maitre, 1997). Most researchers currently agree that GHB meets the criteria necessary to be classified as a neurotransmitter. First, GHB is synthesized in neurons. This occurs when GABA is deaminated to produce succinic semialdehyde (SSA), which is then reduced by SSA reductase to form GHB (see Figure 1 for pathway). The specific SSA reductase enzyme responsible for this conversion is only found in neurons (Vayer, et al., 1987). GHB is rapidly degraded through the reverse of this process: oxidation to SSA by GHB dehydrogenase and either conversion back to GABA or to succinic acid, part of the Krebs cycle. When neurons containing GHB are depolarized by the influx of calcium ions, GHB is released into the extracellular space. Muller et al (2002) have recently demonstrated the existence of a vesicular transport system for GHB. Finally, there are specific receptors for GHB in the synaptic region of neurons.

The search for GHB's binding sites first led researchers to look at the GABA family of receptors, since Xie and Smart demonstrated in 1992 that some of the neuronal action of GHB could be inhibited by GABA_B receptor antagonists. After several years of contradictory reports, Lingenhoehl and his team (1999) used the newly-cloned GABA_B receptor to test the affinity of GHB binding and found GHB to be a weak agonist (EC₅₀ of approximately 5 mM) for these receptors. It appears from these results that these receptors are not a major site of action in the endogenous GHB system since natural GHB concentrations would be too small to overcome the low binding affinity. However, several of the effects of high doses of exogenous GHB have been linked to action at the GABA_B receptors. These effects include increases in extracellular glutamate (Ferraro, et al., 2001) and acetylcholine (Nava, et al., 2001) in the hippocampus, as well as increases in neurosteroid levels in the cortex and hippocampus (Barbaccia, et al., 2002). Also, the study by Lingenhoehl et al disproved a theory about a special high affinity GHB binding site on the GABA_B receptor. The search for GHB binding sites had to continue.

While the research into GHB's interaction with the GABA_B receptor went on, evidence showing the existence of a separate, specific GHB receptor mounted. Snead (1996) hypothesized the existence of presynaptic, G protein-coupled GHB receptors in the brain. Maitre (1997) further proposed that the G proteins coupled to the GHB receptor were from either the G_i or G_o protein family. However, Bernasconi and his team (1999) questioned these conclusions for several reasons. First, they argued that no one has identified a neuronal path that uses GHB. Second, they questioned the role of endogenous GHB in signal transmission. Finally, they pointed out that GHB crosses the blood-brain barrier and is found in its highest concentrations outside the CNS. Then, in 2000, Snead finally published research supporting his earlier hypothesis. He tested the effects of GHB on adenylyl cyclase activity and found that there is a specific G protein-coupled GHB receptor distinct from the GABA_B receptor. This receptor was

found in specific regions of the brain and acted in presynaptic but not postsynaptic cell preparations. It is this receptor that appears to be implicated in the action of GHB when it is present in low concentrations. Overall, the downstream events mediated by the endogenous GHB system are still under investigation. However, when doses of GHB are given externally, it is interaction with the endogenous system of binding and transport that leads to the alteration of several other neurotransmitter systems.

GHB AND THE DOPAMINERGIC SYSTEM

The CNS neurotransmitter dopamine is implicated in the mechanism of action of many drugs of abuse. The effects of GHB on the dopaminergic system are dependent on dose, time, and level of consciousness, and all of them are initiated by binding with either GHB or GABA_B receptors. Roth and Suhr (1970) demonstrated that at doses consistent with anesthesia, GHB produces a significant increase in brain dopamine levels. They hypothesized that the most likely mechanism for this increase was that GHB blocks, by hyperpolarization, the release of dopamine from the nerve terminals. Brain concentrations of dopamine were also found to be higher in an autopsied brain of a woman who died from acute GHB intoxication (Kish, et al., 2001). Morgenroth et al (1976) showed that GHB administration also leads to an increase in dopamine synthesis by a stimulation of tyrosine hydroxylase, the enzyme that catalyzes the rate-determining step. However, the GHB-induced change in dopamine release in the striatum was later shown to be biphasic, with a reduction of dopamine release at low doses and a stimulation of dopamine release at higher doses (Hechler, et al., 1991). The effect of time was also shown to be biphasic in the same study; release of dopamine was reduced in the first 5-10 minutes after GHB administration, but began to increase after 15 minutes. Level of anesthesia affected the firing of dopaminergic neurons in response to administered doses of GHB as well (Diana, et al.,

1991). GHB inhibited the firing of dopaminergic neurons in anesthetized rats but stimulated the firing of these neurons in unanesthetized rats. Thus, changes in dopamine synthesis and transmission were induced by exogenous GHB.

Both the GABA_B and GHB receptors have some role in the alteration of dopamine neuronal function by doses of GHB. Godbout et al (1995) showed that at low doses of GHB, spontaneous cell firings in the prefrontal cortical neurons of rats increased. Dopamine inhibits the firing of these neurons under normal conditions, so the increased firing demonstrated a reduction of dopamine release. Since the response to GHB by these neurons could be blocked by NCS-382, a GHB receptor antagonist, the GHB receptor was implicated as the site of action for the inhibition of dopamine release. However, at higher doses of GHB, an inhibition of the nerve cells was observed, a response shown to be unrelated to NCS-382. These results indicate that the blocking of dopamine release at low doses of GHB is mediated by binding to the GHB receptor. This would then seem to implicate the GABA_B receptor in the stimulation of dopamine release at higher doses. In support of this conclusion, Madden and Johnson (1998) reported that the firing of dopaminergic neurons was blocked in the ventral tegmental area of rat brain by high doses of GHB. This effect was blocked by the GABA_B receptor antagonist CGP-35348, but not by either a GABA_A antagonist or a glycine antagonist. The GHB receptor antagonist NCS-382 was not tested, a test which would have added more weight to the evidence supporting the role of the GABA_B receptor. However, the work of other researchers, such as that of Williams et al (1995) lends credence to the argument that the hyperpolarizing effects of high doses of GHB in dopamine neurons is controlled by binding to the GABA_B receptor.

In a somewhat alternative theory, Maitre (1997) proposed that GHB exerts its effects on GABA_B receptors indirectly by increasing GABA synthesis. He argued that the formation of a “GABA pool” was more likely than direct action since GHB is such a weak agonist at GABA_B

receptors. He even went so far as to say that GHB “does not interact directly with GABA_B receptors.” It would be this GABA pool, then, that would act on the GABA_B receptors and exert the observed effects on the dopaminergic system. This hypothesis was mentioned again in a later study which he co-authored on the increased release of GABA in the frontal cortex of rat brain after GHB administration (Gobaille, et al., 1999). While this hypothesis is not without foundation, it does have some shortcomings. First, although GHB does not bind with particularly high affinity to GABA_B receptors, numerous studies cited above have shown direct binding. Second, the breakdown of GHB can follow two different pathways (see Figure 1), and the succinic acid path seems to be the more dominant (Couper and Marinetti, 2002). A GABA pool seems unlikely if it is not produced in large quantities from GHB decomposition. Third, GABA increase does not occur in all brain regions. In the study of rat brain mentioned above, there was no increase in GABA release in the hippocampus. Finally, one would expect an increase in GABA synthesis and release to stimulate GABA_A receptors as well. However, a literature survey does not reveal much evidence in support of GHB stimulation of GABA_A receptors. While the formation of these GABA pools could be a possible mechanism for the effects of GHB on dopamine neurons, it would seem to be a minor one at best. It is more logical to assert that higher doses of GHB can sufficiently overcome the low binding affinity at GABA_B receptors to initiate dopamine release.

GHB AND THE SEROTONERGIC SYSTEM

Exogenously administered GHB has also been shown to influence the action of serotonin, another CNS neurotransmitter. Spano and Przegalinski (1973) reported the stimulation of serotonin turnover in the entire rat brain after administration of GHB. While their study indicated that serotonin also accumulated in certain regions of the brain, the overall increase did

not appear to be significant. These results were reinforced in later studies. Gobaille et al (2002) showed that the most probable explanation for the changes seen in the serotonin system is the simultaneous increase in tryptophan accumulation in brain tissues (specifically frontal cortex, striatum, and hippocampus) and increasing serotonin metabolism. Tryptophan is the metabolic precursor of serotonin, and both GHB and NCS-356 (a GHB agonist) led to increases in extracellular and tissue concentrations of tryptophan. There are two explanations for the increase in tryptophan concentration. First, GHB may have some effect on the transport of tryptophan across the blood-brain barrier and neuronal membranes. Increases in other neutral amino acid concentrations, including isoleucine, leucine, phenylalanine, and valine were noted in the study, indicating a stimulation of the L-type amino acid transporter. Second, GHB could help to increase the free constituent of tryptophan, making it more available for serotonin synthesis. The albumin binding sites for tryptophan are influenced by the presence of anesthetic substances, so GHB may cause the release of tryptophan from these binding sites.

The increase in serotonin metabolism was monitored by the concentration of 5-hydroxyindole acetic acid (5-HIAA), one of the products of the metabolic breakdown of serotonin. GHB administration led to a decrease in 5-HIAA concentration in the extracellular space and an increase in 5-HIAA concentration in brain tissues. This effect was also induced by the GHB agonist NCS-356. These results are consistent with a GHB-induced increase of serotonin metabolism in the tissues examined, since it does not appear to be due to a blockage of 5-HIAA transport out of the brain (Spano and Przgalinski, 1973). It is the balancing of increased serotonin synthesis and increased serotonin metabolism which results in the overall increase of serotonin turnover caused by GHB.

Unlike the dopaminergic system, the effects of GHB on the serotonergic system appear to be mediated almost exclusively by action at the GHB receptor. Even though the GABA_B

receptor agonists baclofen and CGP-35348 seem to produce similar results to those shown by GHB and its agonists, the GABA_B receptors cannot be implicated in the GHB-induced increase of serotonin turnover. As previously noted, the researchers used the GHB receptor agonist NCS-356 to reproduce the results shown by GHB in the increased tryptophan and 5-HIAA concentrations. In fact, the agonist showed an even greater effect than GHB itself. They were also able to demonstrate the blocking of these effects by the addition of the GHB receptor antagonist NCS-382. It is important to note that neither one of these compounds have any affinity for the GABA_B receptor. Thus, it appears that stimulation of the GHB receptor by exogenous doses of GHB leads to an increase in serotonin turnover in both extracellular fluid and brain tissue.

GHB AND OPIOID MECHANISMS

The interaction of GHB with opioid mechanisms in the brain appears to be a minor and indirect one in comparison with its effects on dopamine and serotonin. Although GHB does not appear to bind directly to several key opioid receptors (Bernasconi, et al., 1999), there is a body of evidence that suggests a role for opioid mechanisms in the action of GHB in the brain. Snead and Bearden (1980) noted that the opioid receptor antagonist naloxone blocked the effect of GHB on striatal dopamine levels. Later, Vayer and Maitre (1989) also noted that naloxone blocked GHB effects on hippocampal second messenger systems. These results were reinforced by the work of Hechler et al (1991) on the effects of GHB in striatal slices. They not only confirmed the GHB-induced blockage of dopamine release and accumulation by naloxone, they also showed an increase in the release of opioid-like substances after direct application of GHB to the striatal slices. These opioid-like substances were not well-defined in the paper except by pointing out that they displaced naloxone. However, the release of these substances was

declared to be significant in that it could indicate a stimulation of opioid interneurons. All of these effects were blocked by the GHB receptor antagonist NCS-382, indicating that the GHB receptor is the site of action for these effects. In order to block the release of the opioid-like substances, however, the time between antagonist application and GHB application had to be reduced. This could indicate that stimulation of the opioid interneurons occurs before the release of dopamine. As the researchers themselves point out, though, the time required in the lab to collect the samples makes it difficult to get a clear picture of the sequence of events. In total, it appears that GHB's mechanism of action includes some intersection with opioid mechanisms, and some direct stimulation of opioid receptors by GHB may remain to be discovered.

CONNECTIONS BETWEEN MECHANISM OF ACTION AND EFFECTS

As mentioned previously, GHB seems to be unique as a neurotransmitter in that it also acts as a drug that has the potential for clinical use, abuse, tolerance, addiction, and withdrawal. Almost all of the potential therapeutic uses of GHB have been connected to its interaction with dopamine. In an early study, Snead (1978) examined the EEG changes in monkeys after administration of GHB and found that these changes involved stimulation of the dopaminergic system. He thus postulated a dopaminergic mechanism for GHB's use in the treatment of seizure disorders, although it seems the drug is not widely used for this purpose. Maitre (1997) provided a body of evidence indicating a possible dopamine-related role for GHB in the treatment of schizophrenia. One hypothesis about this disease implicates changes in dopamine neurotransmission as a possible cause, which led to the interest in using low doses of GHB or its analogues to regulate the firing of dopaminergic neurons. Although the results of studies done to test the effect of GHB on schizophrenia symptoms have been contradictory and inconclusive, a study by Schmidt et al (1991) indicates a possible role for the GHB receptor system in the action

of neuroleptic drugs. Sulpiride, one of the neuroleptics used to treat schizophrenia, seems to possess agonist properties at the GHB receptor and may enhance the effect of GHB itself on the dopaminergic system.

GHB has been tested in the treatment of the addiction and withdrawal symptoms of various drugs of abuse, including ethanol, heroin, and opiates. GHB and ethanol showed cross-tolerance to their mutual side effects in one study by Columbo et al (1995), and GHB eased cravings and reduced consumption in alcoholics. Drugs that stimulate GABA seem to be effective in blocking the effects of ethanol withdrawal, so the binding of GHB to GABA_B receptors is possibly involved in this mechanism (Maitre, 1997). Studies on heroin and opiate withdrawal have also shown a GHB-induced reduction of symptoms. Since alcohol and morphine withdrawal symptoms have been linked to inhibition of dopaminergic activity in specific areas of the brain (Maitre, 1997), GHB action on withdrawal symptoms could be connected to its perturbations of dopamine neurons. The interaction of GHB with opioid interneurons mentioned previously could also play a role in its use in drug addiction therapy. However, some of the early enthusiasm for GHB treatments in this area has faded since it was based on the idea that GHB does not lead to tolerance and addiction in its own right.

In both the United States and Europe, GHB has continued to be investigated for the treatment of narcolepsy and other sleep disorders. GHB functions somewhat differently in this area than other drugs such as benzodiazepines and alcohol in that it produces a sleep pattern that mimics natural sleep and enhances REM sleep (Couper and Marinetti, 2002). There are strong indications that the sedative properties of GHB are regulated by its interaction with the GABA_B receptor (Carai, et al., 2001). The sedation of rats was mimicked by the GABA_B receptor agonist baclofen and blocked by two different GABA_B receptor antagonists. However, there may be a role for the GHB receptor as well, since Schmidt et al (1991) demonstrated that the sedative

properties of GHB could also be blocked by the GHB receptor antagonist NCS-382. The study by Gobaille et al (2002) relating to GHB and serotonin turnover also indicates a role for the GHB receptor. Since the serotonergic system has been implicated in the control of sleep, GHB-induced increases in serotonin turnover may play a role in GHB “sleep.” Roth and Suhr (1970) showed a relationship between GHB-induced sleep and increases in brain dopamine levels as well. Of course, all these systems would also be implicated in the instances of coma observed with acute GHB intoxication.

Thus, many of the same mechanisms that underlie the clinical uses of GHB also seem to be responsible for its effects as a drug of abuse. GHB produces many effects similar to those of ethanol, which has led to several connections between their mechanisms of action as well. Ethanol and GHB appear to have a similar biphasic effect on dopaminergic neurons as well as similar actions in anesthetized and unanesthetized rats (Mereu, et al., 1984). This may not only influence the behavioral effects of ethanol and GHB but also their addictive properties. Ethanol also appears to regulate the increase of the neurosteroids allopregnanolone (AP) and allotetrahydrodeoxy-corticosterone (THDOC), which are thought to play a role in the anxiolytic and sedative/hypnotic actions of ethanol (Barbaccia, et al., 1999). GHB was shown to induce an increase in the same neurosteroids through binding to the GABA_B receptor, implicating them in the psychotropic actions of GHB as well. The enhanced mood, relaxed inhibitions, and loss of anxiety reported by GHB users may be related to the increase in serotonin turnover modulated by GHB, since this system is implicated in the regulation of mood and anxiety (Gobaille, et al., 2002). The GHB-induced changes in acetylcholine (Nava, et al., 2001) and glutamate (Ferraro, et al., 2001) levels in the hippocampus may both play a role in the impairment of cognitive processes such as memory brought on by GHB abuse.

In light of all this, to what class of drugs does GHB belong? Nutt (1996) has argued for a pharmacological classification system for drugs of abuse rather than the traditional physiological or psychological classification. Based on this, Bernasconi et al (1999) created two broad categories for drugs of abuse. Psychostimulants produce their effects by potentiating dopaminergic systems, which serves to increase positive emotions and reduce reward thresholds. Such drugs include opioids, cannabinoids, nicotine, cocaine, and low doses of alcohol. Sedative-hypnotics, including benzodiazepines and barbiturates, work by enhancing GABA responses, which decreases anxiety and other negative emotions. Bernasconi and his team used this framework to classify GHB as a sedative-hypnotic due to its interaction with the GABA_B receptor. However, this classification is limiting for two main reasons. First, although GHB inhibits dopamine release at low doses, it seems to encourage its release at higher doses more consistent with GHB intoxication. Indeed, the effects of GHB on dopaminergic neurons seem to depend on so many variables that it may be premature to rule out GHB action as a psychostimulant. Second, this classification also fails to account for the effects of GHB on other systems such as serotonin. In fact, it may turn out that GHB defies such characterizations and may be placed in both categories. This would not be surprising for a drug that affects so many systems in the brain.