Clinical pharmacology and abuse potential of gamma-hydroxybutyric acid (GHB)

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Under the Direction of:
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Doctoral Thesis

Universitat Autònoma de Barcelona, Spain

Departament of Pharmacology, Therapeutics and Toxicology

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A mis padres

Dosi sola facit venenum,
Paracelso, siglo XVI

Lo último que recuerdo es que estaba bailando….
Paciente tras despertar del coma, Hospital del Mar
Año 2001

Universitat Autònoma de Barcelona

Departament de Farmacologia, de Terapèutica i de Toxicologia

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CERTIFICA:

Que la memòria presentada per Sergio Abanades León amb el títol "Clinical Pharmacology and abuse potential of gamma-hydroxybutyric acid (GHB)", ha estat realitzada sota la seva direcció. La memòria reuneix les condicions per ser presentada per optar al grau de Doctor.

I perquè consti als efectes oportuns signo el present certificat a Barcelona, a 25 dd'Abril de 2008

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Vista la instància presentada per en/na Sergio Abanades León de sol·licitud de presentació de tesi doctoral com a compendi de publicacions,

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RESOLC

Acceptar la presentació de la tesi doctoral de Sergio Abanades León com a compendi de publicacions amb els següents articles:

1. **Gamma-hydroxybutyrate (GHB) in humans: pharmacodynamics and pharmacoknetics**
2. **Disposition of gamma-hydroxybutyric acid in conventional and nonconventional biologic fluids after single drug administration: issues in methodolgy and drug...**
3. **Relative abuse liability of gamma-hydroxybutyric acid, flunitrazepam, and ethanol in club drug users.**

La Subcomissió de Postgrau de la Comissió d'Afers Acadèmics,
Per delegació

Carles JAIME CARDIEL
Delegat del Rector per a Doctorat

Bellaterra (Cerdanyola del Vallès), 6 de març de 2008

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Universitat Autònoma de Barcelona

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Abstract

Despite GHB therapeutic uses being explored and the increasing concern about the toxicity of this drug, little was known about the physiologic and subjective effects and alterations in psychomotor performance induced by GHB in humans. Few studies addressed dose-related effects under controlled administration and the correlation of drug effects and concentrations in biological fluids including alternative matrices. Furthermore, although GHB was being abused by humans, preclinical evaluation of the relative reinforcing effects of GHB was fairly inconsistent. In humans, a number of cases of GHB abuse and dependence have been reported, but no abuse liability studies had been performed. Moreover, previous epidemiological investigations reported an increased abuse of sedative-like drugs in recreational poly-drug users. The reasons for this shift in drug preference and the effects of these drugs in this population remained unclear.

The main aim of this work was thoroughly characterising the actual behavioural effects of GHB in humans. Within this context, we set up a series of controlled studies to evaluate the effects of

GHB and its relative abuse liability in healthy human volunteers previously exposed to this substance. All studies were carried out at the Pharmacology Research Unit at the 'Institut Municipal d'Investigació Mèdica' (IMIM), Barcelona, Spain, and were double blind, randomized, crossover and controlled. Study variables included vital signs (blood pressure, heart rate, oral temperature, pupil diameter), psychomotor performance (digit symbol substitution test, balance, maddox-wing), subjective effects (a set of 13 visual analogue scales, Addiction Research Center Inventory-49 items, and Evaluation of the Subjective Effects of Substances with Potential of Abuse questionnaires), and pharmacokinetics. The studies were conducted in accordance with the 'Declaration of Helsinki', approved by the local Ethical Committee and authorized by the Spanish Ministry of Health.

Firstly, a pilot study was performed as a preliminary phase. As a pilot pharmacology phase I study, different oral doses of sodium GHB (40, 50, 60, and 72 mg/kg) were given to 8 volunteers in a dose escalation schedule. GHB concentrations in plasma, oral fluid, urine, and sweat were analyzed for GHB by gas chromatography–mass spectrometry. GHB stability in plasma was studied at different storage temperatures. The method of detection of GHB in plasma, urine and oral fluid used throughout the studies was validated. No relevant variability due to the effect of storage temperature in GHB plasma concentrations was found. Physiological effects, psychomotor performance, and subjective effects were examined simultaneously.

GHB-mediated physiological and subjective effects were dose dependent and related to GHB plasma concentrations. GHB showed a mixed stimulant-sedative pattern, with initially increased scores in subjective feeling of euphoria, high, and liking followed by mild-moderate symptoms of sedation with impairment of psychomotor performance. GHB was readily absorbed after oral administration and rapidly eliminated. GHB was found in oral fluid at peak value concentrations equivalent to one third to one fourth of those found in plasma. The mean half-life (t½) of GHB was approximately 0.7 hour in plasma and 1.2 hours in oral fluid. GHB urinary excretion was less than 2% of the dose administered. GHB was also detected in sweat at low concentrations.
The information obtained was used to establish the doses to be administered and the optimal design for the final study.

The final study was performed to confirm GHB-induced subjective and physiological effects, and to evaluate its relative abuse liability compared to flunitrazepam and ethanol, and its impact on psychomotor performance in 'Club Drug' users. Twelve healthy male recreational users of GHB

participated in 5 experimental sessions in the framework of a clinical trial. Drug conditions were a single oral dose of GHB (40 or 60 mg/kg), ethanol (0.7 g/kg), flunitrazepam (1.25 mg),and placebo. All active conditions induced positive effects related to their abuse potential. The administration of GHB produced euphoria and pleasurable effects with slightly higher ratings than those observed for flunitrazepam and ethanol. GHB induced a biphasic time profile with an initial stimulant-like effect related to the simultaneous rise of plasma concentrations and a latter sedative effect not related to GHB kinetics. GHB administration also induced dose-dependent mild unpleasant effects, with no within-subject coincidence of positive and negative GHB related effects. GHB plasma concentrations and ethanol blood concentrations were well correlated to subjective effects related with stimulation whereas flunitrazepam plasma concentrations were better correlated to sedative-like effects. GHB plasma concentrations were also well correlated to different VAS related to abuse potential. GHB increased blood pressure and pupil diameter. Ethanol induced its prototypical effects, and flunitrazepam produced marked sedation. GHB and flunitrazepam impaired psychomotor performance (digit symbol substitution test and balance task), whereas ethanol, at the dose tested, induced only mild effects exclusively affecting the balance task.

In conclusion, at the doses tested GHB was capable of inducing euphoria, pleasurable feelings, sedation and slight stimulant-like effects as previously reported by GHB users. GHB induced a biphasic time profile with an initial stimulant-like, euphoric and pleasurable effect related to the simultaneous rise of plasma drug concentrations and ulterior sedative effect collateral to a decrease in GHB plasma concentrations. The three study drugs, GHB, flunitrazepam and ethanol, although known sedate-like drugs, they all induced a mixed sedative-stimulant pattern. GHB tolerability highly differ between subjects with no within-subject coincidence of positive and negative GHB related effects. Following oral administration, GHB is rapidly absorbed and eliminated with high interindiviidual variability. Measurable plasma, urine, oral fluid and sweat were observed. However, oral fluid and sweat appear not to be suitable biological matrices for monitoring GHB consumption. The results suggest a high abuse liability of GHB in 'Club Drug' users, providing the scientific rationale for the increased abuse of the drug in humans.

Resumen

A pesar del uso terapéutico de GHB y de un aumento en la percepción de su toxicidad, el conocimiento sobre los efectos fisiológicos, subjetivos y sobre el rendimiento psicomotor inducidos por el GHB en humanos era limitado. Escasos estudios habían evaluado de forma controlada los efectos dependientes de la dosis y la correlación entre los efectos y la concentración de GHB en diferentes fluidos biológicos, incluyendo matrices alternativas. Además, a pesar del abuso de GHB, los resultados de los estudios preclínicos sobre sus efectos reforzantes eran inconsistentes. Si bien se habían descrito casos de abuso y dependencia, no se habían realizado estudios controlados evaluando el potencial de abuso de la sustancia en humanos. Mientras, los resultados de algunos estudios epidemiológicos apuntaban a un abuso creciente de drogas de tipo sedante en policonsumidores de drogas recreacionales. Tanto las causas de este aumento en el consumo como los efectos de estas sustancias en esta población eran desconocidos.

El objetivo más importante de este trabajo fue la meticulosa caracterización de los efectos del GHB en humanos. En este contexto se pusieron en marcha una serie de estudios controlados para evaluar los efectos del GHB y su potencial de abuso, en sujetos expuestos previamente a la sustancia. Los estudios fueron desarrollados en la "Unitat de Recerca en Farmacologia" del "Institut Municipal d'Investigació Mèdica" (IMIM) de Barcelona, España. Los estudios fueron randomizados, a doble ciego, de tipo cruzado y controlados. Las variables estudiadas incluyeron constantes vitales (presión arterial, frecuencia cardiaca, temperatura oral, diámetro pupilar), efectos sobre el rendimiento psicomotor (test de sustitución de símbolos por dígitos, tarea de balance, ala de maddox) efectos subjetivos (cuestionario Addiction Research Center Inventory reducido de 49 items, 13 escalas visuales analógicas, cuestionario de valoración de efectos subjetivos de sustancias con potencial de abuso-VESSPA) y evaluación farmacocinética. Los estudios se desarrollaron de acuerdo con la "Declaración de Helsinki" y fueron aprobados por el Comité Ético-CEIC de nuestro centro. Además fueron autorizados por la Agencia Española del Medicamento.

En una primera fase se realizó un estudio piloto. Como estudio piloto de farmacología humana de fase I, diferentes dosis de GHB sódico (40, 50, 60 y72 mg/kg) fueron administradas por vía oral a 8 voluntarios en una pauta de dosis ascendente. Las concentraciones de GHB en plasma, fluido oral, orina y sudor fueron analizadas mediante cromatografía de gases acoplada a espectrometría de masas. La estabilidad de GHB en plasma fue estudiada a diferentes temperaturas, no encontrándose una variabilidad significativa. El método de detección de GHB en plasma, fluido

oral y orina fue validado para su posterior uso durante los estudios. Los efectos fisiológicos, subjetivos y sobre el rendimiento psicomotor del GHB fueron evaluados simultáneamente.

Los efectos fisiológicos y subjetivos fueron dosis dependientes y se correlacionaron con las concentraciones plasmáticas de GHB. El GHB produjo efectos de tipo mixto estimulante y sedante, con un incremento inicial de las puntuaciones en los efectos subjetivos agradables de euforia y "colocón", seguido de efectos moderados de tipo sedante asociados a una alteración del rendimiento psicomotor. La administración de GHB se siguió de una rápida absorción y eliminación. Se detectó GHB en fluido oral, con un pico en su concentración equivalente aproximadamente un ¼ del pico de las concentraciones plasmáticas. El tiempo de semivida de eliminación (t½) de GHB fue aproximadamente de 0.7 horas en plasma y 1.2 horas en fluido oral. La excreción urinaria fue inferior a un 2 por ciento de las dosis administradas. Asimismo se halló GHB en sudor a bajas concentraciones. La información obtenida en el estudio piloto fue determinante para la optimización del diseño del estudio definitivo y la selección de las dosis administradas.

El estudio definitivo se realizó con la intención de confirmar los efectos fisiológicos y subjetivos del GHB y su impacto sobre el rendimiento psicomotor, así como pare evaluar su potencial de abuso en comparación con etanol y flunitrazepam, en usuarios de "Club Drugs". Con estos objetivos se evaluaron 12 voluntarios sanos con experiencia previa en el uso de GHB en un ensayo clínico compuesto de 5 sesiones experimentales. Las diferentes condiciones de tratamiento fueron, 2 dosis únicas de GHB (40 o 60 mg/kg), etanol (0.7 g/kg), flunitrazepam (1.25 mg), y placebo, siendo todos los tratamientos administrados por vía oral.

Todos los tratamientos activos indujeron efectos de tipo positivo relacionados con su potencial de abuso. La administración de GHB indujo efectos de tipo euforizantes y placenteros ligeramente superiores a los observados tras la administración de flunitrazepam y etanol. El perfil de efectos inducidos por el GHB fue de tipo bifásico, inicialmente de tipo estimulante-euforizante en relación con el incremento simultáneo de las concentraciones plasmáticas, seguido de un efecto de tipo sedante no relacionado con la cinética plasmática. La administración de GHB produjo asimismo efectos adversos o no deseados dosis dependientes, pero sin una coincidencia intra-sujeto de efectos positivos y negativos. Las concentraciones plasmáticas de GHB y las concentraciones de etanol en sangre se correlacionaron significativamente con los efectos subjetivos estimulantes, mientras que las concentraciones plasmáticas de flunitrazepam se correlacionaron significativamente con efectos de tipo sedante. Las concentraciones plasmáticas de GHB se

correlacionaron significativamente con las variables relacionadas con el potencial de abuso. En cuanto a los efectos fisiológicos, el GHB indujo un aumento significativo de la presión arterial y del diámetro pupilar, mientras el etanol indujo sus efectos prototípicos y flunitrazepam produjo una marcada sedación. GHB y flunitrazepam produjeron un empeoramiento significativo del rendimiento psicomotor (tareas de sustitución de símbolos por dígitos y del balance), mientras que el etanol únicamente indujo un leve empeoramiento de la tarea del balance.

Como conclusión, a las dosis investigadas, la administración de GHB indujo efectos de tipo euforizantes y placenteros, sedación y efectos estimulantes de tipo moderado, similares a los descritos previamente por los usuarios de las sustancia. El perfil de efectos inducidos por el GHB fue de tipo bifásico, inicialmente de tipo estimulante-eufórico y relacionado con el incremento simultáneo de las concentraciones plasmáticas, seguido de un efecto de tipo sedante no relacionado con la cinética plasmática. Si bien los 3 tratamientos activos son reconocidas sustancias de tipo sedante, tanto GHB como flunitrazepam y etanol, indujeron u un patrón de efectos mixto de tipo estimulante y sedante. La tolerabilidad del GHB difirió substancialmente entre los diferentes sujetos, sin coincidencia intra-sujeto de efectos positivos y negativos. La administración de GHB se sigue de una rápida absorción y eliminación con una gran variabilidad inter-indiviudal. Si bien se encontraron concentraciones de GHB todas las matrices biológicas analizadas, tanto el fluido oral como el sudor no parecen convenientes para monitorizar el consumo de GHB. Los resultados sugieren un alto potencial de abuso de GHB en usuarios de "Club Drugs" y aportan los fundamentos científicos del aumento en el abuso de la sustancia en humanos.

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Motivation

During my Clinical Pharmacology medical residency I worked at the emergency department of the '*Hospital del Mar'* in Barcelona. One early morning in January 2001, paramedics brought in a 21-year-old male who had collapsed at a night club. The accompanying persons stated that the incoming patient had earlier consumed a sticky-looking liquid a drug supposedly called 'liquid ecstasy'. However, 3,4-methylendioxymetamphetamine (MDMA), ethanol and cannabis were the only drugs detected in the urine screening test performed. He spontaneously regained consciousness 60 minutes after arriving at the hospital and was discharged, completely recovered, 2 hours later. The patient admitted taking drugs in the preceding hours, including 'liquid ecstasy'.

In the following months an increasing number of cases of overdose following a similar pattern were brought to the emergency department of the 'Hospital del Mar'. We later discovered from patients and relatives that these overdoses were caused by gamma-hydroxybutyrate (GHB, 'liquid ecstasy').

GHB is a naturally occurring short chain fatty acid that possesses unique pharmacological properties. It has been used as a therapeutic drug due to its hypnotic and anesthetic effects and gained significant notoriety in the late 1990s both as a major recreational drug of abuse and public health problem worldwide (Nicholson and Balster, 2001). In 2001, we published one of the first series of cases of suspected intoxication due to GHB in Europe (Abanades et al. 2001). At that time, the work for this thesis was set in motion.

An initial scientific literature review (see Appendix) revealed that while extensive research was being conducted into the basic pharmacology of GHB and its role as a possible neuromodulator, the behavioural effects of GHB in humans remained unclear. Indeed, most of the information available regarding GHB's behavioural/toxicological effects derived from therapeutic studies completed in the 1960s. Additionally, most reports focused exclusively on the pharmacokinetics of GHB. Insight into GHB abuse-related effects came mainly from anecdotal reports and surveys, but these had not been fully characterised using GHB controlled administration. Furthermore, information about the effects of GHB in humans was based on cases of intoxication, which had not been completely clarified due to a lack of confirmed evidence such as through GHB detected in plasma and/or urine. Indeed, the frequent poly-drug use complicated the attribution of clinical features solely to GHB intoxication.

In summary, despite GHB therapeutic uses being explored and the increasing concern about the toxicity of this drug, few studies have previously addressed dose-related effects under controlled administration. Little was known about the physiologic and subjective effects and alterations in psychomotor performance induced by GHB in the range of doses commonly abused. Few studies addressed dose-related effects and the correlation of drug effects and concentrations in biological fluids including alternative matrices. Furthermore, although GHB was being abused by humans, preclinical evaluation of the relative reinforcing effects of GHB has been fairly inconsistent. In humans, a number of cases of GHB abuse and dependence have been reported, but no abuse liability studies had been performed. Moreover previous epidemiological investigations reported an increased abuse of sedative-like drugs in recreational poly-drug users. The reasons for this shift in drug preference and the effects of these drugs in this population remained unclear.

This thesis was born with the aim of thoroughly characterising the actual behavioural effects of GHB in humans. Within this context we set up a series of controlled studies to evaluate the effects of GHB and its relative abuse liability in healthy human volunteers previously exposed to this substance.

1. Introduction

1.1. GHB

1.1.1. History

Gamma-hydroxybutyrate (GHB, Gamma-hydroxybutyric acid, 'liquid ecstasy'), is a naturally occurring short chain fatty acid that possesses unique pharmacological properties. In 1947, γ-butyrolactone, (GBL), a related compound, was shown to have central nervous system depressant properties (Rubin and Giarman, 1947). GHB was later synthesised by Henry Laborit in an attempt to create a more slowly metabolised GABA analogue which would readily cross the blood-brain barrier (Laborit 1964). Soon thereafter, it was discovered that GHB is an endogenous substance. Since then, extensive study has been devoted to determining the function of endogenous GHB in normal brain physiology. GHB was also found to have sedative-like properties similar to those of GBL. GBL has since been shown to be biologically inactive given that all its biological and behavioural effects are due to its rapid conversion to GHB by an active serum lactonase.

In 1962, the first human study of GHB was reported, in which GHB was used as a surgical anaesthetic due to its ability to induce CNS depression with minimal effects on the circulatory and respiratory system and no apparent significant adverse effects (Blumenfeld et al. 1962). Shortly after a correlation between increased blood concentrations of exogenously administered GHB and decreased levels of consciousness was demonstrated. Thus, GHB's ability to induce a sleep-like state was observed in a study where intravenous administration of GHB at doses ranging from 5.9 to 9 g induced sleep in 16 healthy study participants (Helrich et al. 1964).
GHB found significant initial use as an anaesthetic; however it was essentially abandoned in most countries as a useful agent for this purpose because of its lack of analgesia, the difficulties in dosing and adverse effects. While initial evaluation of GHB in animals and humans explored its sleep-promoting or anaesthesia-inducing capacity, most of the more recent human therapeutic research has focused on its use in narcolepsy and alcohol-dependence treatment. In the 1970s, early independent studies established the potential efficacy of GHB (named as sodium oxybate) for the treatment of the sleep disorder narcolepsy. Sodium oxybate recently received approval in the US and Europe for treatment of excessive daytime sleepiness and cataplexy in patients with narcolepsy. This was based on safety and efficacy data established in several randomized, double-blind, placebo-controlled trials (Black and Houghton, 2006). On the other hand, GHB has been proof to be effective both in the management of alcohol withdrawal syndrome and in the maintenance of long-term abstinence. For these reasons, GHB has been approved for the

treatment of alcohol dependence in some European countries (Caputo et al. 2003). In addition, several other potential therapeutic effects of GHB have been explored with divergent results, including: treatment of heroin dependence; efficacy in pain relief, fatigue and sleep fragmentation of fibromyalgia syndrome; treatment for hyperkinetic movement disorders; hypnotic potential in healthy subjects and those with insomnia and neuroprotective effects in ischemia-induced challenges (Teter and Guthrie, 2001).

While GHB therapeutic effects were being explored, the substance and its analogues or precursors GBL and 1,4-butanediol (BD) began to be used with different purposes. GHB was manufactured in the US during the late 1980s and in the beginning of the 1990s was also marketed and used as an over-the-counter dietary supplement, sleep aid and muscle builder (Dyer 1991). Around the same time GHB and its prodrugs GBL and BD began to be abused. Initially, the ability of these compounds to stimulate growth hormone production led to an increased use and abuse by body builders. GHB was also used as a sleep promoter and some cases of intoxication and abuse began to arise. Consequently, the U.S. Food and Drug Administration (FDA) banned the sale of non-prescription GHB in 1990 due to an increase in overdose cases of GHB or its precursors. Interestingly, the prohibition was followed by a spectacular increase in the abuse of this substance during the following years. The drug, initially found over the counter, jumped into the illegal market where it was produced in clandestine laboratories resulting in preparations with a wide range of purity and strength (O'Connell et al. 2000). A few years later the situation worsened as many Internet sites offered instructions for the home production of GHB or advertised the sale of kits that contain the ingredients necessary to produce it. Intriguingly, although GHB is a sedative-like substance, intentional misuse for the purpose of achieving a euphoric state was first reported in the late 1980s. Since then its recreational use has grown significantly and it has been frequently associated to ‘rave’ parties (all-night dance parties with fast-repetitive music often accompanied by elaborate light displays) and to the list of drugs being used at clubs or discotheques. Thus, by the late 1990s, GHB had became a popular ‘Club Drug’ (Abanades et al. 2004-Appendix) and gained significant notoriety both as a major recreational drug of abuse and public health problem all over the world. GHB is abused in this setting because of the euphoria, disinhibition, enhanced sensuality and heightened sexual awareness that are claimed to be associated with its use (Degenhardt et al. 2002; Gonzalez and Nutt, 2005; Miotto et al. 2001). Thus, the U.S. Drug Abuse Warning Network (DAWN) detected dramatic increases in the number of mentions in accident and emergency departments of persons identified as having overdosed on GHB or having GHB related problems: from 56 in 1994 to 4969 in 2000 (1861 in 2005).

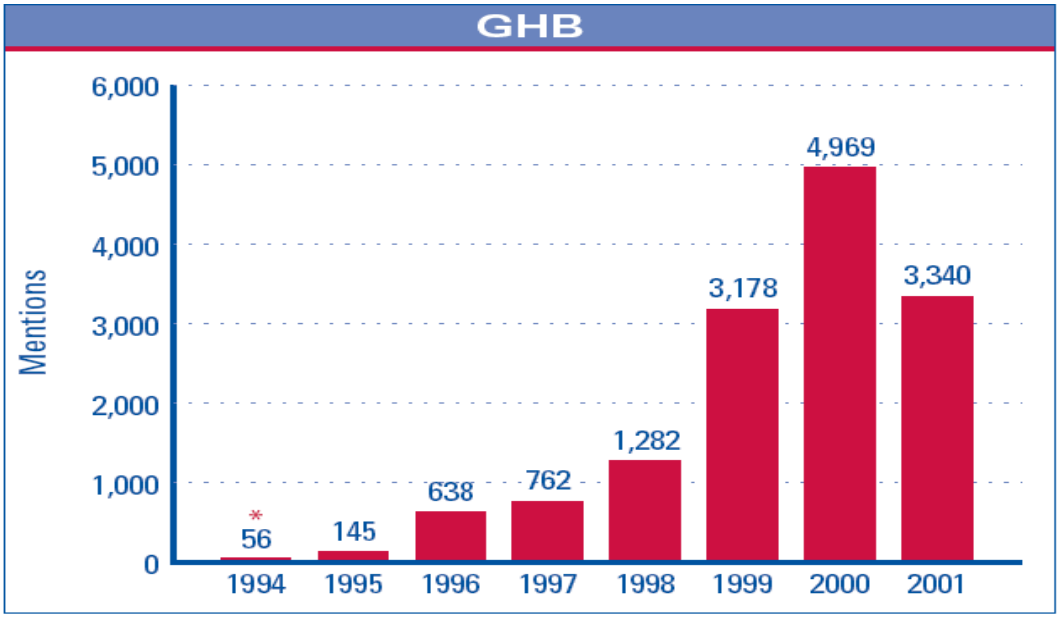


Figure 1. GHB related emergency department visits. SOURCE: Office of Applied Studies, SAMHSA, Drug Abuse Warning Network, 2001 (03/2002 update).

In the last few years we have witnessed a significant increase of intoxications due to GHB and its precursors among 'Club Drugs' users in Europe and remarkably in our setting. In Spain the first cases appeared in the late 1990s and the first series of cases were published in the early part of this decade (Abanades et al. 2001). Furthermore, during the early part of this decade this drug was the main cause of toxic-related admission in the emergency department of several hospitals in Barcelona (Spain) only superseded by alcohol (Abanades et al. 2001; Miró et al. 2002).

GHB gained also significant notoriety as a 'date rape' drug. GHB has been used for narcotizing victims in drug-facilitated sexual assaults possibly because of its capacity to induce short-term antegrade amnesia, increased libido, and suggestibility. (Elsohly and Salamone, 1999; Varela et al. 2004). As a result of both its increasing abuse and intoxication cases and its use as a 'date rape' drug, GHB was classified as a schedule I drug in the US in March 13, 2000. However, despite this increased regulation, illicit forms of GHB remain available under a number of names, such as 'G', 'liquid ecstasy', 'grievous bodily harm', 'scoop', 'cherrymeth', or in Spain 'botes' or 'potes'. In addition, GBL and BD are still available for purchase on the Internet, where they are advertised as mood enhancers, sleep inducers, and for bodybuilding purposes. Nowadays this interesting drug is being tested as a therapeutic agent in potential new indications while its relevance in the CNS function is still being clarified by several investigations. Concurrently, the drug is still being abused as a recreational drug.

1.1.2. Recreational Use

1.1.2.1. Terminology and presentations

GHB has been sold on the street under names such as 'Liquid Ecstasy', 'Liquid E', 'Liquid X', 'GHB', 'Georgia Home Boy', 'Grievous Bodily Harm', 'Soap', 'Scoop', 'Salty Water', 'Organic Quaalude', 'Easy Lay', 'Fantasy', 'G-Riffick' and 'Cherry Meth'. In Spain it is usually available as 'Éxtasis líquido' (Liquid Ecstasy), 'Potes' or 'Botes' (jars) and 'Biberones' (baby's bottles). It is typically available as a sticky-looking, colourless and odourless liquid but it can also be found as a powder or in capsules. It has a mild soapy salty taste which can be easily masked by adding it to drinks. So far, illicit use has only been reported by oral ingestion.

Figure 2. Different presentations of recreational GHB: '*potes', 'botes'* (jars) or '*biberones'* (baby's bottles)'

1.1.2.2. Sources

This product is usually illegally synthesised in small domestic laboratories, mainly through basic precursor GBL hydrolysis (Rhodium 1999). Both GBL and BD metabolise to GHB after their intake, producing virtually the same effects and they are frequently consumed instead of GHB itself. A

recipe for the home production of GHB can be found on the Internet. It consists of mixing sodium hydroxide and butyrol lactate. However, the improper manufacture of the drug can lead to a mixture of GHB and sodium hydroxide, which can be extremely toxic because the caustic effects of sodium hydroxide. Other sources for easily obtaining GHB are a series of new industrial products: gamma-hydroxyvalerate (GHV) and gamma-valerolactone (GVL), the commercial uses of both being similar to those of GBL and BD with much less supervision of their eventual illegal use (Carter et al. 2005).

The synthesis of GHB is relatively simple (Figure 3), and the product found on the street may contain GHB and/or their precursors. As previously argued, GHB is often produced in clandestine laboratories resulting in drug preparations with a wide range of purity and strength. A 99% pure sample of GHB weighs 2.8 g/level teaspoon (5 ml). However, 40 ml of clandestinely produced GHB may weigh from 3-20 g. Therefore, the capful or teaspoon concentration of a street dose could be extremely variable (500 mg-5 g/dose) (Dyer 1991).

Figure 3. Elements and technology required for GHB synthesis (available on the internet).

1.1.2.3. GHB analogues

The restrictions on GHB sales as well as the increasingly tight regulations led to increased interest in alternative supplies of the drug. New sources included not only illegally synthesised GHB but also GHB precursors or analogues. Commercially available products containing GHB analogues have often been marketed for use as an 'ink jet cleaner' or 'weight belt cleaner'. While some GHB-related compounds have legitimate industrial uses, other products containing these compounds promptly became available through sources such as the Internet. Numerous analogues of GHB exist, however the two most widely abused GHB analogues are GBL and BD (Palmer 2004). The synthesis of GHB is relatively simple, and the product found on the street may contain GHB and/or their precursors GBL and BD. Both GBL and BD metabolise to GHB after their intake, producing virtually the same effects. Even if effects are fairly similar, precursors could lead to faster intoxication symptoms. They are frequently consumed instead of GHB itself, and several cases of abuse and intoxication due to these substances have been reported (Ingels et al. 2000; Lora-Tamayo et al. 2003). It has been reported that GBL would exhibit the fastest absorption and greater bioavailability and toxicity, especially in combination with alcoholic beverages (Palmer, 2004). However, there are no clinically significant relevant differences in the management of patients intoxicated with GHB or its analogues. There have also been reports about oesophageal mucosa ulcerations after consumption of these substances from illicit synthesis in an insufficiently neutralised form (Dyer and Reed, 1997). Ingested GBL and BD are metabolised to GHB so urine tests will typically only be positive for GHB and not the analogue ingested.

1.1.2.4. Prevalence of recreational use

In the 1980s, GHB was distributed over-the-counter as a dietary supplement, sleep aid and muscle builder. It was initially used mainly by body builders and health food advocates. However reports from the USA, Australia and Europe later indicated that GHB was being increasingly consumed as a recreational drug (Degenhardt et al. 2002). Even though GHB is namely a sedative drug, its use has been taking place mainly in Clubs and 'Raves'.

Although several indicators point towards an increasing abuse of this substance, its use has not been as widespread as cocaine or MDMA use. It is restricted rather to some populations and is frequently unmentioned in universal surveys concerning drug use. Nevertheless its actual use could be underestimated. First, many of the surveys do not specifically ask for the use of GHB.

Second, an increased GHB use has been allocated to some populations difficult to be approached through surveys. Third, a possible stigma towards GHB has been described due to its toxic effects. It seems to be widespread within the 'Rave' community, thus potentially leading to use that is 'hidden,' and difficult to detect (Palamar and Halkitis, 2006). Therefore only estimations about its actual use can be made, taking into account the results of some surveys and indirect markers of consumption as seizures or the number of intoxication cases.

One of the few general surveys available investigating GHB use is the 'Monitoring the Future Survey' in the USA. This survey is administered to approximately 50,000 students (14-18 year old) and 6000 young adults (18-44 year old) with a response rate of about 90%. In the year 2003, a past-year prevalence of GHB use was found in 1-2% of the students and 0.8% among college students and young adults. The same survey in the year 2006 reflected a stable past-year prevalence of GHB use of 1%. Interestingly the annual prevalence of use rates for males in the senior year (17-18 years old) were 9.5 times higher than females for GHB use (Johnston et al. 2007).

In Europe, there is little mention of GHB in general surveys, however some indirect indicators of increasing use of GHB are available. Thus, several intoxication cases and notable increases in the seizures of precursor chemicals for GHB (GBL and BD) were described in the annual report 2004 of the European Monitoring Centre for Drugs and Drug Addiction (European Monitoring Centre for Drugs and Drug Addiction, 2006). This report detected GHB and ketamine as two synthetic drugs with potential for significant further spread in recreational settings. In addition, in the context of a pill-testing study in Austria, a 12.6 % lifetime prevalence of GHB use among a sample of 225 young people attending raves in Vienna was found (Benschop et al. 2002). During 2003, GHB was also detected in human samples in Belgium, Sweden and Norway and in samples taken from intoxicated subjects and hospital emergency patients in Spain (Barcelona) and the Netherlands. One survey (Winstock et al. 2001) reported that 13% of a sample of 1151 respondents recruited via a dance-culture magazine (mean age of 23.9 years) had previously used GHB (2% within the last month). The mean age of first use GHB in this sample was 22.4 years.

Recently, in an annual magazine-based survey targeting people who use drugs in dance contexts over a 5-year period (335-1151 people, depending of the year of evaluation), an increase in the prevalence of lifetime GHB use from 12.8% in 1999 to 17.5% in 2003, and a similar current use prevalence (3% within the past month) were found (McCambridge et al. 2007)

GHB use in Spain has been described to be similar to other European countries, being mainly associated with 'Raves' and 'Club' users. In addition, this drug has for some time been the main cause of drug-related admission in some hospitals (Miró et al. 2002).

1.1.2.5. Patterns of use

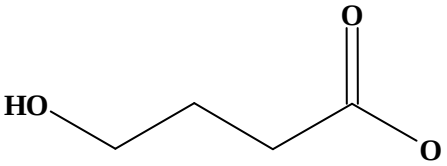
In a sample of 42 GHB users in the USA, GHB was predominately used for 'going out' and having fun. GHB use intentionally to enhance and extend the 'high'' from other drugs was reported by 40% of participants, and 71% reported that they 'usually use GHB with other drugs'. The drugs most frequently used in combination with GHB were 'Ecstasy' (MDMA) (53%), cannabis (50%), cocaine (43%), amphetamines (40%) and alcohol (37%) (Miotto et al. 2001).
In a study of 76 Australian users, reasons for using the drug recreationally included the resultant feelings of euphoria, relaxation, increased sociability and loss of inhibition, pleasurable stimulation as well as heightened sexual interest (Degenhardt et al. 2002). A significant proportion also reported that they liked the fact that the effects of GHB appeared to wear off quickly without a 'come down' period (29%). Notably, 14% reported GHB use to help come down from stimulant drugs (usually 'ecstasy' and amphetamines).

Intriguingly, GHB has also been described as popular among gay and bisexual men where it has been associated with increased sexual risk (Colfax et al. 2001; Mattison et al. 2001). In a survey in 'Club drug' users in New York City (USA), 192 men identified GHB use in the 4 months prior to baseline assessments. Of these 192, 15 men identified GHB as their most frequently used club drug. (Halkitis and Palamar, 2006)

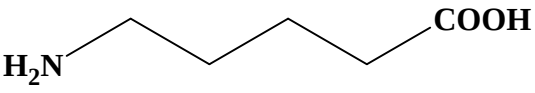
1.1.3. Neurobiology of GHB: basic aspects of GHB physiology and pharmacology

1.1.3.1. GHB chemical structure

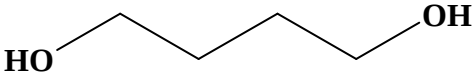
GHB is a short-chained fatty acid found endogenously in the mammalian brain at a concentration of 1–4 mM (Maitre 1997). It is an endogenous metabolite and a precursor of the neurotransmitter gamma-aminobutyric acid (GABA). GHB can be also formed in human peripheral tissues from two precursors, gamma-butyrolactone (GBL) and 1,4-butanediol (BD).



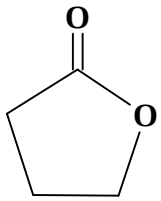
gamma-hydroxybutyrate (GHB)



gamma-aminobutyric acid (GABA)



1,4-butanediol



gamma-butyrolactone (GBL)

Figure 4. Chemical structures of GHB, GABA, BD and GBL.

1.1.3.2. Endogenous GHB in the brain

1.1.3.2.1. Distribution

GHB is present in micromolar concentrations in the brain of mammals, although the concentration differs by region. The highest relative concentrations are found in the striatum reaching concentration of 11-25 μmol/g (Snead and Morley, 1981). Localisation of GHB within

cytosolic and synaptosomal fractions suggests presynaptic accumulation. The concentration of GHB is higher in the developing brain than in the adult brain. GHB is also found in micromolar concentrations in peripheral tissues such as heart, kidney, liver and muscle with the highest concentration being found in the brown fat (37 µmol/g) (Nelson et al. 1981).

1.1.3.2.2. Synthesis and metabolism

The main precursor of GHB is GABA as revealed by a few radiolabelling studies. GHB formation occurs in GABAergic neurons or in neurons that synthesise GABA. GHB serves as both a precursor and a degradation product of GABA. Around 0.05 % (*in vitro*) to 0.16% (*in vivo*) of GABA is ultimately metabolised to GHB. Concentrations of GHB are approximately 0.1% of the concentration of GABA (Roth and Giarman, 1969).

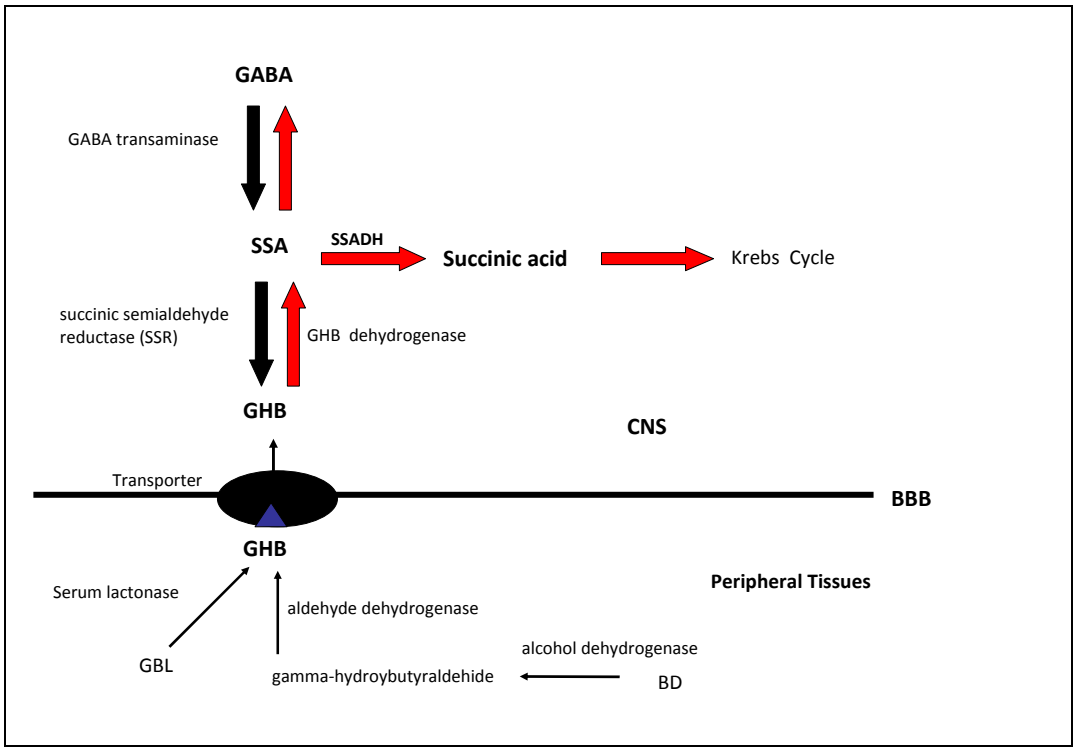


Figure 5. Synthesis of GHB. While GABA can be formed from glutamate by glutamic acid decarboxylase, GABA is degraded to succinic semialdehyde (SSA) by GABA transaminase. At this point, GHB may be formed depending on the activity of the specific enzyme succinic semialdehyde reductase (SSR). GHB can be reconverted back to SSA via GHB dehydrogenase, and the GHB derived SSA can be converted back to GABA. SSA can also be metabolized by succinic semialdehyde dehydrogenase (SSADH) to succinic acid. Mutant mice in which the SSADH enzyme is deleted display high levels of GHB and GABA (Gibson et al. 2003). GHB

can also be formed from its precursors BD and GBL. BD is metabolised to gamma-hydroybutyraldehide by alcohol deshidrogenase. Subsequently gamma-hydroybutyraldehide is metabolised via aldehyde dehydrogenase to produce GHB. GHB can also be produced from GBL via serum lactonases. GHB formed in peripheral tissues crosses the blood brain barrier (BBB) entering the CNS through active transport.

1.1.3.3. GHB as a neurotransmitter

The specific mechanism of GHB's action is still poorly understood. Several findings support that GHB itself serves as an endogenous inhibitory neuromodulator, besides taking part in GABA metabolism. Thus, GHB is synthesised in neurons, can be released in a Ca2+-dependent manner and a subcellular anatomical distribution for GHB and its synthesizing enzyme in neuronal presynaptic terminals have been demonstrated (Drasbek et al. 2006). GHB is released by neuronal depolarization in a Ca2+-dependent fashion and a Na+ dependent GHB uptake system has been demonstrated in the brain. Furthermore, an active vesicular uptake system has been reported that is most probably driven by the vesicular inhibitory amino acid transporter (also responsible for the uptake of GABA and glycine).

GHB action is associated with diverse brain functions such as the control of oxygen consumption, glucose metabolism and temperature regulation. The exact mechanism of action in the brain and peripheral tissues remains partially unknown (Wong et al. 2004).

1.1.3.4. GHB binding sites in brain

It is clear that GHB binds to brain tissues and dose-dependent pharmacological actions can be elicited by the administration of GHB. A specific GHB receptor antagonist known as NCS-382 (sodium salt 6,7,8,9-tetrahydro-5-[H]benzocycloheptene-5-ol-4-ylidene acetic acid) has been isolated. It can displace GHB binding and, in a dose-dependent fashion, it reverses the effects associated with GHB administration such as catalepsy, sedation and an increase in dopamine synthesis. Two interesting receptor primary targets for GHB have emerged: (i) a GHB receptor (GHBR) which is reported to be a G-protein-coupled receptor distinct from $GABA_B$ receptors, and (ii) the $GABA_B$ receptor subtype of GABA receptors, which is found throughout the CNS. GHB binds to the GHB receptor and the $GABA_B$ receptor with high affinity and low affinity, respectively (Drasbek et al. 2006).

1.1.3.4.1. The GHB receptor

The presence of a GHB receptor was suggested after the demonstration of specific, high-affinity [^{3}H]GHB binding sites occurring in the brain, with the highest density found in the hippocampus, dentated gyrus, nucleus accumbens, striatum, ventral tegmental area and in the cortex (prefrontal, frontal, parietal temporal and cingulated). Intermediate concentrations of high-affinity GHB binding are found in the amygdala and thalamus. In the cerebellum, GHB binding is absent or weak although $GABA_B$ receptors are strongly expressed. Two binding sites have been discovered, a high affinity GHB binding site with a dissociation constant (K_D) of 30–580 nM and a low-affinity site with a K_D of 1.5-6.0 mM (Snead and Liu, 1984). Consequently kinetics of these sites correlates with the physiological concentrations of GHB in brain. The GHB high/affinity binding site is absent from peripheral tissues. This could indicate that GHB might act simply a as a metabolic intermediate outside the CNS. 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.

Several studies point to the existence of molecularly distinct entities compatible with GHB receptors. Recently (2003) the cloning of a putative GHBR has been reported. However, this newly cloned receptor displays no affinity for the specific GHB receptor antagonist NCS-382 and was not expressed in expected brain patterns, which suggests the presence of an NCS-382 insensitive subtype of the GHB receptor. A summary of the evidences of GHB actions over GHB receptor is showed in Table 1.

1.1.3.4.2. The $GABA_B$ receptor

GHB activates $GABA_B$ receptors on neurons when its concentration exceeds the physiological micromolar levels in the brain. $GABA_B$ receptors are G-protein-coupled and their activation leads to several events (i) neuronal hyperpolarization by opening postsynaptic G-protein-coupled inwardly rectifying potassium (GIRK) channels, (ii) inhibition of voltage-gated Ca2+ channels and consequent inhibition of Ca^{2+} influx and (iii) lowering the intracellular levels of cyclic AMP (Wong et al. 2004). Consequently, $GABA_B$ receptors exert multiple pre- and postsynaptic actions, including inhibition of neurotransmitter release, postsynaptic silencing, and modulation of intracellular Ca2+ dynamics. Activation of this system has widespread effects on the CNS as $GABA_B$ receptors are found throughout the brain. The micromolar concentrations of GHB that are

normally present in mammalian brain tissue can activate GHB receptors but are insufficient to activate $GABA_B$ receptors, for which GHB has a weak affinity. However, the supraphysiologic (i.e., millimolar) concentrations of GHB that result from systemic administration of this compound have been shown to compete for binding sites at the $GABA_B$ receptor, activate recombinant $GABA_B$ receptor heterodimers, and have an electrophysiological effect that is blocked by a specific $GABA_B$ receptor antagonist but not by a GHB antagonist (Wong et al 2004). In support of this, the specific $GABA_B$ agonist baclofen, resembles in part GHB with regard to *in vivo* effects, such as sedation, reduction in body temperature, and hypolocomotion (Quéva et al. 2003). A summary of the several lines of evidence for the hypothesis that GHB itself directly activates $GABA_B$ receptors is summarized in Table 1.

TABLE 1. Evidence for actions of GHB at $GABA_B$ and GHB receptors*

GHB receptors
GHB binding in rat brain tissues does not overlap with $GABA_B$ receptor distribution, and has a different developmental profile
In mice lacking $GABA_B$ receptors, GHB still binds to brain tissues indicating specific GHB receptors. No changes in GHB[^{3}H]-NCS-382 binding are also found
Behaviourally, rats discriminate between GHB and the $GABA_B$ agonist baclofen
GHB modulates presynaptic cAMP levels in the rat independently of $GABA_B$ receptors
The putative GHB receptor antagonist NCS-382 is more effective than $GABA_B$ antagonists in blocking lethal seizures in epileptic mice
$GABA_B$
GHB is a partial agonist at expressed $GABA_B$ receptors in *Xenopus Oocytes*
GHB inhibits MAP kinase phosphorylation via $GABA_B$ receptors when conversion of GHB to GABA is blocked
GHB depresses electrical activity in the cerebral cortex and inhibits transmitter release via $GABA_B$ receptors
GHB reduces the release of glutamate and GABA via $GABA_B$ receptors in rat cortex as measured by microdialysis
The two lines of $GABA_{B1}$ receptor knockout mice lack the most prominent pharmacological actions of exogenously applied GHB
Novel GHB ligands that are not metabolized to GABA and have no activity at $GABA_B$ receptors are unable to mimic discriminative stimulus effects, and the catalepsy elicited by GHB, effects which are blocked by $GABA_B$ receptor antagonists

* (Modified from Drasbek et al. 2006).

In summary GHB stimulates both $GABA_B$ receptors as well as distinct GHB receptors differentially in the mammalian brain. Both receptor types may be activated when GHB is ingested for abuse purposes. However, experimental evidence to date suggests that the high concentrations of GHB in brain tissue that follow exogenous administration (GHB intoxication, addiction and abuse) may exert their pharmacologic, toxicological and behavioural effects primarily through mechanisms mediated by the $GABA_B$ receptor.

1.1.3.5. Effects on neurotransmitter systems

Although GHB has little or no affinity for receptors other than $GABA_B$ and GHB receptors, it will cause changes in a variety of neuromodulatory systems in the brain.

1.1.3.5.1. Dopaminergic system

Dopamine release is associated with the reward system in the brain. Therefore extensive work has been done to examine the interplay between GHB and dopamine. GHB affects dopamine release in the brain. In animals, GHB can decrease or increase dopamine levels. It seems that GHB biphasically affects the activity of the mesolimbic system, which releases dopamine. At concentrations seen during recreational use (<1 mM), GHB preferentially activates postsynaptic $GABA_B$ receptors on GABAergic neurons, hyperpolarizing them and decreasing their firing rate. This effect would in turn disinhibit the dopamine cells neurons leading to enhanced dopamine output from the VTA, which is typical of drugs of abuse (Cruz et al. 2004). Higher GHB concentrations, however, would also decrease the firing rate of dopaminergic neurons, thus producing an overall reduction in dopamine output from the VTA. This later effect would explain the therapeutically used anti-craving properties of GHB in the treatment of alcohol dependence/withdrawal. These effects seem mediated via postsynaptic $GABA_B$ receptors coupled to K^+ channels. The coupling efficacy of $GABA_B$ receptors to GIRK channels is shown to be lower in GABAergic than in dopaminergic neurons of the VTA, thus providing the first molecular/cellular explanation for the rewarding and anti-craving properties of GHB (Cruz et al. 2004). Lately it has been also shown in dopamine neurons of mice, that the low coupling efficiency reflects the selective expression of heteromeric GIRK2/3 channels and is dynamically modulated by a member of the regulator of G protein signaling (RGS) protein family. Repetitive exposure to GHB would increase the $GABA_B$ receptor-GIRK channel coupling efficiency through downregulation of RGS2. This mechanism might underlie tolerance to GHB and would explain why oral self-administration of GHB at a concentration that is normally rewarding, becomes aversive after chronic exposure (Labouèbe et al. 2007). In addition, putative GHB-producing neurons are surrounded by dopaminergic terminals, suggesting a direct interaction between GHB and dopamine.

1.1.3.5.2. Serotonergic system

GHB seems to increase serotonin turnover in the brain (Gobaille et al. 2002). In adolescent and adult rats GHB stimulated both serotonin synthesis and breakdown and in succinic semialdehyde dehydrogenase deficiency, where GHB accumulates in the brain, serotonin turnover is also enhanced (Gibson et al. 2003).

1.1.3.5.3. Opioid system

GHB itself lacks affinity for mu, kappa or delta opioid receptors. However, the opioid system seems to be indirectly coupled to GHB pharmacology in a manner yet to be determined. Some studies have suggested that the opioid antagonists can block the central effects of GHB (i.e. the effect of GHB on striatal dopamine release was reversed by naloxone in rats). Nevertheless, in another study in rodents naloxone could not reduce the anxiolytic effect of GHB.

1.1.3.5.4. Cholinergic system

GHB decreases the acetylcholine levels in the corpus striatum and brain stem and reduces extracellular levels of acetylcholine in the hippocampus via $GABA_B$ receptors (Nava et al. 2001). This could suggest a possible effect of acetylcholine in cognition impairment effects of GHB.

1.1.3.5.5. Glutamatergic system

GHB has been shown to affect glutamate transmission. A recent elegant study in rats has shown that GHB at intoxicating doses preferentially inhibits N-methyl-D-aspartic acid (NMDA)-mediated cortical synaptic activity more than AMPA-mediated responses, while baclofen exhibited the opposite effect. This would suggest a possible role of NMDA antagonism in the effects of GHB (Li et al. 2007).

1.1.3.5.6. GHB and neurosteroids

In rats the administration of GHB (>300 mg/kg i.p.) increases brain allo-pregnanolone (AP) and allo-tetrahydrodeoxy corticosterone (THDOC) (and its precursors progesterone and pregnenolone) in the cerebral cortex. Both AP and THDOC are neurosteroids and positive modulators at the $GABA_A$ receptor and substances exerting anxiolytic, anticonvulsant, and

hypnotic effects. The effects are blocked by a $GABA_B$ receptor antagonist while the GHB receptor antagonist NCS-382 has no effect. Furthermore, baclofen mimics the effects of GHB on neurosteroid release. The rise in neuroactive steroids *in vivo* by GHB is thought to arise from stimulation of the hypothalamic-pituitary-adrenal axis (Wong et al. 2004).

1.1.3.5.7. GHB and growth hormone

GHB increases the secretion of growth hormone (GH) in humans (Van Cauter et al. 1997) and animals. GHB slow-wave sleep promotion during which GH is strongly released from the pituitary, could partially explain this effect. However, the mechanism by which GHB increases GH is still poorly understood.

1.1.4. Pharmacological effects in humans

When beginning the work for this thesis, knowledge of GHB abuse-related effects mainly came from anecdotal reports and surveys but it had not been fully demonstrated after GHB controlled administration of recreational doses. Information regarding GHB clinical pharmacology and GHB pharmacological related effects at doses that are abused was lacking. The subjective effects and GHB dose related effects will be described separately in sections 1.1.5. and 1.1.6.

1.1.4.1. Cardiovascular effects

Moderate bradycardia was reported after controlled administration of GHB possibly due to central vagal activity. In addition to bradycardia, GHB was able to reduce stroke volume as well as cardiac output. Atropine reversed the decreases in both heart rate and stroke volume. (Virtue et al. 1966). Recent experiments suggest also that GHB elicits sympathomimetic cardiovascular effects that could induce increases in blood pressure following its acute administration (Hicks et al. 2004). The autonomic centres are fully active during GHB-induced coma, and surgical stimuli result in a cardiovascular response, such as tachycardia, hypertension, and raised cardiac output (Vickers 1969; Virtue et al. 1966).

1.1.4.2. Respiratory effects

GHB apparently decreases respiratory frequency while increases breath amplitude (tidal volume) sometimes leading to a Cheyne-Stokes pattern (Vickers 1969). Combined drug use might also increase the risk of hypoventilation. Thus, it has recently been proved that combined administration of alcohol and GHB (at recreational doses) causes a significant blood oxygen saturation drop in healthy volunteers (Thai et al. 2006).

1.1.4.3. Neuroendocrine effects

A few studies have evaluated the effect of GHB on growth hormone (GH) in awakened subjects and during sleep. In a randomized and controlled study in nine male healthy volunteers, GHB induced a significant increase in plasma GH concentrations that was prevented with previous administration of the benzodiazepine receptor antagonist flumazenil (Gerra et al. 1994). These results may suggest that some of the effects of GHB on GH are mediated at $GABA_A$ receptors. Recently the effect of GHB on the secretion of several hormones was studied in a randomized placebo controlled clinical trial. Oral GHB doses of 2.5, 3.0, and 3.5 g were administered at bedtime to eight healthy volunteers (Van Cauter et al. 1997). A significantly increase in the normal secretory pulse of GH during the first 2h after sleep onset was observed. This stimulation of GH secretion was significantly correlated to a simultaneous increase in the amount of sleep stage IV. This effect is believed to be produced by a large pulse in growth hormone secretion during the first stage of slow-wave sleep. Abrupt but transient elevations of prolactin and cortisol were also observed, but did not appear to be associated with the concomitant stimulation of slow-wave sleep. Thyrotropin and melatonin secrectory profiles were not altered by GHB administration. Recently the effect of GHB on GH secretion has been also studied in both normal controls and parkinsonian patients (Volpi et al. 2000). A significant serum GH rise in response to GHB (25 mg/kg body weight p.o.) was observed in controls and in a lesser extent in parkinsonian patients. Interestingly, pretreatment with the anticholinergic drug pirenzepine completely suppressed the GHB-induced GH release in both normal controls and parkinsonian patients. This indicates that the cholinergic system is also implicated in the GH response to GHB in normal men.

GHB has been commonly taken for its proposed anabolic effects (related to the ability of GHB to stimulate the release of growth hormone), especially by the bodybuilding community. However, evidence regarding a possible increase in muscle mass or fat catabolism due to GHB effect of GHB

is lacking. In addition, in patients with chronic alcoholism, long-term administration of GHB did not affect muscular mass (Addolorato et al. 1999)

1.1.4.4. Sedation and Anaesthesia

The anaesthetic effects of GHB are primarily hypnotic as GHB provides little or no analgesia. The transition from wakefulness has been described as being a sudden shift from alertness to unconsciousness (Metcalf et al. 1966). The results of early investigations suggested that GHB appears to act on the cerebral cortex with little or no depression of the reticular activating system (Solway and Sadove, 1965). Depression of the limbic hippocampal structures and subcortical centres could be related to its sedative effects.

1.1.4.4.1. Sleep physiology

Early clinical studies in healthy volunteers examined the ability of the drug to induce a sleep-like state. GHB was able to produce a predictable sequence of dose-dependent EEG changes, decrease sleep onset latency, promote delta activity and enhance sleep maintenance (Metcalf et al. 1966, Yamada et al. 1967).

In further studies in healthy subjects under double-blind conditions, single oral doses of 2.25 g GHB significantly increased stages III and IV and decreases stage I of non-rapid eye movement (NREM) sleep. In addition, GHB improved REM efficiency at night and reduced wake time after sleep onset when administered before a morning nap recording (Lapierre et al. 1990). Furthermore the administration of oral 2.5, 3.0, 3.5 g GHB doses at bedtime to 8 healthy volunteers was able to increase the time spent in slow-wave sleep in healthy volunteers. These subjects showed significant reduced sleep latency at all GHB doses compared with placebo in addition to increasing GH secretion. The effects occurred mainly during the first third of the night after sleep onset, as expected with the single nightly dose administered (Van Cauter et al. 1997).

Sleepwalking events have been described in narcolepsy patients. Despite the fact that these patients generally take 2 doses of GHB while in bed and that they are both capable of inducing slow-wave sleep, this effect has been clearly associated with the second dose rather than the first. Capacity-limited elimination contributing to higher blood levels after the second dose may explain this phenomenon (Scharf et al. 1998).

1.1.4.5. Memory

Most of the information concerning the effect of GHB on memory comes from case reports of GHB abuse and/or overdose. The short and long term use of GHB has been associated with an impairment of human memory. However, the results from the few clinical trials examining cognitive effects are controversial. In an early study, following GHB 10 mg/kg i.v administration subjects exhibited a significant short term memory deficit in a digit recall test at a prolonged delay interval of 20 seconds but not at the shorter interval of 4 seconds. However, this effect was only observed 5 minutes after administration and not later in the experiment (Grove-White and Kelman, 1971). In contrast no changes in short-term memory were found in a study where two oral doses of 12.5 or 25 mg/kg were administered to healthy volunteers (Ferrara et al. 1999). However, both studies presented important drawbacks and differences in the time at which cognitive tests were performed. This could account for the opposite results found. More recently the cognitive effects of supratherapeutic doses of GHB, triazolam and pentobarbital were evaluated in subjects with histories of sedative abuse (Carter et al. 2006). GHB, unlike triazolam and pentobarbital, did not impair participants' recall of eight-digit numbers when they were able to replicate (enter) those numbers. Furthermore, at doses in which participants were able to study a list of words, GHB did not impair the participants' ability to discriminate new and old words 5 h later. Taken together, these data show that at doses that prevented the assessment of cognitive effects (i.e. loss of consciousness) the effects of GHB on these memory tasks were significantly less than those of triazolam and pentobarbital and not significantly different from placebo. Thus, it can be hypothesised that the 'amnesia' that is often attributed to GHB in clinical reports is due to loss of consciousness and not any selective impairment of memory processes.

1.1.4.6. Pupil size

Pupils of GHB suspected intoxicated patients have been described as being miotic or mydriatic and sluggishly reactive to light (Mason and Kerns. 2002). However, the effect of GHB to pupil size has not been previously thoroughly investigated in humans.

1.1.5. Subjective effects

Despite the extensive research of GHB for therapeutic purposes, the information about GHB subjective effects mainly stem from anecdotal reports and surveys. Only few studies have tested GHB elicited subjective effects under controlled experimental settings.

1.1.5.1. Anecdotal reports and surveys

A survey inquiring about the effects of GHB was administered to 42 users in the U.S. GHB was predominately used for 'going out' and having fun. The most frequent reported subjective effects after GHB use were increased feelings of euphoria, well being, happiness, increased sexual interest, augmented tendency to talk, relaxation, pleasant drowsiness and disinhibition. Interestingly, users reported some effects related to stimulant-like effects (i.e. increased energy, euphoria, reduced appetite) and other effects more related to GHB sedative properties (i.e. relaxation and drowsiness) (Miotto et al. 2001).

In a study of 76 Australian users, feelings of euphoria, relaxation, increased sociability and loss of inhibition, as well as heightened sexual interest were reported by the majority of the users (Degenhardt et al. 2003).

1.1.5.2. Controlled studies

A randomized and placebo controlled study was performed in 8 opioid-dependent stabilized patients on levorphanol treatment prior to a naloxone challenge. GHB 30 mg/kg (2.1 g/70 kg) but not 15 mg/kg, was found to significantly increase subjective ratings of 'good mood' 'spaced' 'sluggish' and 'carefree' without significantly affecting psychomotor performance (Rosen et al. 1997). No significant differences were found on any of the scales of the 'Addiction Research Center Inventory' (ARCI) short form questionnaire. However, the design of this study (not specifically planned to measure GHB subjective effects) and the presence of an outlier with an unexpected placebo response might bias the results found.

The effects of GHB on human psychomotor performance and subjective feelings were also studied in 12 healthy volunteers (six males and six females) in a double-blind, cross-over study; two different GHB doses (12.5 and 25 mg/kg), lorazepam (0.03 mg/kg) or placebo were

administered (Ferrara et al. 1999). The only significant changes found were an increased feeling of calmness with the lower GHB dose and a decreased contentedness after both GHB doses. The administration of both GHB doses had no effects on attention or psychomotor performance. However some subjective feelings of dizziness and dullness were recorded as adverse effects, which disappeared 30-60min after GHB administration. Intriguingly the effect of orally administered GHB was tested initially at 15 minutes, but no repeated measures were recored until 60, 120 and 180 minutes following GHB administration. The absence of testing during the 15–60 minute interval following the oral administration could explain the fact that no changes in subjective effects and performance were observed following oral doses of GHB.

In summary, at the time of the initiation of this work most of the information of GHB subjective effects was based in surveys and anecdotal reports and the results of the controlled studies suffered from important drawbacks.

1.1.6. GHB dose related effects

While this thesis was being settled, a review of GHB dose related effects was carried out. The first remarkable fact was that few studies had previously examined GHB dose-related effects under controlled administration. In addition we found several significant discrepancies regarding GHB dose related effects. In particular GHB doses leading to coma induction were fairly unclear. First, numerous publications suggested that GHB doses of 20-30 mg/kg could produce sedation and sleep, and that coma may result when twice this dose is administered. This was partially supported by studies where GHB was administered IV (Yamada et al. 1967). However, there was a strong confusion about the effect of different GHB oral doses in humans. GHB oral doses as little as 10 mg/kg had been associated to the induction of euphoria, amnesia or increased libido. However this was not supported by clinical data. In addition, several discrepancies in reporting the dose of GHB as GHB or sodium GHB interchangeably are consistently found in the literature. This could lead to underestimation of GHB effects in trials where GHB was administered as sodium oxybate or sodium GHB (as 1 mg of sodium GHB is equivalent to 0.83 mg of GHB).
Taking into account these uncertainties GHB dose related effects after oral administration were for the majority uncharacterized previous to this thesis work. Even today, numerous publications in high impact journals continue to wrongly report GHB dose related effects in humans (Snead 3rd and Gibson, 2005). A summary of GHB dose-related effects after oral administration in different clinical studies published is shown in table 2.

TABLE 2. Summary of GHB dose related effects found in previous studies in humans. Manuscript and year of publication, doses given, type of GHB preparation, population and comments.

Manuscript / Year of publication	Doses given Oral GHB / NaGHB	Population	Comments
Metcalf et al. 1966	35-63 mg/kg GHB	20 Healthy Volunteers	-Drowsiness induced after all doses -Doses >50 mg/kg lead to coma. -Myotic Pupils
Mattila et al. 1978	1 g and 2 g * Ethanol 0.5 g/kg ?	12 Healthy Volunteers	-Psychomotor performance impairment observed only in combination with ethanol
Hoes et al. 1980	50, 75 and 100 mg/kg ?	8 Insomniac patients	-Sleep induced at all doses -Subjects awoke at GHB plasma concentrations below 90 mg/dl
Palatini et al. 1993	25 and 50 mg/kg NaGHB	8 Healthy Volunteers	-Mild drowsiness and/or dizziness feeling in all subjects
Van Cauter et al. 1997	2.5, 3.0 and 3.5 ** g ?	8 Healthy Volunteers	-2.5 and 3.0 g indistinguishable from placebo - 3.5 g drunkenness /intoxicated feeling
Ferrara et al. 1999	12.5 and 25 mg/kg NaGHB	12 Healthy Volunteers	-Psychomotor performance was not impaired -25 mg/kg dose induced dizziness
Volpi et al. 2000	25 mg/kg NaGHB	-10 Healthy Volunteers -10 Parkinson's patients	-↑GH -No subjective / side effects reported
Borgen et al. 2003	4.5 g *** NaGHB	36 Healthy Volunteers	-Tmax at 1h -85% Females experienced side effects (Nausea, vomiting and CNS related)

* 14.3 and 28.6 mg/kg for a 70 kg weight person
** 35.7, 42.9 and 50 mg/kg for a 70 kg weight person
*** 64.3 mg/kg for a 70 kg weight person
? Not reported

1.1.7. Pharmacokinetics

The pharmacokinetics of GHB after oral administration have been investigated in healthy volunteers, alcohol-dependent patients, patients with liver impairment and narcoleptic patients (Ferrara et al. 1992; Ferrara et al. 1996; Palatini et al. 1993; Scharf et al. 1998). Consistent with its rapid onset and short pharmacological effect, the data indicated that both GHB absorption into and elimination from the systemic circulation are rapid processes. GHB is rapidly but incompletely absorbed after oral administration. It is eliminated mainly by metabolism with a $t_{1/2}$ of 0.5 to 1h. GHB pharmacokinetics is not significantly altered with repeat dosing and no gender differences are apparent. A summary of GHB main pharmacokinetics parameters is presented in Table 3.

1.1.7.1. Absorption

GHB is absorbed rapidly following oral administration and undergoes extensive first-pass metabolism. The absolute bioavailability of GHB was determined to be about 27% when a dose of 100 mg/kg was compared after intravenous and oral administration (Vree et al. 1978). Plasma concentrations usually reach a peak within 30-60min. The average time to peak plasma concentration (t_{max}) ranged from 0.5 to 1.25h in eight pharmacokinetic studies. The average peak plasma concentrations (1st and 2nd peak) following administration of a 9 g daily dose divided into two equivalent 4.5 g doses given 4h apart were 77.6 and 141.7 µg/mL, respectively (Borgen et al. 2000). Administration of GHB immediately after a high fat meal resulted in delayed absorption (t_{max} increased from 0.75 to 2.0 hr) and a 58% reduction in C_{max} and of systemic exposure (AUC) by 37% (Borgen et al. 2003).

1.1.7.2. Distribution

GHB is a hydrophilic compound with an apparent volume of distribution (V_d) averaging 1.9-3.8 L/kg. Distribution to target tissues occurs rapidly and follows a two-compartment model. The distribution of GHB to various tissues, including the brain, is partially dependent on specific transporters, since at physiological pH, more than 99% of GHB is ionized and can not readily diffuse across cellular membranes. Recent studies have demonstrated that the monocarboxylate transporters (MCT1, MCT2 and MCT4) are important in the membrane transport of GHB (Wang and Morris, 2007). Less than 1% of GHB is bound to plasma proteins at plasma concentrations ranging from 3 to 300 µg/mL.

1.1.7.3. Metabolism

Metabolism is the major elimination pathway for GHB. The primary pathway involves GHB dehydrogenase, a cytosolic NADP+-linked enzyme that catalyses its conversion to succinic semialdehyde (SSA). SSA is then biotransformed to succinic acid by the enzyme succinic semialdehyde dehydrogenase. Succinic acid enters the Krebs cycle where it is metabolized to carbon dioxide and water. A second mitochondrial oxidoreductase enzyme, a transhydrogenase, also catalyses the conversion to succinic semialdehyde in the presence of α-ketoglutarate. An alternate pathway of biotransformation involves β-oxidation via 3,4-dihydroxybutyrate to carbon dioxide and water. No active metabolites have been identified.

GHB does not significantly inhibit the activities of the human isoenzymes: CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A up to the concentration of 3 mM (378 µg/mL), as shown in *In vitro* studies with pooled human liver microsomes (Xyrem 2005).

1.1.7.4. Elimination

On average, less than 5% of unchanged drug appears in human urine within 6 to 8h after dosing. Faecal excretion is negligible. The clearance of GHB is almost entirely by biotransformation to carbon dioxide, which is then eliminated by expiration. Elimination half-life ($t_{1/2}$) is significantly longer in cirrhotic patients than in control subjects (mean $t_{1/2}$ of 59 versus 22min) due mainly to a decreased clearance (Ferrara et al. 1996). GHB can be detected in urine samples up to 8–10h after ingestion, and in blood samples up to 5–6h after single doses up to 4.5 g.

1.1.7.5. Non-linear pharmacokinetics

The systemic exposure of human subjects to GHB increases disproportionately with dose, suggesting capacity limited elimination. Thus, doubling of the nightly oral dose from 4.5 g to 9 g resulted in a 3.8-fold increase in AUC and a significantly longer half life than after the 4.5 g dose (59 vs 35min) (Borgen et al. 2000). The mechanisms underlying GHB non-linear pharmacokinetics is unknown. It may be caused by capacity-limited metabolism, absorption and renal elimination. Interestingly GHB pharmacokinetics exhibits non-linearity only in some subjects.

1.1.7.6. GHB plasma levels in intoxication cases

In the published cases of GHB intoxication including confirmed diagnosis and GHB blood levels, the results have been certainly dissimilar and it has also been impossible to relate GHB plasma levels to clinical state severity (Sporer et al. 2003). The range of plasma concentrations found varies from 45 - 551 mg/L.

TABLE 3. Summary of main pharmacokinetic parameters of GHB

Parameter	Value
Half-life (h)	0.5–1
Oral bioavailability (%)	25
Time to maximum concentration (h)	0.5–1.25
Protein binding (%)	<1
Active metabolites	None known
Renal elimination (%)	<5
pKa	4.7
Apparent volume of distribution (L/kg)	1.9–3.8
CYP450 drug interactions	None known

1.1.8. Interactions

The number of studies assessing the possible interactions of GHB with other drugs is limited. A summary of GHB possible pharmacological interactions is presented in Table 4.

TABLE 4. Possible pharmacological interactions of GHB (Farré and Abanades, 2006; Harrington et al. 1999; Liechti et al. 2006; Sevak et al. 2004; Thai et al. 2006; Uys and Niesink, 2005).

Product / Substance	Interaction Mechanism	Consequence
Alcohol	PK-PD	-Combined use may increase GHB's CNS depressant action, as well as the risk of gastrointestinal adverse effects, arterial hypotension and hypoventilation. -Combined use of BD and alcohol may reduce BD effects due to a reduction of the conversion efficiency of BD into GHB (possible competition for alcohol dehydrogenase). -Pharmacokinetics: possible increase of both plasma GHB concentrations and GHB $t_{1/2}$.
Cannabis	PD	-Combined use may increase GHB's depressant action over CNS
Opiates	PD	-Combined use may increase GHB's depressant action over CNS and respiratory system
Amphetamines, cocaine and other CNS stimulants	PD	-CNS antagonic effects, which may mask GHB action. -Amphetamine users possibly consume higher doses of GHB. -Eventual decrease of convulsion threshold.
Ketamine - phencyclidine	PD	-Possible synergistic effect of GHB's and ketamine's depressant effect over CNS -The NMDA receptor antagonist, dizocilpine (MK-801), amplifies GHB's cataleptic action in rats.
Benzodiazepines / Barbiturates	PD	-Synergistic effect when associated to GHB. -Combined use increases the depressant action over CNS and respiratory system.
Ritonavir, saquinavir	PK	-Coma, bradycardia, respiratory depression, convulsions have been described in a HIV+ patient treated with ritanovir and saquinovir after GHB intake. Possibly due to an inhibition of CYP450 enzymatic system leading to an increase of plasma GHB concentration.
Valproate, phenytoin, ethosuximide	PK	-Through GHB dehydrogenase inhibition. -No reports / studies in humans, however interaction with these drugs is possible
Neuroleptics	PD	-Possible synergistic effect of GHB's depressant action over CNS (effect already proven in rats).
Antidepressants	PD	-Possibility of adding effect, especially in the case of trycilic antidepressants.

1.1.9. Toxicology of GHB in humans

1.1.9.1. Adverse effects

The adverse effects produced by psychoactive drugs clearly differ depending on the population using the drug. Often, psychoactive effects usually described as 'unwanted' by patients (loss of control, 'high') could be in contrast, specifically sought by recreational users. This is also the case of GHB as some of the adverse events described during the clinical trials are on the contrary, sought after effects by recreational users of the drug. The adverse effects described below were found mainly in experimental studies testing GHB as a therapeutic agent and in reports of perceived adverse effects by recreational GHB users. The effects and consequences of GHB intoxication will be described separately. GHB predominantly affects the CNS, cardiovascular system, and respiratory system with lesser impact over the kidneys or the liver.

1.1.9.1.1. Adverse effects in patients

A total of 717 narcoleptic patients had been exposed to GHB (Sodium Oxybate) in clinical trials. The most commonly observed adverse events associated with the use of sodium oxybate were: headache (22%), nausea (21%), dizziness (17%), nasopharyngitis (8%), somnolence (8%), vomiting (8%), confusion (7%) and urinary incontinence (7%). Additional adverse effects were sleep disorders, sleep walking episodes, urinary incontinence, and vomiting. The incidence of adverse effects appears to be related to initiation of therapy and dose and tends to be higher in females than males. In these clinical trials, 10% of patients discontinued because of adverse events compared to 1% receiving placebo. The most frequent reasons for discontinuation (>1%) were nausea (2%), dizziness (2%), vomiting (1%) and confusion (1%) as well as urinary incontinence, dyspnea, hyperesthesia, paresthesia, somnolence, tremor, vertigo, and blurred vision, all occurring in <1% of patients. Some of the adverse events were dose dependent. Thus, in a controlled trial patients were randomized to fixed total daily doses of 3, 6, and 9 g/day or placebo. Confusion was reported at all recommended doses, nonetheless a dose-response relationship for confusion was demonstrated with 17% of patients at 9 g/day experiencing confusion. The confusion resolved soon after termination of treatment and in the majority of cases, it also resolved even through continued treatment.

GHB has also been studied for the treatment of alcohol withdrawal; dizziness, nausea, vertigo and tiredness (in descending order) have emerged during GHB use. Several studies investigated

the pharmacokinetics of GHB in different populations. Although information of adverse events was generally lacking in these publications, the main adverse events observed were dose dependent and involved mainly dizziness, somnolence and nausea (Palatini et al. 1993; Scharf et al. 1998)

1.1.9.1.2. Adverse effects in GHB recreational users

The doses of GHB that elicit adverse effects vary greatly from report to report and range from 1.25 ml to 60 ml. Additionally 40 ml of clandestinely produced GHB may weigh from 3-20 g (Thomas et al. 1997).

A few observational studies have studied GHB perceived adverse effects by GHB users. In 2001 a survey inquiring about the effects of GHB in 42 American users reported episodes of unpredictable loss-of-consciousness lasting minutes to hours in 69% of participants. However, participants considered these episodes as equivalent to 'falling asleep' in contrast to an overdose or toxic state. The most frequent reported adverse effects during GHB use (25-50% users) were nausea, auditory and visual hallucinations and headache and, less frequently (1-24%) amnesia, diarrhoea, stiff muscles and loss of bladder control. The most frequent reported adverse effects after GHB use (30-60%) were confusion, clumsiness and amnesia. Adverse effects occurred more frequently in daily users and polydrug users than in occasional GHB users (Miotto et al. 2001).

In a survey in 76 Australian GHB users (Degenhardt et al. 2002), 99% reported at least one potentially adverse effect (mean number of side effects reported was 6.5). Three quarters reported that they had experienced dizziness and blurred vision while using GHB and two-thirds reported hot/cold flushes. The following adverse effects were reported by around half of the sample: lost consciousness, profuse sweating, vomiting, memory lapses and tremors. Although the vast majority of users felt that these negative side effects were caused by their GHB use, most of them were polydrug users and this could have biased the results. It must be noted that many GHB users in this survey did not appreciate side effects as a negative matter. Furthermore some users considered that GHB overdose was not in itself a dangerous issue.

1.1.9.2. GHB intoxication in humans

The intoxication/overdose effects of GHB (or its precursors) have been extensively reviewed in humans. Nevertheless, the effects of pure GHB intoxication have not yet been duly described due to a lack of publications regarding confirmed cases (GHB detected in plasma and/or urine) and frequent concomitant poly-drug use. These drawbacks make it difficult to assign the clinical features purely to GHB intoxication.

Along with this thesis work, a review of the relevant literature regarding with the effects of GHB intoxication in humans was performed. In an effort to summarize the information available, Table 5 shows an outline of the clinical and epidemiological features of a series of cases published by our group (Abanades et al. 2001), along with the six largest case series previously published. The most relevant data regarding GHB intoxication in humans will be reviewed throughout this chapter.

TABLE 5. Description of the largest series of GHB intoxication cases (Dyer 1991; Chin et al. 1998; Abanades et al. 2001; Liechti and Kupferschmidt, 2004; Miró et al. 2002; Sporer et al. 2003; Elliott 2004).

Author / Country / Date Reference	**DYER / USA /** JUN - OCT 90	**CHIN / USA /** JAN 93 - DEC 96	**LIECHTI / SWISS** JAN 95 - DEC 03	**SPORER / USA /** JUL 98 - JAN 99	**ELLIOT / UK /** MAY 98 - MAY 03	**MIRÓ / SPAIN /** APR 00 - JUN 01	**ABANADES / SPAIN** JAN - JUN 01	**TOTAL**
Number of Cases	16	88	141	16	27	104	11	**403**
socio - demographical data								
Average Age +/- DE	33+/-9	28+/-6	24+/-7	25	26+/-5	23+/-5	22+/-3	**26**
Sex (men %)	14 (88)	61 (69)	103 (73)	11 (69)	25 (92)	69 (66)	9 (82)	**292 (72)**
Weekend (%)	NP	NP	73 (52) *	NP	NP	96 (92) *	9 (82)	**178 (70)** *$_{256}$
Clinical Data								
GCS =3 (%)	NP	25 (28)	32 (23)	11 (69)	4 (15)	18 (17)	2 (18)	**92 (24)** *$_{387}$
GCS < 9 (%)	4 (25)	53 (60)	56 (40)	16 (100) a	6 (22)	52 (50)	5 (45)	**192 (48)**
Myoclonus (%)	2 (13)	2 (2)	18 (13)	NP	NP	6 (6) *	0	**28 (8)** *$_{360}$
Agitation (%)	3 (19)	NP	27 (19) *	NP	NP	5 (5) *	0	**35 (13)** *$_{272}$
Confusion (%)	3 (19)	NP	NP	NP	NP	NP	NP	**3 (19)** *$_{16}$
Pupils / Mydriasis (%)	NP	NP	NP	NP	NP	57 (57) *	5 (45)	**62 (54)** *$_{115}$
Arterial hypotension SBP< 95 mm Hg (%)	NP	10 (11)	11 (8) *	NP	NP	7 (7)	0	**28 (8)** *$_{344}$
Bradycardia < 55 bpm (%)	2 (13)	32 (35)	42 (30) *	5 (31)	NP	20 (20) *	2 (18)	**103 (27)** *$_{376}$
Hypothermia < 36º C (%)	NP	48 (69) *$_{70}$	NP	NP	NP	29 (28)	6 (56)	**83 (41)** *$_{203}$
Sickness (%)	2 (13)	NP	NP	NP	NP	0	1 (9)	**3 (2)** *$_{131}$
Vomiting (%)	7 (44)	26 (30)	23 (16) *	NP	NP	23 (23) *	1 (9)	**80 (22)** *$_{360}$
Hypoventilation / Respiratory acidosis (%)	NP	21(70) *$_{30}$	12 (9)	5 (31)	3 (11)	6 (6)	4 (36)	**51 (13)** *$_{387}$
Mechanical ventilation (%)	0	11 (13)	18 (13)	2 (13)	0	3 (3)	0	**34 (8)**
Bronchial aspiration (%)	NP	NP	NP	1 (6)	NP	0	0	**1 (<1)** *$_{131}$
Drug Use								
Combined Use[b] (%)	1 (6) c	25 (28) d*$_{20}$	48 (34) *	11 (69) d*$_{2}$	15 (55)	89 (86)	10 (91)	**199 (49)**
Pure GHB intoxication (%)	13 (81)	NP	51 (36)	5 (31)	6 (22)	?	0	**75 (36)** *$_{211}$
Alcohol (%)	2 (13)	34 (39)	31 (22)	7 (44)	6 (22) *$_{21}$	76 (73%)	8 (73)	**164 (41)** *$_{397}$
Amphetamine derivatives[e] (%)	0	27 (31)	30(21) *	2 (13%)	11 (41)	45 (43%)	9 (82)	**124 (31)**
Cannabis (%)	1 (6)	2 (2)	23 (16) *	0	4 (15)	8 (8%) *	7 (64)	**45 (11)**
Cocaine (%)	0	4 (5)	11 (8) *	1 (6)	5 (19)	26 (25) *	6 (55)	**53 (13)**
Opiates (%)	0	2 (2)	7 (5) *	1 (6)	3 (11)	1 (1) *	0	**14 (3)**
Hypno - sedatives[g] (%)	0	2 (2)	1 (1) *	1 (6)	5 (19)	3 (3) *	2 (18)	**14 (3)**
LSD (%)	0	1 (<1)	0 *	NR	0	NP	0	**1 (<1)**
Ketamine (%)	0	0	1 (1) *	NR	1 (4)	11 (11) *	0	**13 (3)** *$_{376}$
Laboratory results								
Plasma GHB average +/- DE mg/l (range)	NP	NP	NP	180 (45-295) *$_{15}$	245+/-118 (86-551) *$_{20}$	?	0	**NP**
Urine GHB average +/-DE mg/l (range)	NP	NP	NP	1263 (432-2407)	1732+/-1506 (5-5581) *$_{22}$	NP	885 (130-1680)	**NP**
Plasma GBL	NP	NP	NP	NP	NEG *$_{14}$	NP	0	**NP**
Urine GBL	NP	NP	NP	NP	POS *$_{15}$	NP	0	**NP**
Plasma OH mg/dl average (range)	NP	>40 *$_{15}$	NP	104 (12-295) *$_{7}$	NP	NP	57 (40-80) *$_{10}$	**NP**

a) Inclusion criteria. b) Illegal drugs. c) Only by anamnesis. d) Urine screening e) amphetamines, MDMA, methamphetamine f) methadone, heroine. g) benzodiazepines, barbiturates. NP: not performed. ?: unknown. NEG: negative. POS: positive. * Calculated as a percentage. *$_n$ data available for 'n' patients

1.1.9.2.1. Neurological

GHB intoxication induces a spectrum of CNS changes. A decrease in the level of consciousness is a constant feature in all intoxication cases described whether other drugs are present or not. Patients usually present an abrupt decrease in the level of consciousness that may end in a deep, unreactive coma in up to 1 out of 4 GHB intoxicated patients, depending of the series (48% of total patients scored < 9 in GCS). Lower GCS scores have been associated with a simultaneous use of GHB along with MDMA or cocaine and to a more prolonged coma (Liechti and Kupferschmidt, 2004; Liechti et al. 2006). In series where plasma concentrations were available, no statistically significant correlations between GHB plasma levels and both the GCS score and the time of awakening from coma have been found (Sporer et al. 2003). On the other hand, it should be emphasized that pure GHB intoxication under controlled settings has been described as a spontaneously reversible coma within a few hours and no additional severe adverse effects (Carter et al. 2006).

A confusional state may also be observed mainly in non-severe intoxications and also during the awakening from coma, although this fact has not been mentioned in most of the publications. Minor effects include ataxia, muscular weakness, and urinary/faecal incontinence which are difficult to exclusively assign to GHB. Unusual random clonic movements and uncontrollable shaking associated with GHB have been described as well. However, these symptoms appearing in around 10% of the patients, are currently allocated as non-epileptic myoclonic jerks. These features were also described in earlier clinical trails testing GHB as an anaesthetic (Vickers 1969).

1.1.9.2.2. Psychiatric effects

The presence of agitation and strange behaviours (including self-injury) has been associated to GHB in some intoxication case series and could have been under-reported in earlier series published (Zvosec and Smith, 2005). In addition, the combined consumption of alcohol and GHB has been correlated to the appearance of agitation (Liechti et al. 2006). Psychiatric complications such as delirium, paranoia, depression, and hallucinations have also been reported in a small number of patients

1.1.9.2.3. Ocular effects

Regarding pupil diameter, mydriasis, miosis and also medium pupils have been described in GHB intoxication cases (Mason and Kerns, 2002). Again, the frequent presence of other substances with effects on pupil size makes it difficult to attribute a particular effect on pupil size to GHB.

1.1.9.2.4. Respiratory effects

Respiratory depression, difficulty breathing, and apnea have been reported after the administration of GHB. It appears that GHB decreases respiratory frequency while increases breath amplitude. Nonetheless, hypoventilation might occur if this compensation does not take place. The respiratory depression may be especially severe, and abnormal patterns of breathing such as 'Cheyne-Stokes' breathing may also result. Mild acute respiratory acidosis is a common finding (Mason and Kerns, 2002). About 15% of patients of the GHB intoxication case series presented hypoventilation and/or respiratory acidosis, which could be related to a global CNS inhibition. Combined drug use might also increase the risk of hypoventilation. Prognosis and treatment of GHB intoxication basically relies on the appearance of hypoventilation.

1.1.9.2.5. Cardiovascular effects

No serious cardiovascular effects have been described in most GHB therapeutic studies. Bradycardia has occurred when GHB was given for anaesthesia (Vickers 1969) as well as in overdose situations. About 30% of all patients presented sinusal bradycardia, usually asymptomatic, and up to 10% arterial hypotension along the different series of GHB intoxication cases. However the casual assumption that GHB caused these homodynamic alterations is disturbed with the combined consumption of other drugs. ECG alterations, such as U waves or first grade A-V block have also been described. Nevertheless, ECG alterations are not common during GHB intoxication. In case of appearance are usually associated to the simultaneous use of other cardio-toxic substances, mainly alcohol and cocaine (Li et al. 1998; Mason and Kerns, 2002).

1.1.9.2.6. Gastrointestinal effects

A high frequency of vomiting is associated with the use of GHB, especially at high doses and during induction and on emergence from intravenously induced anaesthesia. Nausea and vomiting have been described in around 20% of all intoxicated patients. These could appear in every stage of the intoxication; as the first intoxicating symptom, during coma and at coma awakening. It has been observed that these adverse effects are more frequently suffered by patients with low levels of consciousness (GCS ≤ 8), which increases the risk of bronchial aspiration (Chin et al. 1998; Liechti and Kupferschmidt, 2004). Vomiting occurs more frequently among those patients who have consumed alcohol simultaneously and also those who used GBL or BD.

1.1.9.2.7. Effects in body temperature

A 20 to 70 per cent of patients described in Table 5, presented a variable grade of mild hypothermia, although general clinically non-significant and difficult to attribute purely to GHB.

1.1.9.2.8. Diagnosis

The suspected diagnosis is clinical and based on patient's anamnesis whenever possible. Currently, there is no duly approved quick drug screening test available that detects GHB and/or its precursors, and only a few medical centres have the technology required (GC-MS) to obtain a definitive diagnosis. For this reason, in most of the cases diagnosis is tentative. In the series including confirmed diagnosis and GHB blood levels, the results have been dissimilar and it has also been impossible to relate GHB plasma levels to clinical state severity. The range of plasma concentrations found varies from 45 - 551 µg/mL (Elliot 2004; Sporer et al. 2003). In addition, only in a few studies a difference is made between the use of GHB or its precursos, GBL and BD.

1.1.9.2.9. Management

Table 6 summarizes the management of patients with a suspected intoxication due to GHB or its precursors.

TABLE 6. Management of patients with reduced level of consciousness when suspecting intoxication by GHB and/or one or more 'Club Drugs'.

1. Permeable air-way and safe recovery position
2. Continued monitoring of vital signs: arterial blood pressure, heart and respiration rate, temperature, pulse-oximetry
3. Activated carbon: only if previous drug use <1h. If GCS < 9 airway protection is mandatory
4. Anamnesis: query accompanying persons to determine possible substances involved and in which amount, as well as pathological (and toxicological) history of the patient
5. Neurological examination (pupils and GCS), heart & lung examination
6. Electro-cardiogram
7. Establish vascular access conduction: blood test samples, arterial blood gasometry, ethanol blood levels. Capillary glycemia
8. Drug screening (blood and urine) and collection of extra samples for later GC-MS analysis
9. Thorax radiography if suspecting bronchial aspiration
10. Computed axial tomography scan (CT scan) or magnetic resonance (MR) in cases of prolonged coma or if intracranial hypertension is suspected
11. If meningitis is suspected, lumbar puncture is mandatory, after cranial CT scan
12. The administration of glucose, thiamine and naloxone is generally recommended
13. Expect a potential withdrawal syndrome after regaining of consciousness

1.1.9.2.10. Treatment

At present, no specific antidote for GHB intoxication is clinically available. Routine treatment is based on clinical supervision and support measures. Several drugs have been used for the treatment of GHB induced coma with dissimilar results. Flumazenil and naloxone are not useful in the reversion of GHB induced coma, although its use is not contraindicated and may direct diagnosis in a coma of unknown origin (Espinosa et al. 2001). The use of flumazenil is contraindicated whenever the presence of cocaine is suspected, since it reduces the convulsion threshold and may result in the latter. Physostigmine, a reversible acetyl cholinesterase inhibitor, has been used for reverting coma with contradictory results. Thus, it has been successfully used for coma reversion during GHB intoxication, but there is no scientific evidence based on controlled clinical trials to support its regular use (Traub et al. 2002). Regarding eventual future treatments, studies in animal models show that the GHB receptor antagonist NCS-382 is not able to revert GHB-induced coma. However, the $GABA_B$ antagonist SCH-50911 remarkably increases survival rates among animals treated with lethal doses of GHB and GBL (Carai et al. 2004; Carai et

al. 2005). These facts support that most of the clinical effects after GHB administration are mediated through $GABA_B$ receptors, and support the eventual clinical use for SCH-50911.

1.1.9.2.11. Complications

As mentioned before, an impaired respiratory function (bradypnoea, apnea) marks intoxication prognosis. The two indicators for mechanical ventilation are a decrease of the level of consciousness and respiratory failure. However, in GHB intoxicated patients there is no agreement on whether intubation is convenient or not. The reversibility of the clinical features makes mechanical ventilation unnecessary except with patients presenting severe hypoventilation which fail to improve through patient stimulation and oxygen supplementation (Michael and Harrison, 2005). On the other hand, endotracheal intubation to avoid bronchial aspiration is not recommended taking into account both the duration of action of GHB and the possible consequent complications (bronchial aspirations usually appear during extubation). Endotracheal intubation would definitely be indicated upon suspicion of combined use of other related drugs which may increase the risk of prolonged coma. Thus, a similar prognosis is observed in all series of cases described to date although the percentage of patients that underwent mechanical ventilation and the number of bronchial aspirations is lower in some case series (Spanish series, Table 5). Bronchial aspiration appears in approximately 1% of all patients and more frequently in cases where alcohol is also present (Liechti and Kupferschmidt, 2004). Complications like arterial hypotension, hypothermia or bradycardia rarely require active treatment since they usually revert fast, with a generally good prognosis. Progression or worsening of these symptoms could be evidence of the masked presence of other drugs or an intercurrent entity. The use of atropine would be restricted to cases of symptomatic bradychardia insensitive to stimulation (Li et al. 1998).

1.1.9.2.12. GHB analogues

It has been reported that GBL would exhibit faster absorption and greater bioavailability and toxicity, especially in combination with alcoholic beverages (Palmer 2004). Even if effects are very much the same, precursors could lead to faster intoxication symptoms. There have also been reports of oesophageal mucosa ulcerations after consumption of these substances from illicit synthesis in an insufficiently neutralized form (Dyer and Reed. 1997).

1.1.9.3. GHB abuse, addiction and withdrawal syndrome

Shortly after the appearance of GHB, it became apparent that heavy GHB use was capable of producing tolerance, dependence and withdrawal. Accordingly, chronic self-administration, compulsive abuse regardless of adverse consequences, as well as drug-seeking behaviours have been shown after GHB abuse (Gonzalez and Nutt, 2005). Data suggest that individuals may become psychologically dependent on GHB. Tolerance to the effects of GHB results in an increase in dosage. Physical dependence may develop, with a withdrawal syndrome occurring on abrupt discontinuation. The withdrawal syndrome appears to range from anxiety, tremor, and insomnia to more severe symptoms such as disorientation, paranoia, hallucinations, tachycardia, and possibly delirium, which may last approximately 1 week. The symptoms of withdrawal may begin within 1-6h after the last dose of GHB and may last from 5-15 days (Tarabar and Nelson, 2004).

Because of the different regimens by which the drug is administered, withdrawal is more common among bodybuilders who use the drug in a several-times-daily dosing schedule for weeks than among club users who generally use GHB on weekends only. However, there are reports of clinically significant withdrawal in patients who used GHB and its analogues as sleep aid (Miotto et al. 2001). The estimated daily dose of GHB, used by the patients who experienced severe withdrawal, ranged between 43 and 144 g/day. Recently, a case of GHB withdrawal after a short, 7-day induction period has been described. Patients who present following frequent use of GHB for even short periods should be therefore evaluated for signs of withdrawal (Perez et al. 2006.)

1.1.9.4. Death

Even though GHB intoxication is usually reversible, several fatalities have been associated with GHB use (Caldicott et al. 2004; Zvosec et al. 2001). In most of the cases, GHB has been consumed together with other substances, being difficult to assume a causal association. Still, there are cases directly relatable to this substance and/or to precursors GBL and BD. It is important to underline that roughly all death cases related to these substances took place out of a health care centre. Most of these cases are in relation with car accidents or victims of some kind of abuse (Caldicott et al. 2004), although some cases of apnea and death in a medical centre have been described after leisure use of GHB (Timby et al. 2000). Indeed, the short life of GHB in both blood (up to 8h) and urine (up to 12 - 24h), have probably caused an underestimation of the number of deaths actually caused by this substance (Caldicott et al. 2004).

1.1.10. GHB abuse liability

The relative abuse liability of GHB in humans was unknown at the beginning of this thesis. Experimental data demonstrating the reasons for abuse was lacking.

1.1.10.1. Preclinical studies

The results of preclinical studies of the reinforcing effects of GHB have been ambiguous. Various drug discrimination studies with GHB have failed to consistently show cross-substitution with abused drugs such as benzodiazepines, barbiturates, opiates, cocaine or *d*-amphetamine (Beardsley et al. 1996; Winter 1981; Woolverton et al. 1999). However, stronger evidence for cross substitution has been reported between GHB and baclofen, a nonabused $GABA_B$ agonist (Lobina et al. 1999), particularly at high doses. Furthermore self-administration studies of GHB show evidence for only weak and inconsistent reinforcing effects. In rhesus monkeys self-administration rates for GHB were below those seen with phencyclidine (PCP) and methohexital, and considerably similar to those obtained with vehicle tests (Beardsley et al. 1996, Woolverton et al. 1999). Rodent studies have been more suggestive of the reinforcing effects of GHB as both oral and IV self-administration has been demonstrated. Nonetheless results were variable and difficult to interpret conclusively (Martellotta et al. 1997; Colombo et al. 1995; Colombo et al. 1998 Martellotta et al. 1998). Thus, the preclinical evaluation of the relative reinforcing effects of GHB have been fairly inconsistent, possibly due to discrepancies in the use of different species, routes of administration, doses, and testing procedures.

1.1.10.2. Abuse liability in humans

Several reports of GHB abuse and dependence in humans has been published and reports of overdoses have increased during the last years. However, overdose data reflect a form of inappropriate use that may have no relation to abuse liability. Interestingly, several overdoses occurred in individuals who regularly used GHB because of their reinforcing effects. This fact in conjunction with the augmented consumption during the last years, have been interpreted as an indirect marker of a high abuse liability of GHB in humans. However, no abuse liability studies had been performed in humans before this thesis work, and few clinical studies have evaluated abuse-related effects of GHB.

In an unblinded outpatient study evaluating the ability of a high dose of GHB (50 mg/kg or 3.5 g/70 kg TID) given three times daily for 24 weeks to promote abstinence from alcohol in alcohol dependent patients, approximately 10% of the patients increased the dose of GHB by 6–7 times the therapeutic dose to 300–350 mg/kg TID (21–24.5 g/70 kg TID), suggesting that GHB was reinforcing in this subset of alcoholic patients (Addolorato et al. 1996).

A randomized and placebo controlled study was performed in 8 opioid-dependent stabilized patients on levorphanol treatment prior to a naloxone challenge. GHB 30 mg/kg (2.1 g for a 70 kg subject) but not 15 mg/kg, was found to significantly increase subjective ratings of 'good mood' and 'carefree' (Rosen et al. 1997). Subject's rating of 'liking' showed a non-significant tendency for the 30 mg/kg dose and no significant differences were found on any of the scales of the Addiction Research Center Inventory (ARCI) short form questionnaire. This study was not able to find significant reinforcing effects. Nonetheless it should be highlighted that it suffered from important drawbacks. Its particular design (not specifically planned to measure GHB subjective effects) and the presence of an outlier with an unexpected placebo response might account for the results found.

After the end of the experimental phase of the present work the first controlled study addressing GHB abuse liability in humans was published. The study was described as a pilot ascending dose sequence trial in subjects with histories of abuse of sedative/hypnotic drugs and no prior exposure to GHB (Carter et al. 2006). A likelihood for GHB to be abused intermediate to triazolam and pentobarbital was reported. GHB produced an intermediate effect (greater than triazolam but lower than pentobarbital) on most measures of likelihood of abuse (i.e. ratings of liking and reinforcing effects). In addition, GHB generally produced greater unpleasant drug effects than the other active conditions and this was interpreted as a possible limit of GHB abuse potential. Nevertheless, alternative explanations could elucidate the rate of GHB abuse potential found in this study. This issue will be further discussed in the discussion chapter of this thesis.

1.1.11. Therapeutic uses

Most of the therapeutic applications of GHB result from its sedative and hypnotic effects on the CNS.

1.1.11.1. Narcolepsy

Although its mechanism of action is unknown, GHB shows efficacy in the treatment of cataplexy associated with narcolepsy. Significant 49–69% median reduction in the number of cataplexy attacks in patients receiving total nightly sodium oxybate doses of 6.0–9.0 g, have been shown (US Xyrem Multicenter Study Group 2002). Recently, extensive research, through multiple large controlled and open-label studies has led to an understanding of GHB as a primary treatment for the complex of narcolepsy symptoms, such as sleepiness, cataplexy and sleep fragmentation (Robinson and Keating, 2007). GHB is now approved in the USA and Europe for the treatment of cataplexy in patients with narcolepsy. GHB ('Sodium oxybate'; 'Xyrem'), taken at bedtime and again during the night, is also effective in decreasing daytime sleepiness in patients with narcolepsy who are also taking stimulants (Black and Houghton, 2006).

1.1.11.2. Alcoholism. Alcohol and opiate withdrawal

GHB has been used for alcoholism, given its many similarities to ethanol the hypothesis is that GHB will act as a substituting compound. GHB 50 mg/kg/day has been given orally to treat the symptoms of acute alcohol withdrawal and to facilitate both short-and long-term abstinence from alcohol. GHB appears to be effective both in the management of alcohol withdrawal syndrome (as effective as benzodiazepines) and in the maintenance of long-term abstinence (Addolorato et al. 1999). GHB has also been approved for the treatment of alcohol dependence in some European countries (i.e. Italy).

GHB has also been used to treat opiate withdrawal, often in higher dosages of 50-300 mg/kg/day (Nimmerrichter et al. 2002). However, patient relapse, non-responders, and the development of a GHB dependency syndrome are potential problems.

1.1.11.3. Anaesthesia

GHB was earlier used for intravenous anaesthesia, due to its CNS depressant effect and minor negative influence on cardiorespiratory function. However, the use of GHB in anaesthesia is complicated by its unpredictable duration of action and lack of analgesia. In addition, nausea and

vomiting greatly reduce the safe use of GHB for this purpose. Even though, GHB still is being used for anaesthesia in some European countries as Germany and Austria (Kleinschmidt et al. 1999).

1.1.11.4. Cellular and cerebral protection

GHB is an endogenous inhibitor of energy metabolism possibly protecting tissues when energy supplies are low. The natural function of GHB may include a role as a tissue protective substance. Evidence suggests that GHB reduces cellular activity, while depressing the utilization of glucose as well as other energy substrates (Ottani et al. 2003). This may result in tissues being less sensitive to the damaging effects of anoxia or during periods of excessive metabolic demand. However, clinical evidence from controlled studies in humans is lacking.

1.1.11.5. Fibromyalgia

In a randomized and controlled clinical trail in 24 female patients, GHB effectively reduced pain and fatigue in patients, possibly through reduction of the sleep abnormalities (alpha intrusion and decreased slow-wave sleep) associated with the nonrestorative sleep characteristic of this disorder (Scharf et al. 2003). Further studies are needed to confirm the role of GHB in this disease.

1.2. Ethanol

Alcohol or ethanol is probably one of the best known psychoactive substances. Because of its long history of use and scientific study, only a summary of the most relevant information of alcohol related to this thesis work is shown below.

1.2.1. History and sources

Alcohol is one of the oldest drugs and the most commonly used drug in world. It is a clear, colourless flammable liquid which absorbs water rapidly from the air. It is generally prepared by the fermentation of sugar by yeast (Various authors 10th Special Report to the U.S. Congress on Alcohol and Health 2000). Since yeast does not survive in greater than 15 percent alcohol, stronger solutions of alcohol are prepared by distillation. Wine and beer generally contain 2 to 20 percent alcohol, while the distilled preparations contain 30 to 60 percent.

1.2.2. Recreational uses

Alcohol is the most consumed drug in the world and more than just a drug for many cultures. A recent report from the UK's Institute of Alcohol Studies shows that adults in Europe consume 11 litres of pure alcohol per head per year (Goddard 2005). Recreational use in Spain was recently addressed in the '*Encuesta Domiciliaria sobre Alcohol y Drogas en España 2005-2006*' a survey reflecting alcohol and drug use in the year 2005. In this survey of 27.934 adults (15-64 year), 48.2% reported drinking alcoholic beverages on a weekly basis and 19.7% reported experiencing drunkenness at least once during the year previous to the survey. A daily consumption of alcohol during the last 30 days was reported by 14.9% of the sample. Alcohol use was higher during the weekend, mainly within the population under 34 years of age. While alcohol use was stabilized within the population of 15-64 in relation to previous surveys, an increase in the heavy use of alcohol within the adolescent population (14-16) was detected (Ministerio de Sanidad y Consumo 2006).

1.2.3. Pharmacology in humans

In science and industry the word alcohol refers to a group of compounds made up of a hydrocarbon chain with a hydroxyl (OH) group attached. Ethanol, a member of the alcohol group,

is a small organic molecule chemical formula C_2H_6O that is both water and lipid soluble (Eckardt et al. 1998).

1.2.3.1. Mechanism of action

Unlike many drugs that have a key site of action, alcohol produces a wide spectrum of effects on brain function via interaction with multiple targets. Alcohol perturbs the balance between excitatory and inhibitory influences in the brain, resulting in anxiolysis, ataxia, and sedation (Various authors 10th Special Report to the U.S. Congress on Alcohol and Health 2000). This is accomplished by either enhancing inhibitory or antagonizing excitatory neurotransmission. Several putative sites at which ethanol may act have been identified, and ethanol most likely produces its effects by simultaneously altering the function of a number of proteins that can affect neuronal excitability (Eckardt et al. 1998). Recent studies show that alcohol exerts powerful and specific effects on ion channel function and these effects may be modulated by the subunit makeup and state of phosphorylation of these ion channels. The most recent evidence shows that it acts through voltage-gated ion-channels and ligand-gated ion channels located at synapses. The results of several studies suggest that alcohol inhibits the two major classes of glutamate activated ion channels N-methyl-D-aspartate (NMDA) and kainate-receptor subtypes in the brain and spinal cord. Because these channels mediate both rapid and prolonged synaptic signalling, inhibition of these responses may underlie some of the intoxicating and sedative, anaesthetic effects of alcohol. In the case of the $GABA_A$ and glycine receptors, compelling evidence exists to suggest that alcohol's effects are mediated by interaction with certain amino acids on the receptor (Various authors 10th Special Report to the U.S. Congress on Alcohol and Health 2000). In addition, ethanol also interacts with serotonin and dopaminergic systems. Ethanol seems to enhance the serotonin-stimulated ion current by its interaction with the $5\text{-}HT_3$ subtype receptor. As with several recreational drugs, ethanol acts throughout the mesolimbic dopamine system stimulating dopamine release in the nucleus accumbens in a $5\text{-}HT_3$ mediated process (Eckardt et al. 1998). Nicotine receptors have been also associated to play a role in the reinforcing effects of ethanol.

1.2.3.2. Pharmacological effects

Alcohol is primarily a CNS depressant, and the degree of depression is directly proportional to the quantity of ethanol consumed. The behavioural and physiological effects are associated with different blood ethanol concentrations. As the blood ethanol concentration begins to increase,

behavioural activation, characterized by euphoria, talkativeness, aggressiveness, and loss of control, generally precedes the overt CNS depression induced by ethanol.

The effect of ethanol in other bodily systems is also dose dependent. A moderate amount causes peripheral vasodilatation, especially of skin vessels, and a consequent increase in the heart rate. It also stimulates the secretion of salivary and gastric fluids. On the other hand, ethanol consumption in high concentrations, as found in undiluted spirits, can induce gastritis and hemorrhagic lesions in the stomach and duodenum, inhibit intestinal brush border enzymes, inhibit the uptake of amino acids, and limit the absorption of vitamins and minerals. Alcohol also inhibits the release of vasopressin from the posterior pituitary gland, resulting in enhanced diuresis (Leppäluoto et al. 1992).

1.2.4. Subjective effects and psychomotor performance

The subjective effects of alcohol and its repercussion in the psychomotor performance have been extensively studied. The determinants of alcohol's effects depend on several factors. Key aspects are the dose of alcohol used, the level of prior alcohol consumption (moderate to heavy drinkers are generally more tolerant to alcohol than light drinkers), gender (in general higher effects in female subjects) and the context in which alcohol is consumed. The most sensitive measure of alcohol action is the self reporting of 'intoxicated' or 'drunkenness' feeling. Doses as low as 0.2 g/kg, have been found to be able to induce a drunkenness feeling. Ethanol administration can induce a biphasic effect. At high doses and during the descending limb of the alcohol dose-response curve, it typically produces sedative-like effects. However, at lower doses (0.2-0.8 g/kg) and during the ascending limb, ethanol has been shown to produce stimulant-like effects after controlled administration (Holdstock and Wit, 1998). This stimulation is expressed as decreased social and psychological inhibition and is most likely the result of a depression of inhibitory pathways in the brain with release of cortical activity. Thus, ethanol can produce marked stimulant-like subjective effects as measured for instance by means of VAS 'stimulated' and the ARCI-A subscale of the ARCI questionnaire (Hernandez-Lopez et al. 2002). The effects of ethanol on psychomotor tasks usually occur at higher blood ethanol concentrations than subjective effects. In addition, in a controlled setting, peak effects of alcohol on subjective measures prelude the decline in a psychomotor task (Schuckit et al. 1991). Nonetheless ethanol produces dose-dependent deterioration of psychomotor performance, and is capable of altering or/and impairing psychomotor performance from blood concentrations as low as 0.3 g/L. The correspondence between ethanol blood concentration and ethanol concentration in breath and their correlation with impact on psychomotor performance and driving abilities is the basis for

the legal limits of ethanol in drivers (Various Authors 10th Special Report to the U.S. Congress on Alcohol and Health 2000).

1.2.5. Pharmacokinetics

After oral administration, ethanol is rapidly absorbed into the bloodstream from the stomach and small intestine. Peak blood levels occur 30-90min after ingestion of ethanol when the stomach is empty. Delays in gastric emptying (as due to the presence of food) slow ethanol absorption, since absorption occurs more rapidly from the small intestine than from the stomach (Holford 1987). After absorption, ethanol is distributed throughout body water. In organs with high blood flow, such as the brain, liver, lungs, and kidney, equilibrium occurs rapidly. It is both water and lipid soluble and its distribution is variable depending in the inter-individual fat and water percentage (V_D of 0.5 to 0.7 L/kg). It displays first-pass metabolism mediated by gastric and liver alcohol dehydrogenase (ADH). Gastric metabolism of ethanol is lower in women than in men, which may contribute to the greater susceptibility of women to the effects of ethanol (Lieber 2000). Ethanol is metabolized largely by sequential hepatic oxidation, first to acetaldehyde by ADH and then to acetic acid by aldehyde dehydrogenase (ALDH). A hepatic cytochrome P450 enzyme, CYP2E1, also can contribute to metabolism (<10%), especially at higher ethanol concentrations and under conditions such as alcoholism, where its activity is induced. Each metabolic step requires NAD^+. NAD^+ availability limits ethanol metabolism to about 8 g or 10 ml per hour in a 70-kg adult, or approximately 120 mg/kg/h. Thus, hepatic ethanol metabolism functionally saturates at relatively low blood levels and ethanol metabolism displays zero-order kinetics (constant amount per unit time) (Holford 1987). Ethanol is mainly eliminated through metabolism (90-98%), nonetheless small amounts are excreted in urine, sweat and breath. Since the ratio of ethanol in end-expiratory alveolar air and ethanol in the blood is relatively consistent, blood alcohol levels can be estimated readily by the measurement of alcohol levels in expired air (blood alveolar air partition coefficient approximately 2000:1).

1.2.6. Toxicology in humans

1.2.6.1. Acute intoxication

Ethanol intoxication is probably the best-known form of drug toxicity. At progressively higher blood ethanol concentrations, the stage of relaxation is transformed into decreased social inhibitions, slurred speech, ataxia, decreased mental acuity, decreased reflexive responses, coma, and, finally, death resulting from respiratory arrest (McIntosh and Chick, 2004). One of the

consequences of ethanol intoxication is difficulty in regulating body temperature. Hypothermia frequently results, with body temperature falling toward that of the ambient environment. High blood levels of ethanol can also prolong the P wave and QTc interval and might be associated with increased risk of arrhythmias (Roldán et al. 2003).

TABLE 7. Ethanol blood concentrations and related clinical features.

Blood concentration gr/L	Outcome	Clinical Features
0.5-1	Euphoria	Euphoria, talkativeness, relaxation, disinhibition, unawareness
1-2	Excitation	Increase in reaction time
2-3	Confusion	Impaired motor and sensory function, impaired cognition
3-4	Stupor	Central nervous system depression, stupefaction.
4-5	Coma	Unconsciousness anaesthesia, hypoventilation hypothermia
> 5	Death	Respiratory Arrest. Apnoea

Generally, no treatment is required for acute ethanol intoxication. Allowing the individual to sleep off the effects of ethanol ingestion is the usual procedure. However, ethanol overdose can result in a medical emergency. For example, prompt treatment is required if the patient is in danger of dying of respiratory arrest, is comatose, has dilated pupils, is hypothermic, or displays tachycardia. Treatment for severe ethanol overdose is generally supportive (Roldán et al. 2003).

1.2.6.2. Addiction and withdrawal syndrome

1.2.6.2.1. Addiction

Chronic alcohol abuse is accompanied by tolerance, dependence, and craving for the drug. About 10% of alcohol drinkers progress to levels of consumption that are physically and socially detrimental. Alcoholism is characterized by compulsive use despite evidently deleterious social and medical consequences. Alcoholism is a progressive illness and chronic alcohol abuse results in shrinkage of the brain leading to loss of both white and grey matter (Kril and Halliday, 1999).

1.2.6.2.2. Withdrawal syndrome

At a cellular level daily alcohol intake induces brain adaptations as a functional increase in NMDA receptor levels. When alcohol is stopped these excess receptors combine to cause a large calcium flux into cells, hyperexcitability, and cell death (Various authors 10th Special Report to the U.S. Congress on Alcohol and Health 2000). Alcohol mediated inhibitory actions via the GABAergic system is also stopped. As a result of the increase in excitatory glutamate combined with a sudden drop in the brain's inhibitory systems, a noradrenergic ´overdrive' appears, leading to an increase in sympathetic activity (McIntosh and Chick, 2004). Patients who stop drinking experience a spectrum of different symptoms ranging from mild sleep disturbance to frank delirium tremens. The severity of these relate to a number of factors, but most importantly the abruptness of withdrawal, level of alcohol intake, and the contribution of residual effects of previous drinking.

Clinical management of alcohol withdrawal syndrome depends on the severity of the symptoms present, however it should be taken into account that this syndrome could lead to death (Mayo-Smith 1997). In patients with minor degrees of alcohol withdrawal there is often no requirement for medication to help with control of symptoms. Benzodiazepines are the treatment of choice for alcohol withdrawal. In clinical practice a long acting benzodiazepine is generally recommended, given in a gradually tapering dose. Some acutely unwell patients may require parenteral treatment, usually with intravenous diazepam (Mayo-Smith 1997). It is important to also remember that acute withdrawal may precipitate Wernicke's encephalopathy.

1.2.7. Abuse liability

Alcohol is one of the most widely abused psychoactive substances. Alcohol abuse is characterized by continued drinking despite adverse effects on family or work, trauma, or negative health consequences (American Psychiatric Association 1994). Ethanol is, in summary, largely recognized as a high abuse liability substance based on vast animal and epidemiological data and several abuse liability trials performed in humans. In experimental studies increases in drug 'liking' and 'drunkenness' scales and drug-induced euphoria have been observed either in healthy volunteers, alcoholics and abusers of several different compounds (Hernandez-Lopez et al. 2002; Holdstock and de Wit, 1998; Walker and Zacny, 2001).

1.3. Flunitrazepam

Flunitrazepam (Rohypnol®, Roche), is a hypnotic benzodiazepine marketed in most countries of the European Union, South America, Asia and Australia. Flunitrazepam is a remarkable drug within its class as it combines a fast rate of onset of effects and high efficacy and affinity for central benzodiazepine site of action (Mattila and Larni, 1980).

1.3.1. History

Swiss pharmaceutical company Hoffman-La Roche first described and developed benzodiazepines in the 1950s. Roche modified the basic benzodiazepine structure and introduced a number of tranquilizers in the 1960s and 1970s, including Rohypnol in 1975 (Hoffman-La Roche). It was introduced as a hypnotic and became one of the leading benzodiazepine hypnotics in many countries. The drug is also used before the induction of anaesthesia (Lader 1994). Reports of flunitrazepam misuse in Europe surfaced in the 1970s, and in the early 1990s, Rohypnol emerged as a drug of abuse and misuse in the United States. In 1995 the United Nations reclassified it from a Schedule IV to a Schedule III drug, which requires thorough record-keeping of its legal distribution.

1.3.2. Recreational uses

Flunitrazepam available on the street comes from manufactured Rohypnol by Roche. The product is available as 0.5, 1 and 2 mg tablets and as a vial for injection. In the USA, street names for flunitrazepam include ‘Mexican Valium’, ‘roofies2, ‘la rocha’, ‘roche’, ‘R2’ or ‘forget-me pill’ (in Spain, a common name is ‘Roches’). Each flunitrazepam tablet could cost 0.5–5 euros on the street. The shape and colour of both legal and counterfeit products vary from country to country. Illegal distribution and possession of flunitrazepam have been documented since 1985. In Spain flunitrazepam was initially mainly abused by heroin users. San et al. found that 80.2% of the heroin addicts admitted to *Hospital del Mar*, Barcelona (Spain) between 1982 and 1991 had a history of benzodiazepine use. Of these, 68.4% reported consuming flunitrazepam (San et al. 1993). However its abuse later spread among ‘Club’ and ‘Rave’ attendees and as with GHB, it has been also classified as a ‘Club Drug’ (The National Institute on Drug Abuse). Thus, in a recent study among 19,084 youths aged 16–23 in the USA one in five (20%) reported having used at least one ‘Club Drug’ in their lifetime and 0.4% reported a previous use of flunitrazepam (Wu et al. 2006). Other reports also pointed out flunitrazepam as a ‘date rape’ drug because of its

hypnotic and amnesic action, as well as the disinhibition that may occur after ingestion (Anglin et al. 1997)

In addition flunitrazepam abuse by the general population could be underestimated. In Sweden, during 1992-1998, 641 fatalities occurred, where the cause of death was attributed to intoxication with flunitrazepam solely (130) or in combination with other drugs, or concomitant conditions (511). The seizures reported by Swedish Customs also revealed a substantial and increasing illegal trade in this period. (Druid et al. 2001).

1.3.2.1. Patterns of use

Flunitrazepam is mainly abused by the oral route, although there are reports of 'snorting' (Bond et al. 1994) and IV injection (Darke et al. 1995). Flunitrazepam is thought to be abused because of its ability to produce euphoria and 'well being' and a relaxed feeling similar to that in alcohol intoxication (Bond et al. 1994; Farré et al. 1996; Simmons and Cupp, 1998). It is often used to enhance the effects of other drugs like alcohol or cannabis. Flunitrazepam is also used in combination with heroin to enhance its effects, to ameliorate heroin withdrawal, and to moderate the stimulant effects of cocaine.

1.3.3. Pharmacology in humans

1.3.3.1. Mechanism of action

Flunitrazepam produces its actions on the benzodiazepine site within the gamma-aminobutyric acid ($GABA_A$) complex. It has been shown to have greater affinity than diazepam, oxazepam, and alprazolam, and less affinity than midazolam and triazolam (Braestrup and Squires, 1978).

1.3.3.2. Pharmacological effects

Flunitrazepam administration reproduces all the pharmacological effects of full benzodiazepine agonists including anxiolytic, anticonvulsant, muscle relaxant, and central sedative-hypnotic effects. Effects occur about 30min after ingestion, peak at 2h, and may last up to 8 to 12h. At therapeutic doses of 1 to 2 mg, it produces memory loss and decrements in psychomotor performance. Higher plasma concentrations are needed to impair attention than those required to impair memory (Bareggi et al. 1998). Recently it has been proved that flunitrazepam improves

sleep in healthy volunteers while it reduces the nocturnal secretion of melatonin (Hajak et al. 1996)

1.3.4. Subjective effects and psychomotor performance

The effects of flunitrazepam in healthy volunteers include dose-dependent sedation, and decrease in concentration. Besides its objective and subjective sedative effects, flunitrazepam induces subjective feelings of intoxication and some pleasurable feelings related to drug abuse potential such as euphoria and well being. On the other hand it also can produce dysphoria and 'unwanted' effects, especially at high doses (Bond et al. 1994; Farré et al. 1996; Roset et al. 2001).

Flunitrazepam may deteriorate psychomotor performance mainly due to its sedative properties. However it has been shown to impair subjective and objective measures of attention and psychomotor activities in the absence of classical objective effects associated with sedation/sleepiness (Lucchesi et al. 2003).

1.3.5. Pharmacokinetics

Flunitrazepam is a 7-nitro-benzodiazepine derivative and its bioavailability is between 80% and 90%. Time to peak concentrations (tmax) is 1-1.5h and the $t_{1/2}$ of flunitrazepam is approximately 20h (Boxenbaum et al. 1978). However, the half-life does not correlate with duration of clinical effect (approximately 8h) because the duration of action is affected by its distribution kinetics more than its elimination kinetics. Once absorbed, flunitrazepam quickly distributes into body tissues from the plasma following a three-compartment open model. It has an estimated volume of distribution of 4-5 L/kg due to its high lipid solubility. In humans, the elimination of flunitrazepam occurs almost exclusively through metabolism: principally, reduction to 7-aminoflunitrazepam (7-AF) and oxidation to N-desmethylflunitrazepam (DMF) and 3-hydroxyflunitrazepam (3-HF) (Cano and Sumirtapura, 1981). The parent compound is thought to be primarily responsible for the hypnotic effects, although DMF may also have some activity. Most metabolites are excreted through the kidneys, with about 10% excreted in the faeces.

1.3.6. Toxicology in humans

1.3.6.1. Adverse effects

Similar to other benzodiazepines, CNS depression is the main adverse effect and is dose related. Toxic effects of flunitrazepam include dizziness, confusion, somnolence, impaired psychomotor performance and amnesia (Mattila and Larni, 1980). Depression of the respiratory drive can be observed, especially when flunitrazepam is used in conjunction with other CNS depressants. Other adverse CNS effects include: visual disturbances, hallucinations, and paradoxical reactions consisting of excitement, stimulation, and hyperactivity (Gahlinger 2004).

1.3.6.2. Acute intoxication

Overdose is similar to other benzodiazepines characterized by slurred speech and ataxia when mild, and by respiratory depression, bradycardia, hypotension, and stupor or coma when severe. These symptoms are rarely fatal unless other CNS-active drugs are used.

Supportive care is generally sufficient for flunitrazepam intoxication. The greatest concern arises when there has been concomitant ingestion of CNS depressants. Activated charcoal and a cathartic can be given to reduce absorption. Intravenous flumazenil, a selective and competitive benzodiazepine antagonist, can be used in certain cases of confirmed benzodiazepine overdose. Flumazenil should be administered with great caution, however. Seizures may be induced in patients receiving benzodiazepines for epilepsy, those experiencing acute withdrawal from flunitrazepam or other benzodiazepines, or those with a cyclic antidepressant overdose. Resedation can be expected rather rapidly, as the effects of flumazenil are short-lived (Ricaurte and McCann, 2005).

1.3.6.3. Addiction and withdrawal syndrome

Physiologic dependence can develops after chronic administration of high doses of flunitrazepam. The emergence of signs of withdrawal has been demonstrated after discontinuation of treatment in both animals and humans. Rebound insomnia has been also described after discontinuation of repeated nightly therapeutic doses of flunitrazepam. Withdrawal syndrome includes headache, tension, anxiety, restlessness, muscle pain, photosensitivity, numbness and tingling of the extremities, as well as increased seizure potential. Treatment for flunitrazepam withdrawal parallels that for ethanol and other benzodiazepines and uses replacement with a long-acting benzodiazepine (i.e. diazepam), often followed by gradual taper (Ricaurte and McCann, 2005).

1.3.7. Abuse liability

At the late 1990s a controversial debate about the actual abuse potential of flunitrazepam in humans took place. Flunitrazepam appeared to be frequently abused by opioid dependent patients and poly-drug abusers, who rated the drug as the most liked benzodiazepine because it was the 'strongest' and gave a good 'high' (Navaratnam and Foong, 1990; Barnas et al. 1992; San et al. 1993). On the other hand a comprehensive review of its abuse potential concluded that it was similar to other benzodiazepines (Woods and Winger, 1997). In experimental studies increases in drug 'liking' scales and drug-induced euphoria have been observed either in normal subjects (Bond et al. 1994; Farré et al. 1996), opioid dependent patients (Farré et al. 1998) and sedative abusers (Mintzer and Griffiths, 1998; Mintzer and Griffiths, 2005). The results of experimental studies and evidence for its augmented abuse in recent years indicate that flunitrazepam is a benzodiazepine with a high abuse liability. The higher abuse liability as well as its preference to other benzodiazepines may be due to its faster onset and stronger effects that make it capable of inducing positive mood changes, pleasurable feelings and euphoria (Roset et al. 2001).

Even after the increased abuse of flunitrazepam 'Club Drug' users, the relative abuse liability of flunitrazepam in 'Club Drug' users have not been previously tested previous to this thesis work.

1.4. ‘Club Drugs’ and ‘Rave’ and club cultures

During the last few years the term ‘Club Drugs’ has been used for defining an heterogeneous group of chemical substances in permanent evolution, that are consumed for recreational purposes (Abanades et al. 2004). These substances have been extensively used at all-night dance or ‘techno’ parties firstly by the ‘Rave’ culture and later by the so called ‘Club culture’ (Weir 2000). These movements are characterized by the search for amplified sensations, by means of the combination of electronic music, marathon dancing and substance abuse. After years of a predominating consumption of designer amphetamines such as MDMA, it seems that the use of other types of substances is increasing, fundamentally drugs with hallucinogenic effects and sedative-like substances. These drugs, at the same time as being drugs of abuse, are medicines with concrete indications in therapeutics, and display an important increase in their consumption in the last few years.

Previous to this thesis work a review of the literature available was undertaken (Abanades et al. 2004). The published report has been included as an Appendix.

1.5. Abuse liability studies

During the second half of the 20th century, the development of psychopharmacology brought an increasing number of new substances with a high potential for abuse by humans. Predicting the potential of abuse for new medications has become a key issue during the development and approval process (Mansbach et al. 2003; Roset et al. 2003). Abuse potential is usually linked with the induction of euphoric feelings, a possible effect of some types of medications that the pharmaceutical developer may want to identify and measure early in development in order to select candidates with the best benefit to drug abuse risk ratio (Brady et al. 2003). This could bring the candidates under drug abuse control if they are shown to have abuse potential. Abuse potential could be approximated through well validated preclinical tools, but this is outside of the scope of the present work. However, even in the case of valid preclinical data, there are limitations in the feasibility and interpretation of certain drugs/dosage forms and the ability to make acurate discriminations in abuse potential from preclinical data. Thus, clinical studies are needed for the following scientific reasons (i) to validate the conclusions of either lesser or greater abuse potential from preclinical data (ii) to evaluate the psychopharmacology of drugs in human populations in situations where the preclinical data are equivocal or species specific (iii) to evaluate the onset, peak, and duration of subjective effects as a function of dose and administration route (iv) to relate the degree of overlap between the therapeutic dose range and the range of doses producing subjective effects (v) to evaluate the generalizability of drug timing in different human subject populations (vi) in the case of new drug classes (e.g., inverse benzodiazepine receptor agonists) (Griffiths et al. 2003; Roset et al. 2003). These ambitions have led to the development of a scientific field of abuse potential assessment (Brady et al. 2003; Griffiths et al. 2003; Mansbach et al. 2003).

TABLE 8. Types of studies for the evaluation of abuse potential in humans

Comparison of subjective, physiologic and psychomotor performance effects
Auto-administration Studies
Preference Studies
Discrimination Studies

Different studies can be performed for the evaluation of the abuse liability of drugs in humans (Roset et al. 2003). However, human abuse liability is usually assessed by comparison of subjective, physiologic and psychomotor performance effects (Griffiths et al. 2003; Roset et al. 2003).

1.5.1. Human abuse liability assessment by comparison of subjective, physiologic and psychomotor performance effects

Estimates of the likelihood that a drug will be abused have generally been based on the subjective effects engendered by that drug. With the development of standardized subjective effects questionnaires in the second half of the last century, self-reported effects of drug have been carefully evaluated, generally making measures before and repeatedly after administration of a single dose of drug (Griffiths et al. 2003; Roset et al. 2003). The use of multiple doses under controlled laboratory conditions in which physiological measures are also taken, and both the investigator and the subject are blind to the dose administered, has been suggested as most likely to yield useful data about the abuse liability of a test compound. Although questions remain about the specific subjective effects measures to be used, there has been general agreement among researchers in this area that scores on scales from the 'Profile of Mood States', 'Addiction Research Center' Inventory, and Visual Analog Scales which include measures of 'high' or 'liking' all provide predictive utility (Griffiths et al. 2003). The addition of a measure of actual drug-taking to this predictive model appears to provide important information about the conditions under which these two behaviours (self-reported effects and drug self-administration) vary, and strengthens the model substantially.

The underlying principles of using subjective and physiologic response measures to predict abuse liability of substances were developed from basic research regarding the addictive process with opioids (Roset et al. 2003). These principles are (i) that subjective effects can be quantitatively assessed, (ii) that subjective responses to drug administration can often be used to predict the reinforcing actions of drugs, and (iii) that subjective and physiologic responses following drug administration reflect specific drug-induced alterations in human brain and body function. A summary of the main features required in a classical abuse liability trial is presented in table 9.

TABLE 9. Features of the classic acute dose-effect abuse liability trial*

Typical trial design characteristics
A complete crossover design in 10-14 subjects
Single doses of drug are evaluated over a period of several minutes to several hours, depending on the time-course of the drugs under study
Intervals between test conditions are typically 1 to several days
Conducted in a controlled clinical pharmacology laboratory setting
Subjects are monitored to prevent use of other drugs
Selection of an appropriate subject population
Usually subjects with histories of polydrug abuse including drugs from the same pharmacological class as the novel compound, such that subjects can meaningfully rate drug effects and categorize drug effects relative to drugs of known abuse liability
The subject population must be one in which the positive control comparison drug tests unequivocally positive
Double-blind, placebo controlled drug administration
To further reduce expectancy, subjects can be blinded to the specific comparison compound(s) and number of active doses to be administered
Selection of appropriate positive control comparison drug(s)
The positive control is usually an abused drug from the same pharmacological class and used for the same medical indication proposed for the novel compound
Consideration may be given to including a negative control comparison compound from the same class which is behaviourally active but not abused
Selection of an appropriate range of doses of the positive control comparison drug
Demonstration of orderly dose effects of the comparison compound on primary outcome measures establishes sensitivity and validity of the trial
Selection of a range of doses of the novel compound, including high supratherapeutic doses
Evaluation of high doses is critical to the validity of the trial
A dose run-up pilot study is useful for selecting maximal doses and matching doses between the novel and comparison compound
If possible, a high dose of the novel compound should produce effects comparable to the highest dose of the positive control comparison compound on one or more outcome measure
Selection of appropriate outcome measures
Measures should be assessed repeatedly to characterize onset, peak and duration/offset of drug effects
Multiple measures should be included that reflect likelihood of abuse (e.g. liking, good effects, estimated monetary street value, drug vs. money choice)
Measures of drug identification and subject-rated side effects and mood changes should be considered in interpreting likelihood of abuse
Additional concurrent measures should be included to assess the equivalency of the novel and comparison compounds on some relevant dimensions of biological activity (e.g. behavioral performance, observer-rated assessments, physiological measures)

* From Griffiths et al. 2003.

2. Hypothesis

1. GHB administration might induce euphoria, stimulation, pleasurable feelings, and sedation. These effects would vary depending on the subject tested and dose administered. Low, medium and high doses of GHB would cause slightly perceptible effects, euphoric related effects and significant somnolence, respectively.

2. The acute administration of GHB would induce significant but indefinite changes in heart rate, blood pressure and pupil diameter.

3. GHB administration could induce an impairment of psychomotor performance.

4. Following its administration, dose dependent measurable GHB concentrations would be observed in plasma, urine, oral fluid and sweat. GHB would be rapidly absorbed and eliminated. GHB would readily distribute to plasma and urine while it might not diffuse to that extent to oral fluid and sweat.

5. GHB might display a high abuse liability potential in humans in comparison to ethanol and flunitrazepam.

3. Aims of the study

3.1. Main aims

1. **The characterization of the clinical effects and pharmacokinetics of GHB in humans under controlled experimental conditions at doses compatible with those being abused.**

2. **Test the relative abuse liability of GHB in comparison with ethanol and flunitrazepam in participants with previous experience with GHB.**

3.2. Specific aims

3.2.1. Pilot study

- To select appropriate doses for the subsequent final clinical trial on GHB clinical pharmacology (dose range: lowest dose with measurable effects and doses leading to significant somnolence).

- To describe the time course of physiological, subjective variables and psychomotor performance following drug administration.

- To investigate the time course of GHB in plasma, urine, oral fluid and sweat after single oral dose administration.

- To evaluate the stability of GHB in plasma under different storage conditions.

- To perform a pharmacokinetic/pharmacodynamic (PK/PD) study of GHB in humans. Concentrations of GHB in different biological matrices will be tentatively correlated with selected subjective effects in a range of doses compatible with those usually consumed by recreational users.

3.2.2. Final study

- To evaluate GHB induced subjective and physiological effects.
- To investigate GHB impact on psychomotor performance.
- To evaluate a possible GHB dose-response relationship in subjective and physiological effects and its impact on psychomotor performance.
- To assess the eventual correlation between drug pharmacokinetics and drug effects in a range of doses compatible with those usually consumed by recreational users (PK/PD study).
- To asses the relative abuse liability of GHB in 'Club Drug' users, by using flunitrazepam and ethanol as reference sedative-like drugs.

4. Summary of the experimental design

As previously discussed little was known about the clinical effects of GHB at doses compatible with those that were being abused. Thus, the present project was undertaken in two different steps described below. Firstly, a pilot study was performed as a preliminary phase. As a pilot pharmacology phase I study, different doses of sodium GHB were given to 8 volunteers in a dose escalation schedule. Two different doses of ethanol were also tested in 2 more volunteers. The information obtained was used to establish the doses to be administered and the optimal design for the final study. A final study was performed in order to test GHB relative abuse liability compared to flunitrazepam and ethanol. All studies were carried out at the Pharmacology Research Unit at the 'Institut Municipal d'Investigació Mèdica' (IMIM), Barcelona, Spain. The studies were conducted in accordance with the 'Declaration of Helsinki', approved by the local Ethics Committee (CEIC-IMAS) and authorized by the Spanish Ministry of Health (Agencia Española de Medicamentos y Productos Sanitarios). The information below is intended to provide a general explanation of the studies performed. More detailed information about material and methods of the studies are further described within the articles that comprise the main part of this thesis work.

4.1. Pilot Study

- **Study design**: double blind, randomized, crossover and controlled, pharmacology phase I study.
- **Participants**: 10 healthy volunteers with previous experience with GHB.
- **Treatments**: increasing single oral sodium GHB (Na GHB) doses 40, 50, 60 and 72 mg/kg (8 subjects) and 2 different ethanol doses (2 subjects)

TABLE 10. GHB pilot study. Different treatments and sessions. Doses given in Na GHB and GHB.

	Session 1		**Session 2**	
Subject number	Na GHB mg/kg	GHB mg/kg	Na GHB mg/kg	GHB mg/kg
1	40.0	33.1	50.0	41.4
2	40.0	33.1	50.0	41.4
3	40.0	33.1	50.0	41.4
4	50.0	41.4	60.0	49.7
5	60.0	49.7	40.0	33.1
6	50.0	41.4	60.0	49.7
7	72.4	60.0	Placebo	Placebo
8	60.3	50.0	72.4	60.0
	Ethanol g/kg		Ethanol g/kg	
9	0.7		0.5	
10	0.7		0.5	

- **Variables studied**:

    - Physiological effects: non-invasive systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate, oral temperature and pupil diameter.
    - Subjective effects: a 49-item short form of the 'Addiction Research Center Inventory' (ARCI) questionnaire, the VESSPA (Evaluation of the Subjective Effects of Substances with Potential of Abuse) questionnaire and a set of 13 different visual analogue scales (VAS).
    - Psychomotor performance: digit symbol substitution test (DSST), balance task and maddox-wing device.
    - Pharmacokinetics: GHB plasma, sweat, oral fluid and urine concentrations. Ethanol blood concentrations.

- **Results in**:

    5.1. Gamma-hydroxybutyrate (GHB) in humans: pharmacodynamics and pharmacokinetics. Ann New York Acad Sci. 2006;1074:559–576.

    5.2. Disposition of GHB in conventional and non-conventional biological fluids after a single drug administration: issues in methodology and drug monitoring. Ther Drug Monit. 2007;29:64-70.

4.2. Final Study. Abuse liability trial

- **Study design**: double blind, double-dummy, randomized according to a balanced 5 × 5 Latin-square design, crossover and controlled.
- **Participants**: 12 healthy volunteers with previous experience with GHB.
- **Treatments**: 5 drug conditions (oral doses)
    - GHB (40 mg/kg)
    - GHB (60mg/kg)
    - Flunitrazepam 1.25 mg
    - Ethanol (0.7 g/kg)
    - Placebo

- **Variables studied**:

    - Physiological effects: non-invasive systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate, oral temperature and pupil diameter
    - Subjective effects: 'Addiction Research Center Inventory' (ARCI), the VESSPA (Evaluation of the Subjective Effects of Substances with Potential of Abuse) questionnaire and a set of 13 different visual analogue scales (VAS).
    - Psychomotor performance: digit symbol substitution test (DSST), balance task and maddox-wing device.
    - Pharmacokinetics: GHB plasma concentrations. Flunitrazepam plasma concentrations and ethanol blood concentrations.

- **Results in**:

    5.3. Relative abuse liability of gamma-hydroxybutyric acid (GHB), flunitrazepam and ethanol in Club Drug users. J Clin Psychopharmacol. 2007;27:625-638.

5. Results

5.1. Gamma-hydroxybutyrate (GHB) in humans: pharmacodynamics and pharmacokinetics. Ann New York Acad Sci. 2006;1074:559–576.

γ-Hydroxybutyrate (GHB) in Humans

Pharmacodynamics and Pharmacokinetics

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ABSTRACT: **Despite γ-hydroxybutyrate (GHB) therapeutic uses and the increasing concern about its toxicity, few studies have addressed GHB dose-related effects under controlled administration and their relationship with its pharmacokinetics. The study design was double-blind, randomized, crossover, and controlled. As a pilot pharmacology phase I study, increasing doses of GHB were given. Single oral sodium GHB doses (40, 50, 60, and 72 mg/kg) were administered to eight volunteers. Plasma and urine were analyzed for GHB by gas chromatography–mass spectrometry. Physiological effects, psychomotor performance, and subjective effects were examined simultaneously. GHB produced dose-related changes in subjective effects as measured by questionnaires and VAS. GHB showed a mixed stimulant-sedative pattern, with initially increased scores in subjective feeling of euphoria, high, and liking followed by mild-moderate symptoms of sedation with impairment of performance and balance. Mean peak GHB plasma concentrations were 79.1, 83.1, 113.5, and 130.1 μg/L for 40, 50, 60, and 72 mg/kg, respectively. GHB-mediated physiological and subjective effects were dose dependent and related to GHB plasma concentrations. GHB urinary excretion was mainly related to administered doses. GHB-mediated**

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subjective and physiological effects seem dose dependent and related to GHB plasma concentrations. Results suggest a high abuse liability of GHB in the range of dose usually consumed.

KEYWORDS: **γ-hydroxybutyric acid; γ-hydroxybutyrate; GHB; subjective effects; abuse liability**

INTRODUCTION

γ-hydroxybutyrate (GHB, "liquid ecstasy") is also known as γ-hydroxybutyric acid, 4-hydroxybutyiric acid, 4-hydroxybutanoic acid, and oxybate (sodium oxybate is the United States approved name). GHB is a short chain fatty acid that can be considered both an endogenous metabolite and a precursor of the neurotransmitter γ-aminobutyric acid (GABA). GHB acts in the central nervous system as a neuromodulator. GHB can be formed in human peripheral tissues from two precursors, γ-butyrolactone and 1,4-butanediol.[1] GHB is marketed in the United States with the name of Xyrem® (Jazz Pharmaceuticals, Palo Alto, CA) for the treatment of cataplexy in patients with narcolepsy,[2] and in some European countries as an anesthetic agent and for the treatment of alcohol withdrawal (e.g., Alcover®; CT Laboratorio Farmaceutico, SRL, San Remo, Italy). On the other hand, GHB is a recreational drug commonly consumed at nightclubs and "raves" in conjunction with alcohol, cannabis and drugs, such as 3,4-methylenedioxymethamphetamine (MDMA, ecstasy), or ketamine, also known as "club drugs."[3] In humans, γ-butyrolactone and 1,4-butanediol have also been abused. During the last years, GHB has become a major concern in emergency rooms of some countries due to an important increase in the number of cases of intoxications.[4–6] GHB has also been used for narcotizing victims in drug-facilitated sexual assaults because its capacity to induce short-term antegrade amnesia, increased libido, and suggestibility.[7,8] Recreational users of GHB experience euphoria, relaxation, reduction of social inhibitions, decreased motor skills, and other effects similar to those reported for a moderate alcohol intoxication.[9] These effects can explain its abuse liability in humans, but this aspect has not been yet characterized under controlled administration. After GHB ingestion, a mild intoxication may be observed with nausea, dizziness, and difficulty in focusing the eyes.[10] Acute severe intoxications have been reported, where individuals may experience vomiting, extreme dizziness, disorientation, amnesia, and unconsciousness[4–6,11] that may evolve to convulsions, deep coma, and rarely death.[12] The range between high recreational doses and overdose is narrow; and acute intoxications are quite common in humans.

GHB has been detected in blood and urine of subjects after acute intoxications and in the context of clinical trials.[13–16] Results from these reports indicate that GHB is eliminated from the body very rapidly; being detection

dependent on the time elapsed between consumption and sample collection. Because GHB is an endogenous compound, there are measurable baseline concentrations in blood and urine.[17–20]

The range of GHB doses typically abused by subjects range between 2 and 6 g (25–75 mg/kg). Most reports available are focused on the pharmacokinetic of GHB in humans.[15–17,21–23] Nevertheless, little is known on the physiological and subjective effects and alterations in psychomotor performance induced by this drug in the range of doses commonly abused. In addition, despite its therapeutic uses and the increasing concern about the toxicity of GHB, few studies addressed dose-related effects under controlled administration and the correlation of drug effects and concentrations in biological fluids.

The aims of this article were as follows: (*a*) to select appropriate doses for a series of GHB clinical pharmacology studies (interval: lowest dose with noticeable effects and doses that lead to significant somnolence); (*b*) to describe time course of physiological, subjective variables, and psychomotor performance following drug administration; (*c*) to investigate the presence and the time course of GHB in plasma and urine; and (*d*) to assess the eventual correlation between GHB pharmacokinetics and drug effects in a range of doses compatible with those usually consumed by recreational users.[24]

MATERIALS AND METHODS

Human Subjects

Eight male subjects were recruited by ''word of mouth'' and included in the study. Eligibility criteria required the recreational use of GHB on at least five occasions. Exclusion criteria included daily consumption of more than 20 cigarettes and more than 30 g of ethanol (3 units per day). All subjects gave their written informed consent before inclusion and were economically compensated for inconveniences caused by their participation in the study. The study was conducted in accordance with the Declaration of Helsinki, approved by the local Ethical Committee (CEIC-IMAS), and authorized by the Spanish Ministry of Health (Agencia Española del Medicamento). Eligible subjects were interviewed by a psychiatrist (structured clinical interview for Diagnostic and Statistical Manual version N [DSM-IV-TR] in order to exclude psychiatric disorders, including schizophrenia, psychosis, and major affective disorders. Each participant underwent a general physical examination, routine laboratory tests, urinalysis, and a 12-lead electrocardiogram. The participants had a mean age of 28.1 years (range 25–32), mean weight of 71.9 kg (range 60.5–84.2), and mean height of 179.1 cm (range 167.5–194.0). Participants were non-smokers ($n = 7$) except for one, and their average consumption of alcohol was 9 units per week. All of them had previous experience with the consumption of alcohol, cannabis,

sedatives, stimulants, and "club drugs," with at least five previous consumptions of GHB. None had history of abuse or drug dependence according to DSM-IV criteria (except for nicotine dependence), nor had experienced any medical or psychiatric adverse reaction following GHB consumption.

Study Design

This study is a preliminary phase of a series of clinical trials of GHB administration in humans. As a pilot pharmacology phase I study, increasing doses of GHB were given. The study design was double-blind, randomized, crossover, and controlled. Subjects participated as outpatients in two different randomly assigned 6-h study sessions with a washout period of 7 days, in which they were given single doses of 40 (33.1), 50 (41.4), 60 (49.7), and 72 (60.1) mg/kg of sodium (GHB) or placebo by the oral route. Participants and evaluators (two physicians) were blind to treatments although they were told that GHB or placebo would be given during the sessions. Thus, in a dose escalation schedule, two different doses of sodium GHB were given to every subject (40 mg/kg dose was given in four occasions, 50 mg/kg given in four occasions, 60 mg/kg given in five occasions, 72 mg/kg given in two occasions, and placebo given in one occasion). Participants were requested to abstain from consumption of any drug of abuse during the study period and urine drug testing was performed before each study session for cannabinoids, cocaine, opiates, amphetamine/methamphetamine, barbiturates, benzodiazepines, and phencyclidine. For all groups of substances, participants tested negative before each experimental session. In each session, subjects arrived at the laboratory at 8 AM after an overnight fast and had an indwelling intravenous catheter inserted into a subcutaneous vein in the forearm of the non-dominant arm. Thereafter, they remained seated in a quiet room throughout the session. GHB (Alcover OS® sodium GHB, 17.5% syrup, CT Laboratorio Farmaceutico) or matched placebo (syrup, CT Laboratorio Farmaceutico) were orally administered around 9:00 AM in a fasting state. The different doses corresponding to appropriate volumes of syrup and placebo were diluted to 250 mL of a soda orange-based drink. Participants were told to drink the beverage as soon as possible (mean of 10.2 s; range: 5–20 s). Placebo consisted of syrup diluted to the same 250 mL of a soda orange-based drink.

Collection of Blood and Urine Samples

Blood was collected at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, and 6 h after GHB administration in heparinized tubes and immediately centrifuged. Urine samples were collected before, and at 0–3 and 3–6 h after drug administration. All biological specimens were frozen at −20°C until analysis. No preservatives were added to the specimens.

Determination of GHB Concentrations in Biological Fluids

Frozen, plasma, and urine samples were allowed to thaw at room temperature. Aliquots of 100 μL plasma and urine were added with 5 μg (5 μL of the 1 mg/mL methanolic solution) of GHB hexadeuterated analoge, GHB-d_6 as internal standard, and 200 μL of acetonitrile. After 30-s vortex and 5-min centrifugation at 1400 rpm, 150 μL of the organic phase were transferred to a clean extraction tube and evaporated to dryness. The dried extracts were derivatized with 50 μL of BSTFA-1% TMCS for 30 min at 70°C.

A 1-μL aliquot of derivatized samples was injected onto a Hewlett-Packard (HP) 6890 gas chromatograph coupled to a HP5973 quadrupole mass spectrometer (Agilent, Palo Alto, CA, USA). The capillary column was a cross-linked 5% phenyl-methylsilicone (12 m × 0.2 mm i.d. and 0.33-μm film thickness, Ultra-2, Agilent). The samples were injected in split-less mode and helium gas was used as carrier at a flow rate of 1.2 mL/min (measured at 180°C). The injector and detector temperatures were both maintained at 280°C, respectively. The temperature program was initially set at 60°C for 2 min and increased to 180°C at 20°C/min, then 35°C to 250°C, and then held for 4 min, being the total run time 14 min. The mass spectrometer was operated in the electron impact ionization and selected ion monitoring (SIM) acquisition mode and the following ions were monitored (underlined ions used for quantification): GHB-bis-TMS: m/z 233, 204, 117; GHB-d_6-bis-TMS: m/z 239. Under these analytical conditions, the limit of quantification (LOQ) was 0.5 μg/mL and the intra-day precision and accuracy were always better than 4.2% and 13.4%. Similarly, inter-day precision and accuracy were lower than 13.4% and 12.1% at the GHB LOQ.

PHARMACOLOGICAL EFFECTS

Physiological Measures

Non-invasive systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate, oral temperature, and pupil diameter were recorded at −15 min and immediately before drug administration (time 0, baseline) and at 0.25, 0.50, 0.75, 1, 1.50, 2, 3, 4, 5, and 6 h after GHB administration using a DinamapTM 8100-T vital signs monitor (Critikon, Tampa, FL). Pupil diameter was recorded with a Haab pupil gauge.[25]

Psychomotor Performance Measures

The psychomotor performance battery included the digit symbol substitution test (DSST), the Maddox-wing device, and the balance task. This battery has

been used previously in the evaluation of psychostimulants and sedatives.[26,27] The DSST is a subtest of the Wechsler Adult Intelligence Scale-Revised.[28] A computerized version was used and scores were based on the number of correct patterns keyed in 90 s (correct responses). The Maddox-wing device measures the balance of extraocular muscles and quantifies exophoria, as an indicator of extraocular musculature relaxes, and esophoria. Results were expressed in diopters along the horizontal scale of the device.[29] The Balance Task assessed the participant's ability to stand upright for a maximum of 30 s on each foot.[30] The score was the total number of seconds the participant was able to balance (maximum of 60 s). The DSST and balance task were performed at −15 min and immediately before drug administration (time 0, baseline) and at 0.50, 1, 1.50, 2, 3, 4, and 6 h after drug administration. Measurements with the Maddox-wing device were performed at −15 min and at 0.25, 0.50, 0.75, 1, 1.50, 2, 3, 4, 5, and 6 h after drug administration.

Subjective Effects Rating Scales

Subjective effects were measured using the Addiction Research Center Inventory (ARCI), the VESSPA (Evaluation of the Subjective Effects of Substances with Potential of Abuse) questionnaire, and a set of 13 different visual analog scales (VAS). ARCI is a true–false questionnaire with empirically derived scales that are sensitive to the effects of a variety of classes of drugs of abuse.[31] The Spanish validated version of a 49-item short form of ARCI was used.[32] The questionnaire included five scales: PCAG (pentobarbital-chlorpromazine-alcohol group, a measure of sedation); MBG (morphine-benzedrine group, a measure of euphoria); LSD (lysergic acid diethylamide group, a measure of dysphoria and somatic symptoms); BG (benzedrine group, a stimulant scale consisting mainly of items relating to intellectual efficiency and energy); and A (amphetamine, an empirically derived scale sensitive to the effects of D-amphetamine). ARCI was administered at −15 min (immediately before drug administration), and at 0.50, 1, 1.50, 2, 3, 4, 5, and 6 h after GHB administration. VESSPA (evaluation of the subjective effects of substances with potential of abuse) is an in-house developed and validated questionnaire specifically created to measure changes in subjective variables caused by MDMA.[33] It contains six scales: sedation (SED), psychosomatic anxiety (ANX), changes in perception (PER), pleasure and sociability (SOC), activity and energy (ACT), and psychotic symptoms (PSY). Each scale consists of six questions with a five-point Likert response (0 to 4 depending on the intensity of the effect). VESSPA scales were administered at −15 min (before drug administration), and at 1, 2, 3, 4, and 6 h after GHB administration.

A total of 13 VAS (100 mm) labeled with different adjectives marked at opposite ends with "not at all" and "extremely" were used. Subjects were asked to rate effects of "stimulated," "high", "any effect," "good effects,"

"bad effects," "liking," "content," "drunkenness," "drowsiness," "dizziness," "confusion," "depression or sadness," and "relax." Scales were administered at −15 min (immediately before drug administration), 0.25, 0.50, 0.75, 1, 1.50, 2, 3, 4, 5, and 6 h after GHB administration.

Pharmacodynamic and Pharmacokinetics Parameters

The following parameters were determined from GHB plasma concentrations over time: peak concentration (C_{max}), time to reach peak concentrations (t_{max}), area under the concentration-time curve from 0 to 6 h (AUC_{0-6}), elimination half-life ($t_{1/2}$), and elimination constant (Ke). Taking into account the duration of GHB physiological and subjective effects, area under the effects-time curve from 0 to 2 h (AUC_{0-2}), and maximal effect (E_{max}) were also determined. AUCs were calculated by the linear trapezoidal rule.

Pharmacokinetic parameters were obtained with use of specific functions of computer program (PK Functions for Microsoft Excel, Microsoft Corporation, USA).

Data Analysis: Statistical Methods

Data are represented as mean ± standard deviation (SD) or median. The nonparametric Kruskal–Wallis test was applied to compare the distributions of AUC_{0-6} and C_{max} values (pharmacokinetics) and AUC_{0-2} and E_{max} values (pharmacodynamics) between the different sodium GHB doses assayed without assuming any parametric form of these distributions. The Kruskal–Wallis test was applied to test the null hypothesis, which assumes equal distribution functions versus the alternative that, at least, two distributions differ with respect to the median. Since the power of the test was low because of only two observations in the GHB dose group of 72 mg/kg, posterior comparisons of GHB doses groups of 40 and 60 mg/kg were also carried out. In this latter case, the Wilcoxon Mann-Whitney test was applied. Given the small sample sizes, tests were carried out using the add-on module SPSS Exact of the statistical software package SPSS, version 12.0 (SPSS Inc., Chicago, IL).

RESULTS

A summary of results for pharmacological effects and pharmacokinetics showing statistical significant differences between treatments is presented in TABLE 1.

Pharmacological Effects

Time course of several GHB pharmacological dose-related effects in humans are presented in FIGURES 1 (physiological effects and Maddox wing),

TABLE 1. Variables showing statistically significant differences between treatments. Mean, minimum, maximum values for the four doses tested and comparisons performed

		40 mg/kg ($n = 4$)		50 mg/kg ($n = 5$)		60 mg/kg ($n = 4$)		72 mg/kg ($n = 2$)		
	Criteria	Mean	Median	Mean	Median	Mean	Median	Mean	All	40 and 60
GHB Plasma concentrations	$AUC_{0\text{-}6h}$	106.5	108.5	143.7	133.9	183.9	185.8	301.1	0.004	0.029
Pupil diameter	$AUC_{0\text{-}2h}$	1.2	1.2	.65	.69	.22	.13	1.4	0.014	
VAS any effect	$AUC_{0\text{-}2h}$	14.2	12.2	46.1	40.9	61.8	57.1	67.2		0.029
VAS drunkenness	$AUC_{0\text{-}2h}$	3.7	3.6	33.6	21.9	49.7	38.8	33.1		0.029
VAS good effects	$AUC_{0\text{-}2h}$	13.4	11.7	45.6	48.6	60.2	58.1	64.8		0.029
VAS high	$AUC_{0\text{-}2h}$	15.3	14.4	47.5	49.1	59.4	55.4	65.2		0.029
VAS content	$AUC_{0\text{-}2h}$	13.9	11.8	39.5	13.4	53.3	53.5	23.1		0.029
VAS stimulated	$AUC_{0\text{-}2h}$	7.2	6.4	34.3	16.6	53.3	57.4	68.1	0.040	0.029
	E_{max}	10.2	10.5	30.4	33.0	43.2	46.5	61.5	0.020	0.029
VAS liking	$AUC_{0\text{-}2h}$	14.1	12.6	44.6	47.1	54.2	54.2	58.9		0.029
VAS bad effects	$AUC_{0\text{-}2h}$	0.7	0.6	15.1	0.0	30.2	27.9	16.9		0.029
	E_{max}	1.7	1.5	15.4	0.0	33.2	29.0	20.0		0.029
VAS dizziness	$AUC_{0\text{-}2h}$	7.8	5.4	34.4	19.9	67.3	68.2	56.6		0.029
	E_{max}	10.0	9.5	32.6	32.0	63.0	67.0	62.0	0.049	0.029
VAS relax	$AUC_{0\text{-}2h}$	7.3	3.4	29.8	9.1	59.3	57.1	19.4		0.029
	E_{max}	7.7	5.5	25.6	15.0	49.2	48.0	9.7		0.029
ARCI A	E_{max}	1.7	2.0	3.6	4.0	4.0	3.5	2.0	0.048	0.029

[a] P values < 0.05.

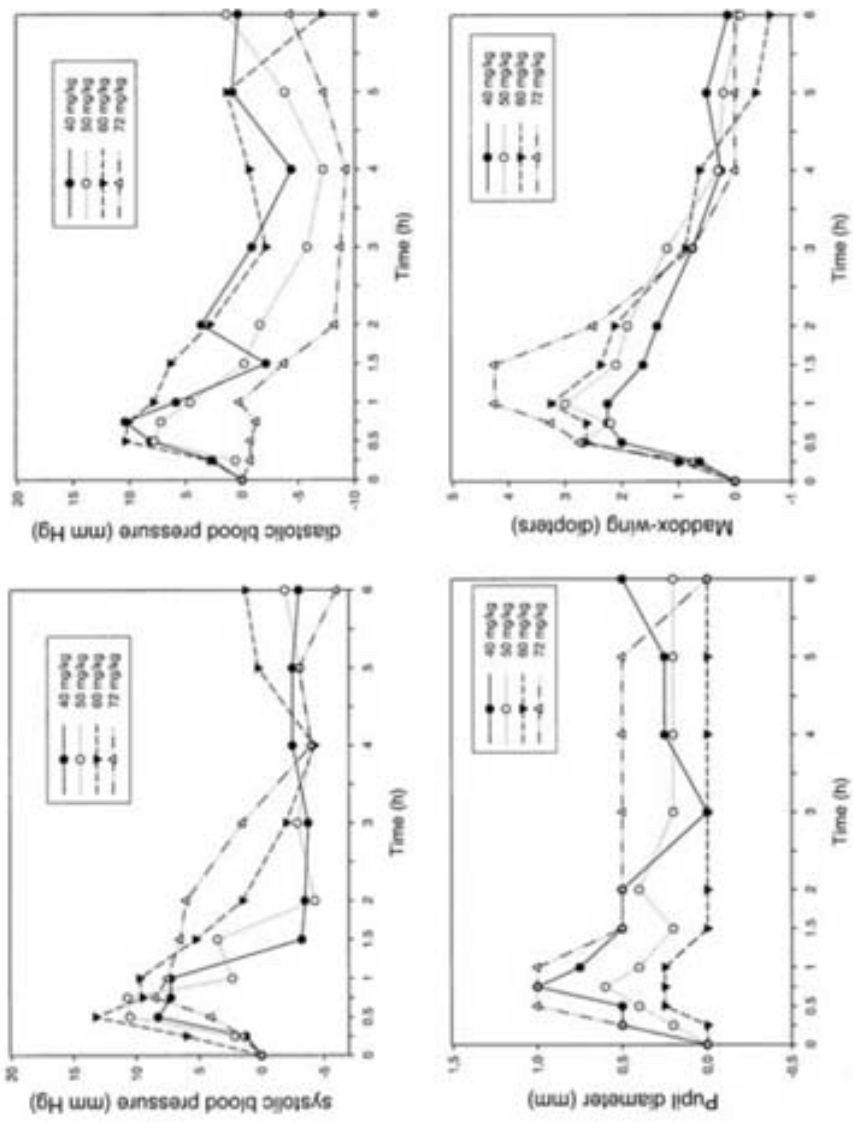

FIGURE 1. Time course of GHB dose-related physiological effects (mean values) in humans (SBP, DBP, pupil diameter, and Maddox wing).

2 (subjective effects: VAS), and 3 (subjective effects: ARCI and VESSPA and DSST performance).

Physiological Effects

GHB produced a slight increase in systolic and diastolic blood pressure for all doses tested, with a peak effect between 30 and 45 min and a return to previous values at 3 h after GHB administration, without reaching statistical significance between different doses (FIG. 1). No apparent changes in heart rate or oral temperature were observed. GHB produced a significant (AUC 0–2 h comparison) dose-related pupil diameter increase from 0.5 mm to 1 mm between 30 min and 1-h post-administration.

Subjective Effects and Psychomotor Performance

GHB administration produced dose-related changes in subjective effects as measured by questionnaires and VAS. GHB showed a mixed sedative-stimulant pattern mediating both types of effects (see FIGS. 2 and 3). GHB produced marked stimulant-like effects as measured by VAS "stimulated," ARCI A, and VESSPA activity and energy (ACT) scale. GHB produced euphoria as measured by VAS "high," "liking," and "good effects," ARCI MBG questionnaire and VESSPA pleasure and sociability (SOC) scale. Most stimulant-like effects and euphoria were dose dependent (see TABLE 1) and peaked at 45 min post-drug administration.

GHB produced objective and subjective sedation effects as reflected in VAS "drowsiness," "dizziness," and "drunkenness" scores, ARCI PCAG questionnaire and VESSPA sedation (SED) scale. Peak effects were achieved between 1 and 1.5 h after drug administration and lasted for 3 h. GHB also produced a slight deterioration of psychomotor performance apparently dose dependent with a peak effect at 30 min after administration for lower doses and at 1.5 h post-administration for the 72 mg/kg dose. GHB produced a decrease in DSST total responses (FIG. 3) ranging from −5 to −11 total responses for the E_{max} values, and in DSST correct responses, ranging from 0 to −2.75 for the E_{max} values, while there was an increase in DSST errors at the same time. Doses of 60 and 72 mg/kg were associated with an impairment of the balance task with a peak effect at 1 h post-administration ranging from −15 s to −19 s decrease for the E_{max} values from a maximum of 60 s standing on both feet. At all administered doses, GHB induced exophoria, a typical effect for sedatives, as measured by the Maddox-wing device. Thus, GHB produced an apparent dose-dependent increase in diopters with a peak effect at 1 h post-administration (E_{max} 2.25 40 mg/kg, 2.7 50 mg/kg, and 3.25 60 mg/kg), that lasted 4 h post-administration (see FIG. 1).

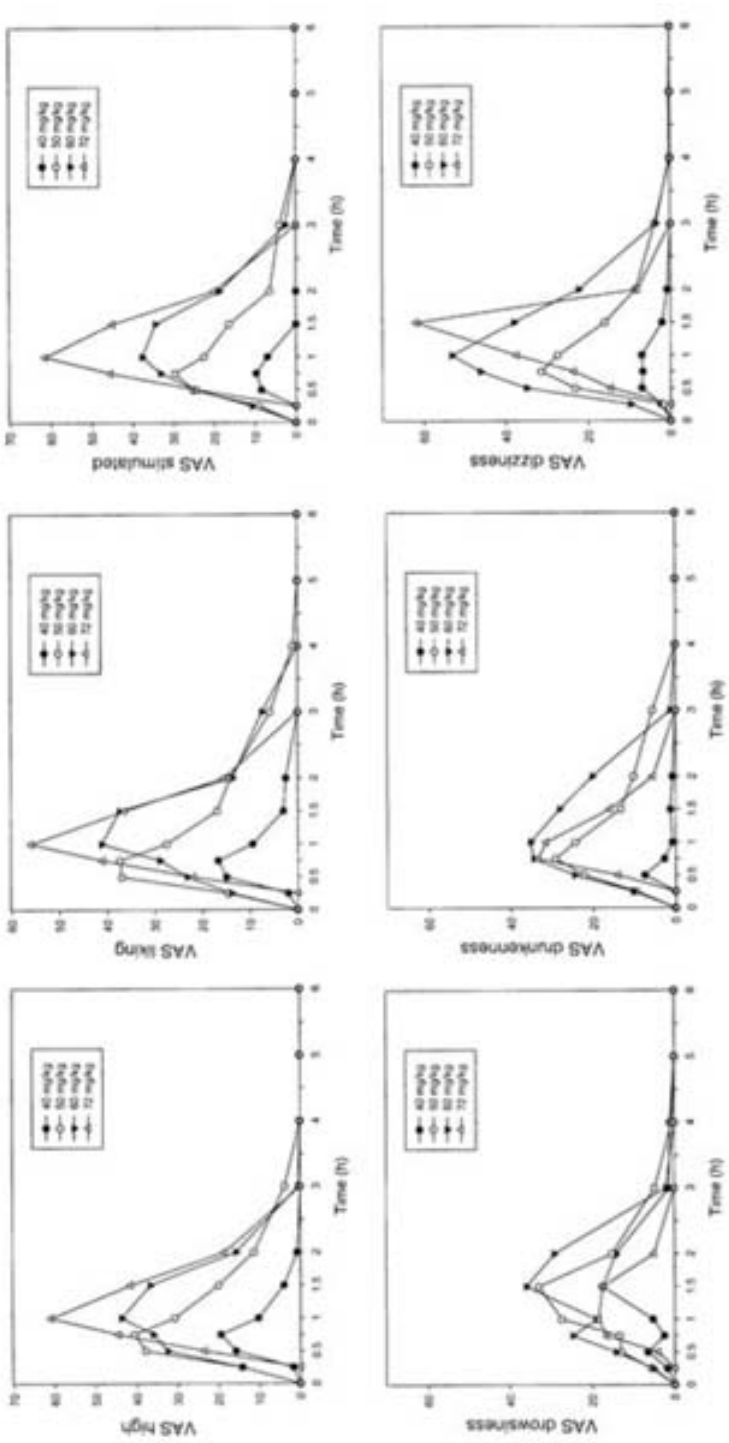

FIGURE 2. Time course of GHB dose-related subjective effects (VAS).

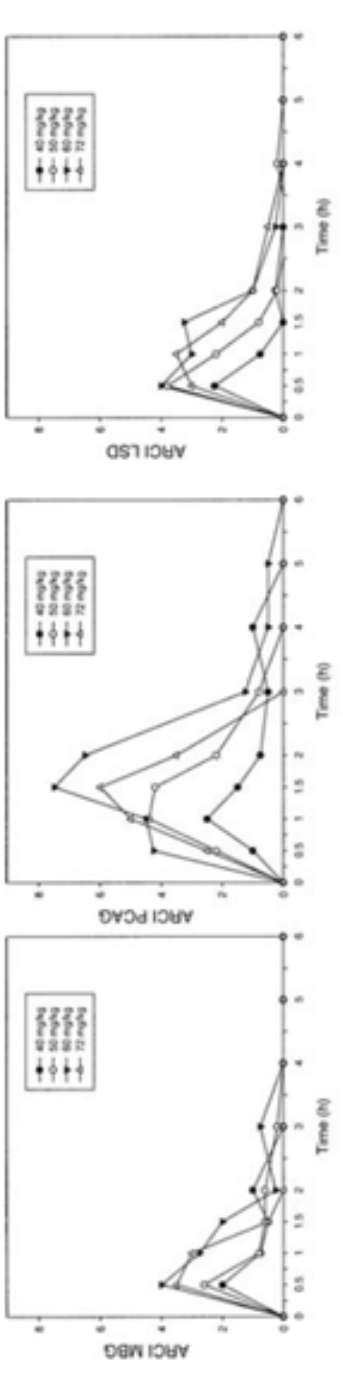

FIGURE 3. Time course of GHB dose-related subjective effects (ARCI and VESSPA questionnaires) and psychomotor performance (DSST total responses).

TABLE 2. Pharmacokinetic parameters of GHB in plasma (mean ± SD values given)

Parameter	40 mg/kg[a]	50 mg/kg[a]	60 mg/kg[a]	72 mg/kg[a]
C_{max} (μg/mL)	79.1 ± 26.4	83.1 ± 28.8	113.5 ± 20.1	130.1 ± 10.7
t_{max} (h)	0.7 ± 0.2	0.6 ± 0.1	0.6 ± 0.14	0.9 ± 0.2
AUC_{0-6} (μg/mL h)	106.5 ± 6.7	143.7 ± 36.4	183.9 ± 47.0	301.1 ± 11.4
K_e (h^{-1})	0.98 ± 0.21	1.01 ± 0.18	1.06 ± 0.18	1.23 ± 0.11
$t_{1/2}$ (h)	0.73 ± 0.17	0.71 ± 0.15	0.67 ± 0.12	0.57 ± 0.05

[a]Sodium GHB doses.

None of the participants required specific therapy or special care during the study. Serious adverse events were not observed, although one subject vomited 1 h after the administration of the 60 mg/kg dose. No hallucinations, psychotic episodes, or any other psychiatric symptoms were observed during the experimental sessions.

Concentration-Time Profiles of GHB in Plasma

Pharmacokinetic parameters of GHB in plasma after oral doses of 40, 50, 60, and 72 mg/kg of sodium GHB are presented in TABLE 2.

GHB was detected in all baseline plasma samples, with mean concentrations of 0.04 ± 0.01 μg/mL. After drug administration, concentrations peaked between 30 and 60 min after drug administration (C_{max} range: 45.8–109.3 μg/mL for 40 mg/kg; 51.0 – 123.5 μg/mL for 50 mg/kg, 90.3 – 134.7 for 60 mg/kg, and 122.5 – 137.6 for 72 mg/kg) (TABLE 2). Following the absorption phase, concentrations declined to a mean values at 6 h that ranged from 0.5 to 0.9 mg/mL for all doses tested.[13,34] GHB was readily absorbed after oral administration and rapidly eliminated (t_{max} and $t_{1/2} < 1$ h). AUC_{0-6h} derived from plasma GHB concentrations were significantly different upon comparing the four different doses ($P < 0.05$) (TABLE 1). No significant differences were observed for C_{max}, t_{max}, Ke, and $t_{1/2}$ between different drug doses. Normalized (1 mg/kg) values for AUC were similar for 40, 50, and 60 mg/kg doses (2.7, 2.9, and 3.1). For the 72 mg/kg dose, this value was higher (4.2). Normalized C_{max}, values were similar for all doses tested.

Excretion of GHB in Urine

A summary of GHB urinary excretion is shown in TABLE 3. GHB was detected in urine at baseline samples, with mean concentrations of 0.21 ± 0.14 μg/mL. The highest GHB recovery was found in the 0–3 h urine samples. Less than 2% of doses tested were recovered in urine for the 0–6 h collection period.

TABLE 3. Urinary excretion of GHB following different oral doses

		Urinary collection period		
Dose[a] mg/kg	Mean dose given (g)	0–3 h	3–6 h	0–6 h
40	2.9 ± 0.4	305.8 ± 121.4[b]	125.3 ± 214.4[b]	431.2 ± 202.4 (1.60%)[c]
50	3.7 ± 0.3	593.1 ± 297.9[b]	113.2 ± 155.0[b]	706.3 ± 433.8 (1.98%)[c]
60	3.9 ± 0.4	440.1 ± 114.1[b]	104.9 ± 118.6[b]	545.0 ± 106.8 (1.50%)[c]
72	5.2 ± 1.2	821.8 ± 149.8[b]	96.9 ± 33.3[b]	918.7 ± 116.5 (1.90%)[c]

[a]As sodium GHB.
[b]Mean ± SD, values given in micromoles.
[c]Recoveries (0–6 h) as a percentage of the administered dose.

DISCUSSION

Results of the study provide new insights on GHB pharmacodynamics and pharmacokinetics in humans. To our knowledge, there are no previous reports of the evaluation of GHB physiological and subjective effects after controlled administration of doses compatible with those consumed by recreational users. The main finding of the study is that GHB-mediated physiological and subjective effects are dose dependent and related to GHB plasma concentrations.

Our study, although somehow limited by both a dose escalation schedule design and the number of volunteers tested, provides new knowledge about its induced subjective effects and alterations of psychomotor performance. GHB produced dose-related changes in subjective effects as measured by specific questionnaires and VAS. GHB showed a mixed stimulant-sedative pattern with a biphasic time profile as described for other sedatives (alcohol or cannabis).[35,36] Psychostimulant effects were predominant in the first hour while sedative effects initiate more slowly and predominate in the second hour after drug administration. In reference to its abuse liability, GHB induced euphoria, well being, pleasurable effects, and liking effects that are on the basis of its misuse as recreational drug and similar to those reported by GHB abusers.[9,37] In the case of the sedative effects, they were similar to those elicited by low doses of benzodiazepines and alcohol[26,27] and included subjective feelings of sedation, a decrease in psychomotor performance, ataxia, and exophoria.

Regarding physiological effects, GHB administration produced a constant slight increment in SBP and DBP for all doses tested, lasting for 1–2 h post-administration that did not reached statistical significance when comparing doses assayed. Interestingly, this effect was not reported in none of the therapeutic studies previously performed. Furthermore, following GHB intoxication hypotension is frequently reported.[10] However, recent results suggest that GHB has also sympathomimetic cardiovascular effects that could induce increases

in blood pressure following its acute administratrion.[38] Further studies with a larger population of subjects are needed to confirm these findings.

GHB given by the oral route is rapidly absorbed and eliminated. Drug consumption can be differentiated from GHB endogenous concentrations both in plasma and urine in a time-window of 6 h post-ingestion. Our results are in agreement with those obtained in healthy subjects administered with 4.5 g of oxybate[13] (equivalent to 50–60 mg/kg of GHB) and lower to those observed in severe acute intoxications.[39] GHB elimination follows a nonlinear process as suggested by comparison of normalized AUCs. In the dose range of 40–60 mg/kg, elimination is linear in agreement with previous reports,[13,17] although nonlinearity can be observed at the 72 mg/kg dose. GHB elimination appears to be capacity-limited at higher doses as it has been observed in some narcoleptic patients administered at a fixed dose of 3 g twice nightly at a 4-h interval.[23] The accumulation of GHB in the body as a result of a nonlinear disposition of GHB might have some implications in the susceptibility of some subjects to develop acute intoxications.

GHB urinary excretion was mainly related to administered doses and in accordance with previous reports.[13,16,40] However, there was a slightly higher recovery of GHB for the 50 mg/kg dose than for 60 mg/kg dose. Higher mean weights of the subjects (resulting in similar total mean dose given), and an increased percentage of the administered dose recovery in the 50 mg/kg dose group, account for these results. In line with early reports, less than 2% of doses administered were recovered in the collection period.

Plasma concentrations reached in the range of doses administered are lower than that observed in acute intoxications and therefore is not surprising that, in our study, strong sedation or coma were not observed.[39] GHB plasma concentrations correlated better with psychostimulant-like effects rather than with sedative ones. At higher doses, sedative effects would predominate leading to the observed effects in acute intoxications. Our results suggest a high abuse liability of GHB in the range of doses usually abused. Further studies are needed to confirm these results.

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REFERENCES

1. NICHOLSON, K.L. & R.L. BALSTER. 2001. GHB: a new and novel drug of abuse. Drug Alcohol Depend. **63:** 1–22.
2. U.S. XYREM® MULTICENTER STUDY GROUP. 2003. A 12-month, open-label, multicenter extension trial of orally administered sodium oxybate for the treatment of narcolepsy. Sleep **26:** 31–35.
3. ABANADES, S., A.M. PEIRO & M. FARRE. 2004. Club drugs: old medicines as new party drugs. Med. Clin. (Barc.) **123:** 305–311.
4. MIRO, O., S. NOGUE, G. ESPINOSA, J. TO-FIGUERAS & M. SANCHEZ. 2002. Trends in illicit drug emergencies: the emerging role of gamma-hydroxybutyrate. J. Toxicol. Clin. Toxicol. **40:** 129–135.
5. CHIN, R.L., K.A. SPORER, B. CULLISON, *et al*. 1998. Clinical course of gamma-hydroxybutyrate overdose. Ann. Emerg. Med. **31:** 716–722.
6. LIECHTI, M.E. & H. KUPFERSCHMIDT. 2004. Gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL): analysis of overdose cases reported to the Swiss Toxicological Information Centre. Swiss. Med. Wkly. **134:** 534–537.
7. ELSOHLY, M.A. & S.J. SALAMONE. 1999. Prevalence of drugs used in cases of alleged sexual assault. J. Anal. Toxicol. **23:** 141–146.
8. VARELA, M., S. NOGUE, M. OROS, *et al*. 2004. Gamma hydroxybutirate use for sexual assault. Emerg. Med. J. **21:** 255–256.
9. DEGENHARDT, L., S. DARKE & P. DILLON. 2002. GHB use among Australians: characteristics, use patterns and associated harm. Drug Alcohol Depend. **67:** 89–94.
10. MASON, P.E. & W.P. KERNS 2nd. 2002. Gamma hydroxybutyric acid (GHB) intoxication. Acad. Emerg. Med. **9:** 730–739.
11. ABANADES, S., M.L. IGLESIAS, J.L. ECHARTE, *et al*. 2001. Gammahydroxibutyrate: a novel toxicological emergency. Meth. Find. Clin. Exp. Pharmacol **23:** 326.
12. FERRARA, S.D., L. TEDESCHI, G. FRISON, *et al*. 1995. Fatality due to GHB and heroin intoxication. J. Forensic Sci. **40:** 501–504.
13. PALATINI, P., L. TEDESCHI, G. FRISON, *et al*. 1993. Dose-dependent absorption and elimination of gamma-hydroxybutyric acid in healthy volunteers. Eur. J. Clin. Pharmacol. **45:** 353–356.
14. MCCUSKER, R.R., H. PAGET-WILKES, C.W. CHRONISTER, *et al*. 1999. Analysis of gamma-hydroxybutyrate (GHB) in urine by gas chromatography-mass spectrometry. J. Anal. Toxicol. **23:** 301–305.
15. FERRARA, S.D., S. ZOTTI, L. TEDESCHI, *et al*. 1992. Pharmacokinetics of gamma-hydroxybutyric acid in alcohol dependent patients after single and repeated oral doses. Br. J. Clin. Pharmacol. **34:** 231–235.
16. BRENNEISEN, R., M.A. ELSOHLY, T.P. MURPHY, *et al*. 2004. Pharmacokinetics and excretion of gamma-hydroxybutyrate (GHB) in healthy subjects. J. Anal. Toxicol. **28:** 625–630.
17. BORGEN, L.A., R.A. OKERHOLM, A. LAI, *et al*. 2004. The pharmacokinetics of sodium oxybate oral solution following acute and chronic administration to narcoleptic patients. J. Clin. Pharmacol. **44:** 253–257.

18. LEBEAU, M.A., R.H. CHRISTENSON, B. LEVINE, *et al*. 2002. Intra- and interindividual variations in urinary concentrations of endogenous gamma-hydroxybutyrate. J. Anal. Toxicol. **26:** 340–346.
19. ELLIOTT, S.P. 2003. Gamma hydroxybutyric acid (GHB) concentrations in humans and factors affecting endogenous production. Forensic Sci. Int. **133:** 9–16.
20. YEATMAN, D.T. & K. REID. 2003. A study of urinary endogenous gamma-hydroxybutyrate (GHB) levels. J. Anal. Toxicol. **27:** 40–42.
21. CAPUTO, F., G. ADDOLORATO, F. LORENZINI, *et al*. 2003. Gamma-hydroxybutyric acid versus naltrexone in maintaining alcohol abstinence: an open randomized comparative study. Drug Alcohol Depend. **70:** 85–91.
22. BORGEN, L.A., R. OKERHOLM, D. MORRISON, *et al*. 2003. The influence of gender and food on the pharmacokinetics of sodium oxybate oral solution in healthy subjects. J. Clin. Pharmacol. **43:** 59–65.
23. SCHARF, M.B., A.A. LAI, B. BRANIGAN, *et al*. 1998. Pharmacokinetics of gammahydroxybutyrate (GHB) in narcoleptic patients. Sleep **21:** 507–514.
24. GONZALEZ, A. & D.J. NUTT. 2005. Gamma hydroxy butyrate abuse and dependency. J. Psychopharmacol. **19:** 195–204.
25. PICKWORTH, W.B., R.V. FANT & E.B. BUNKER. 1998. Effects of abused drugs on papillary size and the light reflex. *In* Drug Abuse Handbook. S.B. Karch, Eds.: 266–275. CRC Press. Boca Raton, FL.
26. FARRE, M., M.T. TERAN & J. CAMI. 1996. A comparison of the acute behavioral effects of flunitrazepam and triazolam in healthy volunteers. Psychopharmacology (Berl.) **125:** 1–12.
27. HERNANDEZ-LOPEZ, C., M. FARRE, P.N. ROSET, *et al*. 2002. 3,4-Methylenedioxymethamphetamine (ecstasy) and alcohol interactions in humans: psychomotor performance, subjective effects, and pharmacokinetics. J. Pharmacol. Exp. Ther. **300:** 236–244.
28. WECHSLER, D. 1958. The Measurement and Appraisal of Adult Intelligence. Williams & Wilkins. Baltimore.
29. HANNINGTON-KIFF, J.G. 1970. Measurement of recovery from out patient general anesthesia with a simple ocular test. Br. Med. J. **3:** 132–135.
30. EVANS, S.M., J.R. TROISI II & R.R. GRIFFITHS. 1994. Tandospirone and alprazolam: comparison of behavioral effects and abuse liability in humans. J. Pharmacol. Exp. Ther. **271:** 683–694.
31. HAERTZEN, C.A. 1974. An overview of the Addiction Center Research Inventory: an appendix and manual of scales DHEW Pub. no. (ADM) 79. Department of Health, Education and Welfare, Washington.
32. LAMAS, X., M. FARRÉ, M. LLORENTE, *et al*. 1994. Spanish version of the 49-item short version of the Addiction Research Center Inventory (ARCI). Drug Alcohol Depend. **35:** 203–209.
33. POUDEVIDA, S., M. FARRÉ, P.N. ROSET, *et al*. 2003. Construcción de un cuestionario para la Valoración de los Efectos Subjetivos de Sustancias con Potencial de Abuso (VESSPA): Evaluación del éxtasis. Adicciones **15:** 115–126.
34. ELIAN, A.A. 2001. GC-MS determination of gamma-hydroxybutyric acid (GHB) in blood. Forensic Sci. Int. **122:** 43–47.
35. DAVIDSON, D., K. HUTCHISON, C. DAGON, *et al*. 2002. Assessing the stimulant effects of alcohol in humans. Pharmacol. Biochem. Behav. **72:** 151–156.
36. HEISHMAN, S.J., K. ARASTEH & M.L. STITZER. 1997. Comparative effects of alcohol and marijuana on mood, memory, and performance. Pharmacol. Biochem. Behav. **58:** 93–101.

37. MIOTTO, K., J. DARAKJIAN, J. BASCH, *et al*. 2001. Gamma-hydroxybutyric acid: patterns of use, effects and withdrawal. Am. J. Addict. **10:** 232–241.
38. HICKS, A.R., D.R. KAPUSTA & K.J. VARNER. 2004. Mechanisms underlying the sympathomimetic cardiovascular responses elicited by [gamma]-hydroxybutyrate. J. Cardiovas. Pharmacol. **44:** 631–638.
39. SPORER, K.A., R.L. CHIN, J.E. DYER, *et al*. 2003. Gamma-hydroxybutyrate serum levels and clinical syndrome after severe overdose. Ann. Emerg Med. **42:** 3–8.
40. KAVANAGH, P.V., P. KENNY, J. FEELY. 2001. The urinary excretion of gamma-hydroxybutyric acid in man. J. Pharm. Pharmacol. **53:** 399–402.

5.2. Disposition of GHB in conventional and non-conventional biological fluids after a single drug administration: issues in methodology and drug monitoring. Ther Drug Monit. 2007;29:64-70.

ORIGINAL ARTICLE

Disposition of Gamma-Hydroxybutyric Acid in Conventional and Nonconventional Biologic Fluids After Single Drug Administration: Issues in Methodology and Drug Monitoring

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Abstract: Little controlled drug administration data are available to aid in the interpretation of gamma-hydroxybutyric acid (GHB) distribution in conventional and nonconventional fluids and the potential correlation between the pharmacokinetics of GHB and drug effects. Single oral sodium GHB doses of 50 mg/kg were administered to five volunteers. Plasma, oral fluid, urine, and sweat were analyzed for GHB by gas chromatography–mass spectrometry. GHB stability in plasma was studied at different storage temperatures. Subjective effects were measured using a set of 13 different visual analog scales. Mean peak GHB plasma concentrations at 30 minutes were 83.1 μg/mL. After the absorption phase, concentrations declined to mean values of 0.9 μg/mL at 6 hours. GHB was found in oral fluid at peak value concentrations equivalent to one third to one fourth of those found in plasma. The oral fluid-to-plasma ratio varied two fold in the 1- to 6-hour time range but always was lower than unit. The mean half-life ($t_{1/2}$) of GHB was approximately 0.7 hour in plasma and approximately 1.2 hours in oral fluid. GHB urinary excretion is less than 2% of the dose administered. GHB was also detected in sweat at low concentrations. GHB showed a mixed sedative–stimulant pattern with subjective effects peaking between 1 and 1.5 hours after drug administration and lasting for 2 hours. Oral fluid and sweat appeared not to be suitable biologic matrices for monitoring GHB consumption. GHB -mediated subjective effects are related to GHB plasma concentrations.

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INTRODUCTION

Gamma-hydroxybutyric acid (GHB, "liquid ecstasy," gamma-hydroxybutyrate, 4-hydroxybutiric acid, 4-hydroxybutanoic acid, oxybate) is a short chain fatty acid. It is an endogenous metabolite and a precursor of the neurotransmitter gamma-aminobutyric acid. GHB can be formed in human peripheral tissues from two precursors, gamma-butyrolactone and 1,4-butanediol (Fig. 1) and acts in the central nervous system as a neuromodulator.[1] GHB is marketed in the United States under the name Xyrem for the treatment of cataplexy in patients with narcolepsy and in some European countries as an anesthetic agent and for the treatment of alcohol withdrawal (eg, Alcover in Italy). GHB has also emerged as a major recreational drug and health problem all over the world. By the late 1990s, GHB had become a popular drug used in clubs and gained significant notoriety as a major drug of abuse and as a date rape drug. Gamma-butyrolactone and 1,4-butanediol, both GHB metabolic precursors, have also been abused in humans. During the last years, it has become a major concern in emergency departments of some countries as a result of an important increase in the number of cases of intoxication. GHB overdose frequently results in nonreactive coma associated with bradycardia, hypothermia, agitated delirium, myoclonus, and rarely seizure-like activity.[2]

Although GHB has been successfully detected in urine and blood of consumers,[3–12] drug measurement in biologic fluids to assess both voluntary and involuntary consumption has some drawbacks. First, GHB is eliminated from the body rapidly, making identification dependent on the time elapsed between consumption and biologic matrix collection. This fact addressed investigations toward the use of biologic matrices with time windows for drug detection wider than blood and urine such as hair.[13,14] Second, a compounding difficulty resides in the fact that GHB is an endogenous compound present in the human body with measurable baseline concentrations both in blood and urine.[15–18] Hence, different authors proposed cutoff (ie, 10 μg/mL for urine samples) concentrations to identify exogenous GHB exposure.[15,16,19]

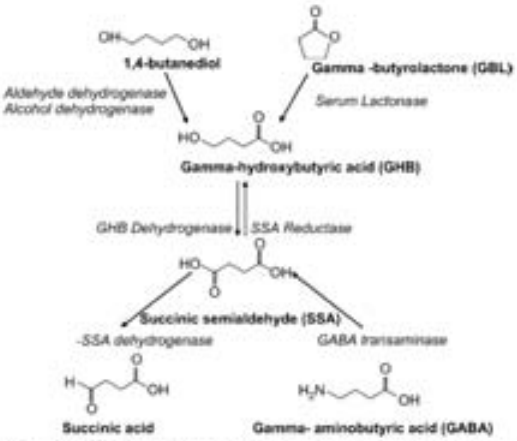

FIGURE 1. Biosynthesis of gamma-hydroxybutyric acid in vivo from its precursors and enzymes involved in its biotransformation and biosynthesis.

Finally, several studies underlined that endogenous GHB concentrations can be elevated both in antemortem and postmortem biologic samples resulting from storage conditions and enzymes responsible for biotransformation of GHB into the body (Fig. 1).[15,19,20–23] Whereas the possible influence of storage conditions and microorganisms in the production of GHB in postmortem specimens has been clarified,[22,24] data on GHB stability in stored biologic fluids from administration studies and intoxication cases are scarce.

Although several studies have addressed the measurement of GHB in plasma and oral fluid after single and repeated administrations,[5,8,25–27] there remain many questions on disposition of GHB biologic fluids other than plasma and urine, on the stability of the compound in stored biologic samples, and on the subjective effects induced by this drug in the range of doses commonly abused. In addition, few studies addressed the correlation of drug effects and concentrations in biologic fluids, including alternative matrices.

The aims of this study were as follows: 1) to investigate the presence and the time course of GHB in plasma, urine, oral fluid, and sweat after single oral administration; 2) to evaluate the stability of GHB in plasma under different storage conditions; and 3) to explore the potential correlation between the pharmacokinetics of GHB in different biologic matrices and some subjective effects.

MATERIALS AND METHODS

Human Subjects

Five male subjects were recruited by "word of mouth" and included in the study. Eligibility criteria required the recreational use of GHB on at least five occasions. Exclusion criteria included daily consumption of more than 20 cigarettes and more than 30 g of ethanol (3 units per day). All subjects gave their written informed consent before inclusion and were economically compensated for inconveniences caused by their participation in the study. The study was conducted in accordance with the Declaration of Helsinki, approved by the local ethical committee, and authorized by the Spanish Ministry of Health (Agencia Española del Medicamento). Eligible subjects were interviewed by a psychiatrist (structured clinical interview Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition) to exclude psychiatric disorders such as schizophrenia, psychosis, and major affective disorders. Each subject underwent a general physical examination, routine laboratory tests, urinalysis, and a 12-lead electrocardiogram. Participants had a mean age of 28.2 years (range, 25–32 years), mean weight of 74.4 kg (range, 66.8–84.0 kg), mean height of 184.2 cm (range, 176.0–194.0 cm), and a mean body mass index of 22.0 kg/m^2 (range, 18.5–25.7 kg/m^2). Three participants were nonsmokers and two smokers, and their average consumption of alcohol was 1.5 units per day. All of them had previous experience with the consumption of alcohol, cannabis, sedatives, stimulants, and designer drugs. None had a history of abuse or drug dependence according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition criteria (except for nicotine dependence) nor experienced any medical or psychiatric adverse reaction after GHB consumption.

Study Design

Subjects participated as outpatients in a 6 hour study session in which they were given a single dose of 50 (equivalent to 41.4 mg/kg of GHB) mg/kg of sodium GHB by the oral route. Participants were requested to abstain from consumption of any drug of abuse during the study period and urine drug testing (applying the cutoff values in parentheses) was performed before each study session for cannabinoids (50 ng/mL), cocaine (300 ng/mL), opiates (2000 ng/mL), amphetamine/methamphetamine (1000 ng/mL), MDMA (500 ng/mL), barbiturates (200 ng/mL), benzodiazepines (300 ng/mL), and phencyclidine (25 ng/mL) (Instant-View; Alfa Scientific Designs, Poway, CA). For all groups of substances, participants tested negative before each experimental session. Subjects arrived at the laboratory at 8 AM after an overnight fast and had an indwelling intravenous catheter inserted into a subcutaneous vein in the forearm of the nondominant arm. Thereafter, they remained seated in a quiet room throughout the session. GHB (Alcover OS sodium GHB, 17.5% syrup; CT Laboratorio Farmaceutico, SRL, San Remo, Italy) was orally administered around 9:00 AM in a fasting state. The dose (corresponding to appropriate milliliters of syrup) was diluted to 250 mL of a soda orange-based drink. Participants were told to drink the beverage as soon as possible (mean, 12 seconds; range, 5–20 seconds).

Collection of Blood, Oral Fluid, Urine, and Sweat Samples

Blood was collected at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5 and 6 hr after GHB administration in heparinized tubes and immediately centrifuged. Samples of oral fluid were collected without any stimulation over a 1 min period at 0, 0.5, 1, 1.5, 2, 3, 4, 5 and 6 hr after drug administration. Immediately after drug administration, mouth was washed (100 mL of water) to minimize oral fluid contamination. Urine samples were

collected before and at 0–3 and 3–6 hours after drug administration. Sweat samples were obtained by wiping the forehead with cotton fleece at 0, 0.5, 1, 2, 3, 4, 5, and 6 hours after drug administration. Additionally, a sweat patch (PharmChem Laboratories, Menlo Park, CA) was applied to the back of each participant after the skin was cleaned with a 70% isopropyl alcohol swab and removed 6 hours after administration. All biologic specimens were frozen at –20°C until analysis. No preservatives were added to the specimens.

Pharmacologic Effects

In the context of a dose-finding study for the performance of clinical trials intended at evaluating the pharmacology of GHB in humans, a number of physiological and subjective effects as well as psychomotor performance of subjects under the effects of the drug were measured. Results have been reported elsewhere.[28]

In this article, some representative subjective effects induced by GHB are included to explore the potential correlation between its pharmacokinetics in different biologic matrices and drug effects.

Subjective effects were measured using a set of 13 different visual analog scales (VAS) of 100 mm labeled with different adjectives marked at opposite ends with "not at all" and "extremely" were used. Subjects were asked to rate effects of "stimulated," "high," "any effect," "good effects," "bad effects," "liking," "content," "drunkenness," "drowsiness," "dizziness," "confusion," "depression or sadness," and "relax." Scales were administered at –15 minutes (immediately before drug administration), 0.25, 0.50, 0.75, 1, 1.50, 2, 3, 4, 5, and 6 hours after GHB administration.

Chemicals

Pure standards of GHB sodium salt and N,O-Bis(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane (BSTFA-1% TMCS) were obtained from Sigma Chemicals (St. Louis, MO). GHB-d_6 methanolic solution (1 mg/mL) was purchased from Cerilliant (Austin, TX). Ultrapure water and all other reagents of analytic grade were from Carlo Erba (Milano, Italy).

Determination of Gamma-Hydroxybutyric Acid Concentrations in Biologic Fluids

Frozen oral fluid, plasma, and urine samples were allowed to thaw at room temperature. Before analysis, oral fluid was centrifuged to discard the mucous part, which accumulated at the bottom. Aliquots of 100 μL plasma, oral fluid, and urine were added with 5 μg of GHB-d_6 (as internal standard, 5 μL of the 1 mg/mL methanolic solution) and 200 μL of acetonitrile. After 30 second vortex and 5 minute centrifugation at 1000*g*, 150 μL of the supernatant was transferred to a clean extraction tube and evaporated to dryness under a nitrogen steam (40°C, 10 minutes). The dried extracts were derivatized with 50 μL of BSTFA-1% TMCS for 30 minutes at 70°C. For GHB determination in sweat, the cotton wipe or the sweat patch spiked with 5 μg of GHB-d_6 was extracted with 5 mL of acetonitrile (covering the whole cotton fleece or the patch). The acetonitrile extract was evaporated to dryness and derivatized as described previously.

A 1 μL aliquot of derivatized samples was injected onto a Hewlett-Packard 6890 gas chromatograph coupled to a Hewlett-Packard P5973 quadrupole mass spectrometer (Agilent, Palo Alto, CA). The capillary column was a cross-linked 5% phenyl-methylsilicone (12 m × 0.2 mm internal diameter and 0.33 μm film thickness, Ultra-2; Agilent). The samples were injected in splitless mode and helium gas was used as carrier at a flow rate of 1.2 mL/minute (measured at 180°C). The injector and detector temperatures were both maintained at 280°C, respectively. The temperature program was initially set at 60°C for 2 minutes and increased to 180°C at 20°C/minute, then 35°C to 250°C, and then held for 4 minutes. The total run time was 14 minutes. The mass spectrometer was operated in the electron ionization and selected ion monitoring acquisition mode. The following ions were monitored (underlined ions were used for quantification): GHB-bis-TMS: m/*z* 233, 204, 117; GHB-d_6-bis-TMS: m/*z* 239.

Before application to real samples, the analytical method was tested for plasma, oral fluid, and urine after a 4 day validation protocol. Linearity, precision, accuracy, stability (freeze/thaw cycles), limits of detection, and quantification were assessed.

Calibration standards containing 1, 25, 50, 100, 200, 300 μg/mL of GHB were prepared in duplicate daily for each analytic batch by adding suitable amounts of methanol working solutions (10 μg/mL, 100 μg/mL, 1 mg/mL) to glass tubes and evaporated to dryness (40°C, 3 minutes). Residue was reconstituted with 100 μL of prechecked drug-free (see further in this section) oral fluid, plasma, and urine samples. Quality control (QC) samples of 5 (low control), 100 (medium control), and 200 μg/mL (high control) GHB in plasma, oral fluid, and urine were prepared, distributed in aliquots, and stored at −20°C. QC samples were included in each analytic batch to check the daily quality of the analytic process.

Peak area ratios between GHB and internal standard were used for calculations using a weighted (1/concentration) least square regression analysis. Standard deviation of the analytic background response was used to estimate the lower limit of quantification (LLOQ = 10 standard deviations) and the limit of detection (LOD = 3 standard deviations). Because GHB is an endogenous compound, a number of replicates (n = 8) of different biologic matrices (plasma, oral fluid, and urine) under basal conditions from subjects participating in the clinical studies as well as those used for the preparation of calibrators were analyzed by isotopic dilution mass spectrometry using the deuterated analog of GHB at the concentration of 0.25 μg/mL and aliquots of 200 μL of the corresponding matrix. The purpose of this approach was to check that basal GHB endogenous concentrations were well below the LLOQ of the analytic method.

Extraction recoveries were calculated by comparing the peak areas of four replicates of QC samples after the previously mentioned extraction procedure with matched methanolic dilutions of GHB and IS.

Nine replicates of the three QC samples were analyzed for the determination of intraday precision and accuracy, whereas the interday precision and accuracy were determined for three different assays of three replicates of the previously mentioned QC samples. Precision was expressed as the

relative standard deviation of concentrations calculated for QC samples. Accuracy was expressed as the relative error of the calculated concentrations. Interday precision has been estimated through analysis of variance of determinations in three consecutive days estimating components of precision with the formula given in Krouwer and Rabinowitz.[29]

The effects of three freeze–thaw cycles (storage at –20°C) on the compound stability in different biologic fluids were evaluated by repeated analysis (n = 3) of QC samples. The stability was expressed as a percentage of the initial concentration of the analyte spiked in different matrices and quantified just after preparation.

Effect of Storage Temperature

Plasma samples were distributed in aliquots and stored at different temperatures [room temperature (25°C), refrigerated (4°C), frozen (−20°C), and heated (37°C)]. The storage subsets were analyzed in triplicate at 1, 5, 24, and 48 hours after preparation and compared with a triplicate immediately analyzed. Differences from the initial concentration higher than the precision of the method were considered relevant.

Pharmacokinetics Parameters

The following parameters were determined: with regard to plasma and oral fluid concentrations of GHB, peak concentration (C_{max}), time to reach peak concentrations (t_{max}), area under the concentration–time curve from 0 to 6 h (AUC_{0-6}), elimination half-life ($t_{1/2}$), and elimination constant (Ke). AUCs were calculated by the linear trapezoidal rule.

Pharmacokinetic parameters were obtained with use of specific functions of a computer program (PK Functions for Microsoft Excel; Microsoft Corp., Redmond, WA).

RESULTS

Method Validation

Calibration curves were linear over the concentration range 0.2 (LLOQ) to 300 μg/mL GHB in the case of plasma, oral fluid, and urine samples and determination coefficients, r^2, for all the calibration curves were always greater than 0.990. Mean extraction efficiency of GHB was always greater than 95% in all biologic fluids under investigation.

Table 1 shows the results obtained for intraday and interday precision and accuracy calculations for GHB in biologic matrices under examination. These results satisfactorily met the internationally established acceptance criteria.[30,31]

No relevant degradation was observed after any of the three freeze–thaw cycles with differences in the initial concentration lower than 10%.

Effect of Storage Temperature in Gamma-hydroxybutyric Acid Plasma Concentrations

GHB was stable at all temperatures tested. A higher variability in GHB plasma concentrations was mainly observed at 25°C and 37°C; nevertheless, changes were never higher than the precision of the analytic method.

Concentration–time Profiles and Pharmacokinetics of GHB in Plasma and Oral Fluid Samples

Mean concentration–time curves of GHB in plasma and oral fluid after 50 mg/kg of sodium GHB are presented in Figure 2.

GHB was detected in all baseline plasma samples with mean estimated concentrations of 0.04 ± 0.01 μg/mL. GHB was readily absorbed after oral administration and rapidly eliminated (t_{max} and $t_{1/2}$ < 1 hour). After drug administration, concentrations peaked between 30 and 60 minutes after drug administration (C_{max} range, 51.0–123.5 μg/mL) (Table 2). After the absorption phase, concentrations declined to a mean values at 6 hours of 0.9 μg/mL.

GHB was detected in all baseline oral fluid samples with mean estimated concentrations of 0.12 ± 0.02 μg/mL. The time course of drug concentration in oral fluid was similar to that seen in plasma with maximum concentrations achieved at 0.5 hour (C_{max} range, 11.6–49.0 μg/mL); then concentrations decreased to mean values of 0.5 μg/mL at 6 hours.

Oral fluid GHB mean concentrations time course paralleled those found in plasma. However, individual values for oral fluid and plasma did not correlate. The oral fluid to plasma ratio (S/P ratio) after drug administration was lower

TABLE 1. Intraday and Interday Precision and Accuracy for Gamma-hydroxybutyric Acid in Plasma, Urine, and Saliva

		Intraday			Interday		
Biologic Fluid	Target Concentration (μg/mL)	Calculated Concentration (μg/mL)	Precision (RSD)	Accuracy (% error)	Calculated Concentration (μg/mL)	Precision (RSD)	Accuracy (% error)
Plasma	5	5.0 ± 0.7*	13.5	10.4	4.8 ± 0.7*	8.4	12.1
	100	99.1 ± 3.7	3.7	2.9	94.5 ± 8.1	2.2	6.7
	200	215.9 ± 5.6	2.6	7.8	189.0 ± 10.3	0.9	6.7
Urine	5	5.1 ± 0.4	6.6	17.3	5.8 ± 0.5	6.9	16.6
	100	107.6 ± 5.9	5.5	8.0	111.1 ± 4.4	1.6	11.1
	250	266.9 ± 10.0	3.7	6.7	281.1 ± 21.3	5.2	12.4
Saliva	5	5.6 ± 0.2	4.2	12.1	5.7 ± 0.3	5.4	14.6
	100	108.4 ± 3.6	3.3	8.4	105.4 ± 10.8	4.3	10.7
	200	208.3 ± 8.5	4.1	4.8	188.8 ± 16.0	3.7	7.1

*Mean ± standard deviation, n = 9 for each concentration level.
RSD, relative standard deviation.

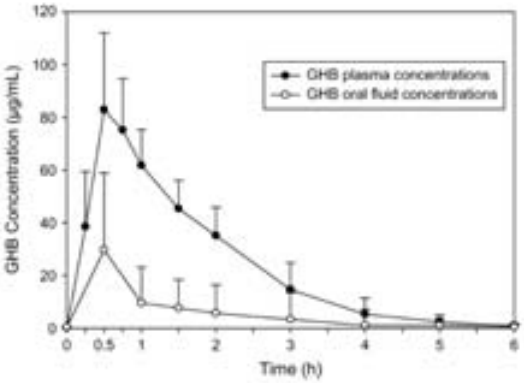

FIGURE 2. Gamma-hydroxybutyric acid plasma and oral fluid concentrations (mean ± standard deviation) over time after administration of an oral dose of 50 mg/kg sodium gamma-hydroxybutyric acid in five subjects.

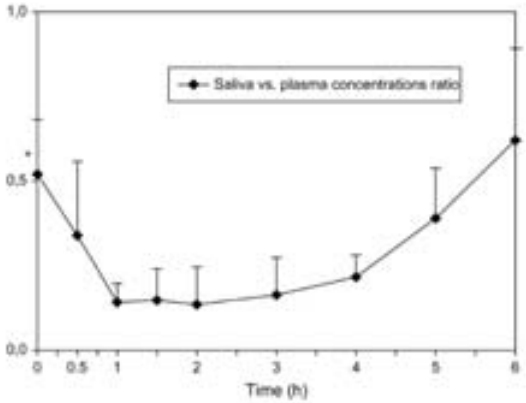

FIGURE 3. Oral fluid to plasma concentration ratio (oral fluid to plasma ratio) after administration of an oral dose of 50 mg/kg sodium gamma-hydroxybutyric acid in five subjects (mean ± standard deviation). *Baseline ratio was only estimated by isotopic dilution because basal concentrations of gamma-hydroxybutyric acid in both matrices were quite low.

than unit at all times tested (Fig. 3) and ranged between 0.5 and 0.2. A high interindividual variability in S/P ratio was observed among subjects.

Excretion of Gamma-Hydroxybutyric Acid in Urine

A summary of GHB urinary excretion is shown in Table 3. GHB was detected in urine at baseline samples with estimated concentrations of 0.21 ± 0.14 µg/mL. The highest GHB recovery was found in the 0- to 3-hour urine samples. Concentrations of GHB in urine (median and percentiles 25/75) for the two collection periods evaluated (0–3 hours and 3–6 hours) were, respectively, 584.0 (435.8–763.0) and 60.5 (12.8–107.5) µg/mL. Less than 2% of the dose tested was recovered in urine for the 0- to 6-hour collection period.

Excretion of Gamma-Hydroxybutyric Acid in Sweat

Sweat samples were analyzed following an analytic approach similar to the one applied for other fluids evaluated in the present report. A preliminary study for the cotton wipe collection of blank samples made in 20 drug- free male healthy volunteers showed a very high variability in GHB endogenous basal concentrations. There was a more than a 100 fold intersubject difference in sweat GHB values. The range of concentrations were 0.03 to 5.46 µg per wipe. Tentatively, these differences were attributed to the fact that those subjects, displaying higher concentrations, presented acne and oily skin. This was an empiric and plausible observation that should be verified experimentally in further studies. Proper validation of GHB in sweat was not possible as a result of the inability to find a representative blank sample for preparation of calibrators and controls.

A preliminary assessment of baseline amounts of GHB measured in cotton wipes swabbed for 10 seconds on the armpit and forehead of subjects participating in the study showed high variability. This observation was biased by a subject with basal sweat GHB concentrations of 3.6 µg per wipe. Concentrations measured overtime never increased more than 1 to 2 µg per wipe over basal values. GHB was also found in the sweat patches (0- to 6-hour accumulation) in amounts ranging between 0.9 µg and 1.3 µg.

TABLE 2. Pharmacokinetic Parameters of Gamma-hydroxybutyric Acid in Plasma and Oral Fluid After an Oral Dose of 50 mg/kg Sodium Gamma-hydroxybutyric Acid (mean ± Standard Deviation Values Given)

	50 mg/kg	
Parameter	Plasma	Oral Fluid
C_{max} (µg/mL)	83.1 ± 10.7	29.6 ± 17.5
t_{max} (h)*	0.6 ± 0.1	0.50
AUC_{0-6} (µg/mL/h)	143.7 ± 36.0	33.3 ± 23.8
K_e (h^{-1})	1.01 ± 0.18	0.56 ± 0.12
$t_{1/2}$ (h)	0.71 ± 0.15	1.27 ± 0.27

Pharmacologic Effects

Subjective effects corresponding to VAS "stimulated" and "drowsiness" are presented in Figure 4. GHB showed a mixed sedative–stimulant pattern mediating both types of effects. GHB produced slight stimulant-like effects as measured by VAS "stimulated" that peaked at 45 minutes after drug administration and objective sedation effects as reflected in VAS "drowsiness." The latter peaked between 1 and 1.5 hours after drug administration and lasted for 2 hours.

DISCUSSION

The results of the study provide new insights on GHB pharmacokinetics in nonconventional matrices in humans.

TABLE 3. Urinary Excretion of Gamma-hydroxybutyric Acid After Oral Dose of 50 mg/kg Sodium Gamma-hydroxybutyric Acid (median and Percentiles 25/75 Given)

		Urinary Collection Period		
Dose* mg/kg	Mean Dose given (g)	0–3 h	3–6 h	0–6 h
50	3.7 ± 0.3	584.0 (435.8–763.0)†	60.5 (12.8–107.5)	588.8 (496.3–870.5) (1.98%)‖
		60. 8 (45.3–79.4)‡	6.3 (1.3–11.2)‡	61.3 (51.6–90.6) ‡

*As sodium gamma-hydroxybutyric acid.
†Values given in micromoles.
‡Values given in milligrams.
‖Recoveries (0–6 hours) as a percentage of the administered dose.

GHB given by the oral route is rapidly absorbed and eliminated. Drug consumption can be differentiated from GHB endogenous concentrations both in plasma and urine in a time window of 6 hours after ingestion. Our results are in agreement with those obtained in healthy subjects administered with 4.5 g of oxybate (equivalent to 50–60 mg/kg of GHB)[3] and in terms of C_{max} value, barely double those observed after the administration of a single dose of 25 mg/kg of GHB.[27] In contrast, present results are lower than those observed in severe acute intoxications.[32]

GHB was found in oral fluid at peak value concentrations equivalent to one third to one fourth of those found in plasma with an even quicker return to baseline values than that observed in plasma. Therefore, oral fluid and plasma kinetics are poorly related. In early experiments,[27] in which a single oral dose of 25 mg/kg was given, a t_{max} at 15 minutes in oral fluid was reported. Oral fluid samples were collected at 15 minutes, but despite using a drinking device, the authors themselves did not exclude a possible contamination, because GHB concentrations exceeded more than five times those observed in plasma. Taking into account this previous report, in the present study, oral fluid sample collection was started at 0.5 hour after a mouthwash and concentrations observed are closer to those expected following Henderson-Hasselbach modified equation for weak acidic substances (estimated pKa of GHB is approximately 5) excreted into a matrix (oral fluid) more acidic than plasma.[33] Indeed, the saliva plasma ratio was always lower than 1, which indicates that GHB, an acidic drug, did not diffuse to a large extent into oral fluid in contrast with basic drugs such as MDMA, which appears in saliva in concentrations remarkably higher than those in plasma.[33] Furthermore, differently from MDMA and other basic drugs, a large intersubject variability in S/P ratio was found, which prevents any possible correlation of dose–oral fluid concentration. Because GHB oral fluid concentrations are lower than those observed in plasma, they quickly reach baseline concentrations at 3 hours after ingestion, limiting somewhat the time window for detecting its consumption in this biologic matrix and its usefulness in GHB forensic toxicology.

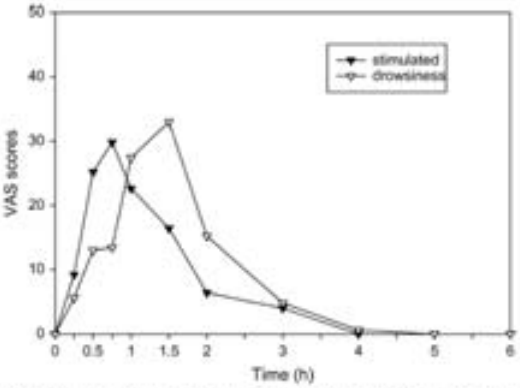

FIGURE 4. Time course of gamma-hydroxybutyric acid induced subjective effects on visual analog scale "stimulated" and "drowsiness."

GHB urinary excretion was in accordance with previous reports.[3,27,34] In line with early reports, less than 2% of doses administered were recovered in the collection period. Highest recoveries were found in the 0- to 3-hour urine samples.

Concerning GHB detection in sweat, it appears that drug diffusion in this matrix has even more limitations than those found for oral fluid. After drug intake, GHB concentrations both in cotton wipes (which represent a punctual measure of excretion) and in sweat patches (which represent an accumulative measure of excretion) are marginally higher than baseline values when peak concentrations are achieved in plasma. In addition, taking into account the variability in basal concentrations, concentrations of GHB in sweat higher than reported basal values cannot be attributed to its consumption. Therefore, similar to oral fluid, sweat does not appear to be a suitable biologic matrix for monitoring GHB consumption.

GHB was stable during storage conditions and no biotransformation toward GHB formation was observed in the collected matrices.

GHB showed a mixed stimulant–sedative pattern with a biphasic time profile as described for other sedatives (alcohol or cannabis).[35,36] Psychostimulant effects were predominant in the first hour, whereas sedative effects initiate more slowly and predominate in the second hour after drug administration. GHB plasma and oral fluid concentrations correlated better with psychostimulant-like effects rather than with sedative ones.

CONCLUSION

In summary, the time course of GHB concentrations in oral fluid was similar to that seen in plasma; nevertheless, concentrations were more than three times lower in oral fluid.

Oral fluid and sweat appear not to be suitable biologic matrices for monitoring GHB consumption. Some GHB-mediated subjective effects are related to plasma concentrations.

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REFERENCES

1. Nicholson KL, Balster RL. GHB: a new and novel drug of abuse. *Drug Alcohol Depend.* 2001;63:1–22.
2. Chin RL, Sporer KA, Cullison B, et al. Clinical course of gamma-hydroxybutyrate overdose. *Ann Emerg Med.* 1998;31:716–722.
3. Palatini P, Tedeschi L, Frison G, et al. Dose-dependent absorption and elimination of gamma-hydroxybutyric acid in healthy volunteers. *Eur J Clin Pharmacol.* 1993;45:353–356.
4. Ferrara SD, Zotti S, Tedeschi L, et al. Pharmacokinetics of gamma-hydroxybutyric acid in alcohol dependent patients after single and repeated oral doses. *Br J Clin Pharmacol.* 1992;34:231–235.
5. McCusker RR, Paget-Wilkes H, Chronister CW, et al. Analysis of gamma-hydroxybutyrate (GHB) in urine by gas chromatography-mass spectrometry. *J Anal Toxicol.* 1999;23:301–305.
6. LeBeau MA, Montgomery MA, Miller ML, et al. Analysis of biofluids for gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL) by headspace GC-FID and GC-MS. *J Anal Toxicol.* 2000;24:421–428.
7. Couper FJ, Logan BK. Determination of gamma-hydroxybutyrate (GHB) in biological specimens by gas chromatography–mass spectrometry. *J Anal Toxicol.* 2000;24:1–7.
8. Frison G, Tedeschi L, Maietti S, et al. Determination of gamma-hydroxybutyric acid (GHB) in plasma and urine by headspace solid-phase microextraction and gas chromatography/positive ion chemical ionization mass spectrometry. *Rapid Commun Mass Spectrom.* 2000;14:2401–2407.
9. Elian AA. A novel method for GHB detection in urine and its application in drug-facilitated sexual assaults. *Forensic Sci Int.* 2000;109:183–187.
10. de Vriendt CA, van Sassenbroeck DK, Rosseel MT, et al. Development and validation of a high-performance liquid chromatographic method for the determination of gamma-hydroxybutyric acid in rat plasma. *J Chromatogr B Biomed Sci Appl.* 2001;752:85–90.
11. Chen M, Andrenyak DM, Moody DE, et al. Stability of plasma gamma-hydroxybutyrate determined by gas chromatography-positive ion chemical ionization–mass spectrometry. *J Anal Toxicol.* 2003;27:445–448.
12. Villain M, Cirimele V, Ludes B, et al. Ultra-rapid procedure to test for gamma-hydroxybutyric acid in blood and urine by gas chromatography-mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2003;792:83–87.
13. Kintz P, Cirimele V, Jamey C, et al. Testing for GHB in hair by GC/MS/MS after a single exposure. Application to document sexual assault. *J Forensic Sci.* 2003;48:195–200.
14. Verstraete AG. Detection times of drugs of abuse in blood, urine, and oral fluid. *Ther Drug Monit.* 2004;26:200–205.
15. Elian AA. Determination of endogenous gamma-hydroxybutyric acid (GHB) levels in antemortem urine and blood. *Forensic Sci Int.* 2002;128:120–122.
16. LeBeau MA, Montgomery MA, Morris-Kukoski C, et al. A comprehensive study on the variations in urinary concentrations of endogenous gamma-hydroxybutyrate (GHB). *J Anal Toxicol.* 2006;30:98–105.
17. Tedeschi L, Carai MA, Frison G, et al. Endogenous gamma-hydroxybutyric acid is in the rat, mouse and human gastrointestinal tract. *Life Sci.* 2003;72:2481–2488.
18. Yeatman DT, Reid K. A study of urinary endogenous gamma-hydroxybutyrate (GHB) levels. *J Anal Toxicol.* 2003;27:40–42.
19. Elliott SP. Gamma hydroxybutyric acid (GHB) concentrations in humans and factors affecting endogenous production. *Forensic Sci Int.* 2003;133:9–16.
20. Kerrigan S. In vitro production of gamma-hydroxybutyrate in antemortem urine samples. *J Anal Toxicol.* 2002;26:571–574.
21. Sakurada K, Kobayashi M, Iwase H, et al. Production of gamma-hydroxybutyric acid in postmortem liver increases with time after death. *Toxicol Lett.* 2002;129:207–217.
22. Elliott S, Lowe P, Symonds A. The possible influence of micro-organisms and putrefaction in the production of GHB in post-mortem biological fluid. *Forensic Sci Int.* 2004;139:183–190.
23. Wong CG, Gibson KM, Snead OC 3rd. From the street to the brain: neurobiology of the recreational drug gamma-hydroxybutyric acid. *Trends Pharmacol Sci.* 2004;25:29–34.
24. Elliot S. The presence of gamma-hydroxybutyric acid (GHB) in postmortem biological fluids. *J Anal Toxicol.* 2001;25:152.
25. Kintz P, Goulle JP, Cirimele V, et al. Window of detection of gamma-hydroxybutyrate in blood and saliva. *Clin Chem.* 2001;47:2033–2034.
26. Caputo F, Addolorato G, Lorenzini F, et al. Gamma-hydroxybutyric acid versus naltrexone in maintaining alcohol abstinence: an open randomized comparative study. *Drug Alcohol Depend.* 2003;70:85–91.
27. Brenneisen R, Elsohly MA, Murphy TP, et al. Pharmacokinetics and excretion of gamma-hydroxybutyrate (GHB) in healthy subjects. *J Anal Toxicol.* 2004;28:625–630.
28. Abanades S, Farré M, Segura M, et al. Gamma-hydroxybutyrate (GHB) in humans: pharmacodynamics and pharmacokinetics. *Ann N Y Acad Sci.* 2006;1074:559–576.
29. Krouwer JS, Rabinowitz R. How to improve estimates of imprecision. *Clin Chem.* 1984;30:290–292.
30. Guidance for Industry, Bioanalytical Method Validation. US Department of Health and Human Services, Food and Drug Administration; May 2001 Available at: www.fda.gov/cder/guidance/4252fnl.htm.
31. The European Agency for the evaluation of Medicinal Products: ICH Topic Q 2 B Validation of Analytical Procedures: Methodology. Available at: www.emea.eu.int/htms/human/ich/quality/ichfin.htm.
32. Sporer KA, Chin RL, Dyer JE, et al. Gamma-hydroxybutyrate serum levels and clinical syndrome after severe overdose. *Ann Emerg Med.* 2003;42:3–8.
33. Navarro M, Pichini S, Farre M, et al. Usefulness of saliva for measurement of 3,4-methylenedioxymethamphetamine and its metabolites: correlation with plasma drug concentrations and effect of salivary pH. *Clin Chem.* 2001;47:1788–1795.
34. Kavanagh PV, Kenny P, Feely J. The urinary excretion of gamma-hydroxybutyric acid in man. *J Pharm Pharmacol.* 2001;53:399–402.
35. Davidson D, Hutchison K, Dugon C, et al. Assessing the stimulant effects of alcohol in humans. *Pharmacol Biochem Behav.* 2002;72:151–156.
36. Heishman SJ, Arasteh K, Stitzer ML. Comparative effects of alcohol and marijuana on mood, memory, and performance. *Pharmacol Biochem Behav.* 1997;58:93–101.

5.3. Relative abuse liability of gamma-hydroxybutyric acid (GHB), flunitrazepam and ethanol in Club Drug users. J Clin Psychopharmacol. 2007;27:625-638.

ORIGINAL CONTRIBUTION

Relative Abuse Liability of γ-Hydroxybutyric Acid, Flunitrazepam, and Ethanol in Club Drug Users

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Objectives: Despite the increasing concern about γ-hydroxybutyric acid (GHB) toxicity, there are few studies examining the clinical pharmacology of GHB and its abuse potential. To evaluate GHB-induced subjective and physiological effects, its relative abuse liability and its impact on psychomotor performance in club drug users.

Materials and Methods: Twelve healthy male recreational users of GHB participated in 5 experimental sessions in the framework of a clinical trial. The study was randomized, double-blind, double-dummy, and crossover. Drug conditions were a single oral dose of GHB (40 or 60 mg/kg), ethanol (0.7 g/kg), flunitrazepam (1.25 mg), and placebo. Study variables included vital signs (blood pressure, heart rate, oral temperature, pupil diameter), psychomotor performance (digit symbol substitution test, balance, Maddox-Wing), subjective effects (a set of 13 visual analogue scales, Addiction Research Center Inventory–49 items, and Evaluation of the Subjective Effects of Substances with Potential of Abuse questionnaires), and pharmacokinetics.

Results: All active conditions induced positive effects related to their abuse potential. The administration of GHB produced euphoria and pleasurable effects with slightly higher ratings than those observed for flunitrazepam and ethanol. γ-Hydroxybutyric acid induced a biphasic time profile with an initial stimulantlike effect related to the simultaneous rise of plasma concentrations and a latter sedative effect not related to GHB kinetics. γ-Hydroxybutyric acid increased blood pressure and pupil diameter. Ethanol induced its prototypical effects, and flunitrazepam produced marked sedation. γ-Hydroxybutyric acid and flunitrazepam impaired psychomotor performance, digit symbol substitution test, and balance task, whereas ethanol, at the dose tested, induced only mild effects exclusively affecting the balance task.

Conclusions: Our results suggest a high abuse liability of GHB and flunitrazepam in club drug users.

(*J Clin Psychopharmacol* 2007;27:625–638)

γ-Hydroxybutyric acid (GHB, "liquid ecstasy") is a short-chain fatty acid that has been suggested to act as a neuromodulator.[1] γ-Hydroxybutyric acid was detected in the brain and subsequently synthesized in 1960, during the search for a γ-aminobutyric acid (GABA) analog that would readily cross the blood-brain barrier for therapeutic uses.[2] Despite its early use as a general anesthetic, it has not found widespread clinical use. γ-Hydroxybutyric acid has been found useful in the management of alcohol withdrawal.[3] However, the only Food and Drug Administration–approved indication for GHB is the treatment of narcolepsy, where trials have shown amelioration of symptoms and improved sleep patterns.[4] Apart from its therapeutic uses, it has emerged as a major recreational drug and public health problem all over the world. γ-Hydroxybutyric acid was initially distributed over the counter as a dietary supplement, sleep aid, and muscle builder. By the late 1990s, it had gained significant notoriety as a major recreational drug and as a date rape drug.[5–7] γ-Hydroxybutyric acid abuse has risen because of the euphoria, disinhibition, enhanced sensuality, and heightened sexual awareness that are claimed to be associated with its use.[8–10]

Although it has been abused by humans, the results of preclinical studies of the reinforcing effects of GHB have been ambiguous. Various drug discrimination studies with GHB have failed to consistently show cross substitution with abused drugs such as benzodiazepines, barbiturates, opiates, cocaine, or *d*-amphetamine.[11–13] However, stronger evidence for cross substitution has been reported between GHB and baclofen, a γ-aminobutyric acid-B agonist non-abused drug,[14] particularly at high doses. Furthermore, self-administration studies of GHB show evidence for only weak and inconsistent reinforcing effects. In rhesus monkeys, self-administration rates for GHB were below those seen with

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phencyclidine and methohexital, and considerably similar to those obtained with vehicle tests.[11,13] Rodent studies have been more suggestive of the reinforcing effects of GHB as both oral and intravenous self-administration has been demonstrated. Nonetheless, results were variable and difficult to interpret conclusively.[15–18] Thus, the preclinical evaluation of the relative reinforcing effects of GHB have been fairly inconsistent, possibly because of discrepancies in the use of different species, routes of administration, doses, and testing procedures.

In humans, a number of cases of GHB abuse and dependence have been reported, but no abuse liability studies had been performed until recently. In an ascending-dose sequence study in subjects with histories of abuse of sedative/hypnotic drugs and no prior exposure to GHB, a likelihood of abuse for GHB intermediate to triazolam and pentobarbital has been suggested.[19]

γ-Hydroxybutyric acid is presently a controlled substance classified by the US National Institute on Drug Abuse as a club drug.[20] Club drugs are a loosely defined category of drugs from different classes grouped together because of their use at dance clubs and raves.[21] In these circumstances, GHB and also flunitrazepam are abused while dancing to electronic music. Users seek their euphoric effects and possibly both sedative and stimulantlike effects.[22]

Most of the information concerning the effects of GHB in humans has been obtained in therapeutic studies. Knowledge of abuse-related effects mainly comes from anecdotal reports and surveys but has not been fully demonstrated after GHB-controlled administration. A small number of clinical trials have examined GHB dose-related effects,[19,23–25] but little is known about GHB clinical pharmacology at doses that are abused.[19] In addition, few studies have addressed the effects of GHB in club drug users (the most important current population of GHB abusers) or the association of drug effects with plasma concentrations.[26] In addition, previous epidemiological investigations reported an increased abuse of sedativelike drugs by club drug users.[27] The reasons for this shift in drug preference and the effects of these drugs in this population remain unclear. Furthermore, no previous research has been done to test the relative abuse liability of sedativelike drugs in recreational polydrug users.

A study was carried out to evaluate GHB-induced subjective and physiological effects, relative abuse liability, and impact of GHB on psychomotor performance in club drug users. These participants should provide low false-positive rates as measured by response to placebo administration and are less likely to provide false-negative results than subjects without histories of drug use. These reasons could make this population preferable to test GHB abuse liability than GHB-naive drug users.[28] The relative abuse liability evaluation included the comparison with flunitrazepam and ethanol, 2 well-known recreational drugs with well-characterized abuse potential and similar behavioral profiles to GHB.[28] To our knowledge, there are no previous controlled studies evaluating flunitrazepam abuse potential in club drug users, a population claimed to be increasingly abusing this drug.[29] The present study tries also to assess the eventual correlation between drug pharmacokinetics and drug effects in a range of doses compatible with those usually consumed by recreational users.

MATERIALS AND METHODS

Participants

Twelve white male subjects were recruited by word of mouth and included in the study. Eligibility criteria required the recreational use of GHB on at least 5 occasions. Exclusion criteria included daily consumption of more than 20 cigarettes and/or more than 30 g of ethanol (3 standard units per day). All subjects gave their written informed consent before inclusion and were economically compensated for their participation in the study. The study was conducted in accordance with the Declaration of Helsinki, approved by the local ethical committee (Comité Ético de Investigación Clínica del Instituto Municipal de Asistencia Sanitaria), and authorized by the Spanish Ministry of Health (Agencia Española del Medicamento). Eligible subjects were interviewed by a psychiatrist (structured clinical interview *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*) to exclude psychiatric disorders, including schizophrenia, psychosis, and major affective disorders. Each participant underwent a general physical examination, routine laboratory tests, urinalysis, and a 12-lead electrocardiogram. The participants had a mean age of 27.9 years (range, 22–33 years), mean weight of 71.2 kg (range, 58.7–83.1 kg), and mean height of 179.2 cm (range, 168.0–194.5 cm). Among participants, there were current cigarette smokers (n = 8), and the average consumption of alcohol was 9 units per week. All of them had previous experience with the consumption of the following drugs: ethanol, cannabis (average of 112 previous experiences; range, 28–240), benzodiazepines (average of 5 previous experiences; range, 1–10), and club drugs including cocaine and amphetamine (average of 57 previous experiences; range, 27–107) and ketamine, lysergic acid diethylamide (LSD), and hallucinogenic mushrooms (average of 10 previous experiences; range, 3–20). Participants had at least 5 previous consumptions of GHB (average of 15 previous experiences; range, 5–34). None had history of abuse or drug dependence according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* criteria (except for nicotine dependence) or had experienced any medical or psychiatric adverse reactions after GHB consumption.

Study Design and Procedures

The study was conducted as double-blind, controlled, crossover, and randomized according to a balanced 5 × 5 Latin-square design. Subjects participated as outpatients in 5 different randomly assigned 6-hour study sessions with a washout period of 7 days. Before study sessions, volunteers completed 1 practice session to familiarize themselves with the behavioral measures and daily laboratory routine and to obtain a steady score in the performance tasks. No medications were administered on this day. Volunteers then completed a total of 5 experimental sessions. The 5

drug conditions were as follows: 2 single doses of GHB (40 and 60 mg/kg), flunitrazepam (1.25 mg), ethanol (0.7 g/kg), and placebo. Volunteers were informed that during their participation, they would receive different drugs, administered orally, and that these could include placebo, sedatives like GHB or benzodiazepines, and possibly other club drugs. Other than receiving this general information, volunteers were blind to the type of drug administered. Participants were requested to abstain from consumption of any drug of abuse during the study period, and urine drug testing was performed before each study session for cannabinoids, cocaine, opiates, amphetamine/methamphetamine, barbiturates, benzodiazepines, and phencyclidine (InstantView, Alfa Scientific Designs, Poway, Calif). An ethanol breath test (Dräger Alcotest 7310, Drägerwerker AG, Lübeck, Germany) was also performed. For all groups of substances, participants tested negative before each experimental session. In each session, subjects arrived at the laboratory at 8:00 am after an overnight fast and had an indwelling intravenous catheter inserted into a subcutaneous vein in the forearm of the nondominant arm. Thereafter, they remained seated in a quiet room throughout the session. A light meal was provided 5 hours after drug administration. Tobacco smoking was permitted 6 hours after drug administration.

Drugs

Pertinent doses of GHB were selected in a pilot study in which single increasing oral doses of 40 (33.1), 50 (41.4), 60 (49.7), and 72 (60.1) mg/kg of sodium GHB (GHB) were given to 8 volunteers.[26] Taking into account previous studies in healthy volunteers,[30,31] and in view of the results of the pilot study, a flunitrazepam dose was selected to find a GHB behavioral equivalent dose. Ethanol dose was selected after a pilot study in which 2 doses of 0.5 and 0.7 g/kg were given to 2 subjects familiar with both ethanol and GHB effects. The chosen ethanol dose elicited subjective effects comparable to those corresponding to GHB selected doses. γ-Hydroxybutyric acid doses of 40 and 60 mg/kg of GHB (corresponding to 48.3 and 72.4 mg/kg of sodium GHB, respectively) were prepared from commercially available syrup (Alcover OS sodium GHB, 17.5%, CT Laboratorio Farmaceutico, SRL, San Remo, Italy). Matched placebo was also obtained from the same company. Flunitrazepam dose (1.25 mg) was acquired from commercially available vials (flunitrazepam 2 mg, Rohipnol, Laboratorios Roche SA, Madrid, Spain). Ethanol (0.7 g/kg of body weight) was obtained from an alcoholic beverage (vodka Absolut, Ahus, Sweden). The different doses were diluted in 300 mL of a soda orange–based drink. This volume was distributed in 5 cool glasses containing 60 mL each. Treatments were orally administered around 9:00 AM in a fasting state. Participants were told to drink each glass in a maximum of 2 minutes, and consequently, the total volume (300 mL, 5 glasses of 60 mL) was taken during a 10-minute period. Ethanol was diluted throughout the 5 glasses to mask its presence, whereas GHB, matched placebo, or flunitrazepam was presented only in the first glass. This approach permitted synchronization of the peak effects of GHB, flunitrazepam, and ethanol based on previous pharmacokinetic and behavioral data.[26,30–32]

Pharmacological Effects

Physiological Measures

Noninvasive systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate, oral temperature, and pupil diameter were recorded at −15 minutes, immediately before drug administration (time 0, baseline), and at 0.25, 0.50, 0.75, 1, 1.50, 2, 3, 4, 5, and 6 hours after GHB administration using a DinamapTM 8100-T vital signs monitor (Critikon, Tampa, Fla). Pupil diameter was recorded with a Haab pupil gauge.[33]

Psychomotor Performance Measures

The psychomotor performance battery included the digit symbol substitution test (DSST), the Maddox-Wing device, and the balance task. This battery has been previously used in the evaluation of psychostimulants and sedatives.[30,34] The DSST is a subtest of the Wechsler Adult Intelligence Scale–Revised.[35] A computerized version was used, and scores were based on the number of correct patterns keyed in 90 seconds (correct responses). The Maddox-Wing device measures the balance of extraocular muscles and quantifies exophoria as an indicator of extraocular musculature relaxation, and esophoria, as an indicator of extraocular musculature contraction. Results were expressed in diopters along the horizontal scale of the device.[36] The balance task assessed the participant's ability to stand upright with his eyes closed for a maximum of 30 seconds on each foot.[37] Balance was measured for each foot and summed across both feet. The score was the total number of seconds the participant was able to balance (maximum of 60 seconds). If a participant could not perfoem the task, a score of 0 was recorded. The DSST and balance task were perfomed at −15 minutes (immediately before administration) and at 0.50, 1, 1.50, 2, 3, 4, and 6 hours after administration. Measurements with the Maddox-Wing device were performed at −15 minutes and at 0.25, 0.50, 0.75, 1, 1.50, 2, 3, 4, 5, and 6 hours after administration.

Subjective Effects Rating Scales

Subjective effects were measured using the Addiction Research Center Inventory (ARCI), the Evaluation of the Subjective Effects of Substances with Potential of Abuse (VESSPA) questionnaire, and a set of 13 different visual analogue scales (VAS). The ARCI is a true-false questionnaire with empirically derived scales that are sensitive to the effects of a variety of classes of drugs of abuse. The Spanish-validated version of a 49-item short form of ARCI was used.[38] The questionnaire included 5 scales: the pentobarbital-chlorpromazine-alcohol group (PCAG), a measure of sedation; the morphine-benzedrine group (MBG), a measure of euphoria; the LSD group, a measure of dysphoria and somatic symptoms; the benzedrine group (BG), a stimulant scale consisting mainly of items relating to intellectual efficiency and energy; and the amphetamine group (A), an empirically derived scale

TABLE 1. Summary of Significant Drug Effects and Comparisons Between GHB 40 mg/kg, GHB 60 mg/kg, Flunitrazepam, Ethanol, and Placebo on Study Measures

	Tukey Multiple Comparison Test							
	Comparisons of Drug vs Placebo*				Comparisons Between Drugs†			
Dependent Measures	GHB60 vs PL	GHB40 vs PL	ETH vs PL	FLU vs PL	GHB60 vs ETH	GHB60 vs FLU	GHB60 vs GHB40	FLU vs ETH
Physiological								
SBP	NS	NS	NS	NS	NS	GHB > FLU[‡]	NS	NS
DBP	NS	NS	NS	−[1]	NS	GHB > FLU[‡]	NS	NS
Heart rate	NS	NS	+[1]	NS	ETH > GHB[‡]	NS	NS	NS
Oral temperature	NS	NS	NS	−[1]	NS	NS	NS	ETH > FLU[‡]
Pupil diameter	+[1]	+[1]	+[1]	−[1]	GHB > ETH[‡]	GHB > FLU[‡]	NS	ETH > FLU[‡]
VAS								
Stimulated	+[1]	+[1]	+[1]	NS	NS	GHB > FLU[‡]	NS	ETH > FLU[‡]
High	+[1]	+[1]	NS	+[1]	GHB > ETH[‡]	NS	NS	NS
Any effect	+[1]	+[1]	+[1]	+[1]	NS	NS	GHB60 > GHB40[‡]	FLU > ETH[‡]
Good effects	+[1]	+[1]	+[1]	+[1]	NS	NS	NS	NS
Bad effects	+[1]	NS	NS	NS	NS	NS	GHB60 > GHB40[‡]	NS
Liking	+[1]	NS	+[1]	+[1]	NS	NS	NS	NS
Content	+[1]	NS	+[1]	+[1]	NS	NS	NS	NS
Drunkenness	NS	NS	+[1]	NS	ETH > GHB[‡]	NS	NS	ETH > FLU[‡]
Drowsiness	+[1]	NS	NS	+[1]	GHB > ETH[‡]	FLU > GHB[‡]	NS	FLU > ETH[‡]
Depression or sadness	NS	NS	NS	NS	NS	NS	NS	NS
Dizziness	+[1]	NS	NS	+[1]	GHB > ETH[‡]	NS	GHB60 > GHB40[‡]	NS
Confusion	+[1]	NS	NS	+[1]	GHB > ETH[‡]	NS	GHB60 > GHB40[‡]	NS
Relax	+[1]	NS	NS	+[1]	NS	FLU > GHB[‡]	NS	FLU > ETH[‡]
ARCI								
PCAG	+[1]	+[1]	NS	+[1]	GHB > ETH[‡]	FLU > GHB[‡]	NS	FLU > ETH[‡]
MBG	+[1]	NS	+[1]	+[1]	NS	NS	NS	NS
LSD	+[1]	NS	NS	+[1]	GHB > ETH[‡]	NS	GHB60 > GHB40[‡]	FLU > ETH[‡]
MBG	−[1]	NS	NS	−[1]	ETH > GHB[‡]	GHB > FLU[‡]	NS	ETH > FLU[‡]
A	+[1]	+[1]	+[1]	+[1]	NS	NS	NS	ETH > FLU[‡]
VESSPA[§]								
SED	+[1]	+[1]	NS	+[1]	GHB > ETH[‡]	FLU > GHB[‡]	GHB60 > GHB40[‡]	FLU > ETH[‡]
ACT	NS	NS	+[1]	NS	NS	NS	NS	NS
Psychomotor								
DSST total	−[1]	NS	NS	−[1]	ETH > GHB[‡]	GHB > FLU[‡]	GHB40 > GHB60[‡]	ETH > FLU[‡]
DSST correct	−[1]	NS	NS	−[1]	NS	GHB > FLU[‡]	GHB40 > GHB60[‡]	ETH > FLU[‡]
DSST errors		NS		+[1]	NS	FLU > GHB[‡]	NS	FLU > ETH[‡]
Maddox-Wing	+[1]	+[1]	+[1]	+[1]	GHB > ETH[‡]	FLU > GHB[‡]	NS	FLU > ETH[‡]
Balance task	−[1]	−[1]	−[1]	−[1]	ETH > GHB[‡]	GHB > FLU[‡]	GHB40 > GHB60[‡]	ETH > FLU[‡]

*These 4 columns show the results of comparisons of the effects of placebo with GHB 60 mg/kg, GHB 40 mg/kg, flunitrazepam, and ethanol. Symbol (+ or −) indicates significant differences from placebo ($P < 0.05$); symbol (+ or −) also indicates the direction of the drug effect relative to placebo. NS indicates that the drug was not different from placebo.

†These 4 columns show the results of comparisons between drugs. The first 3 columns show comparisons between GHB 60 mg/kg, ethanol, flunitrazepam, and GHB 40 mg/kg. The rightmost column shows the comparison between flunitrazepam and ethanol. The drug to the left of the symbol (>) produced a significantly greater effect. Comparisons between GHB 40 mg/kg and either ethanol or flunitrazepam are not shown. NS indicates that the effects produced by the 2 drugs were not significantly different (Tukey test not significant).

‡Emax comparison.

§AUC comparison.

||VESSPA changes in perception, pleasure and sociability, and psychotic symptoms are not shown because ANOVA was not significant.

ETH indicates ethanol; FLU, flunitrazepam; GHB40, GHB 40 mg/kg; GHB60, GHB 60 mg/kg.

sensitive to the effects of *d*-amphetamine. The ARCI was administered at −15 minutes (immediately before administration) and at 0.50, 1, 1.50, 2, 3, 4, 5, and 6 hours after administration. The VESSPA is an in-house–developed and validated questionnaire specifically created to measure changes in subjective variables caused by 3,4-methylenedioxy methamphetamine (MDMA).[39] It contains 6 scales: sedation (SED), psychosomatic anxiety, changes in perception, pleasure and sociability, activity and energy (ACT), and psychotic symptoms. Each scale consists of 6 questions with a 5-point Likert response (0 to 4 depending on the intensity of the effect). The VESSPA scales were administered at −15 minutes (before administration), and at 1, 2, 3, 4, and 6 hours after administration.

A total of 13 VAS (100 mm) labeled with different adjectives marked at opposite ends with "not at all" and "extremely" were used. Subjects were asked to rate effects of "stimulated," "high (feeling good)," "any effect," "good effects," "bad effects," "liking," "content," "drunkenness," "drowsiness," "dizziness," "confusion," "depression or sadness," and "relaxed." Scales were administered at −15 minutes (before administration) and at 0.25, 0.50, 0.75, 1, 1.50, 2, 3, 4, 5, and 6 hours after administration.

At the end of each study session, subjects filled out a drug class identification questionnaire in which the class of drug they believed had been given (placebo, benzodiazepines, alcohol, designer drugs such as MDMA, amphetaminelike stimulants, cocaine, cannabis, hallucinogens, ketamine, GHB, and others) was indicated.

Analytical Assays

Collection of Blood Samples

Blood was collected at each session to preserve the double-blind masking of the study. Blood was collected before administration and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, and 6 hours after administration in heparinized tubes and immediately centrifuged. All biologic specimens were frozen at −20°C until analysis.

Determination of GHB Plasma Concentrations

γ-Hydroxybutyric acid in plasma was determined by gas chromatography coupled to mass spectrometry using a validated method previously published.[40] Aliquots of 100 μL of plasma were added to 5 μg of GHB-d6 and 200 μL of acetonitrile. After a 30-second vortex and a 5-minute centrifugation at 1000 *g*, 150 μL of the supernatant was transferred to a clean extraction tube and evaporated to dryness under a nitrogen steam. The dried extracts were derivatized with 50 mL of N,O-bis (trimethylsilyl) trifluoroacetamide–1% trimethylchlorosilane (BSTFA with 1% TMCS). A 1-μL aliquot of derivatized samples was injected onto a Hewlett-Packard 6890 gas chromatograph coupled to a Hewlett-Packard 5973 quadrupole mass spectrometer (Agilent, Palo Alto, Calif). The capillary column was a cross-linked 5% phenylmethylsilicone. The mass spectrometer was operated in the electron ionization and selected ion monitoring acquisition mode. The following ions were monitored: GHB-bis-TMS: m/z 233, 204, 117; GHB-d6-bis-TMS: m/z 239.

Determination of Ethanol Concentrations in Biologic Fluids

Ethanol determination in total blood was performed using a validated method previously published.[41] Blood (1 mL) was added to an 8-mL vial containing 1 mL of Milli-Q water and 243 ng of *n*-butanol as internal standard. A gas chromatograph (HP 5890; Hewlett-Packard, Palo Alto, Calif) fitted with a headspace injector HP 19395A and equipped with a flame ionization detector was used for ethanol quantification in blood. Analyses were performed in a cross-linked polyethylene glycol capillary

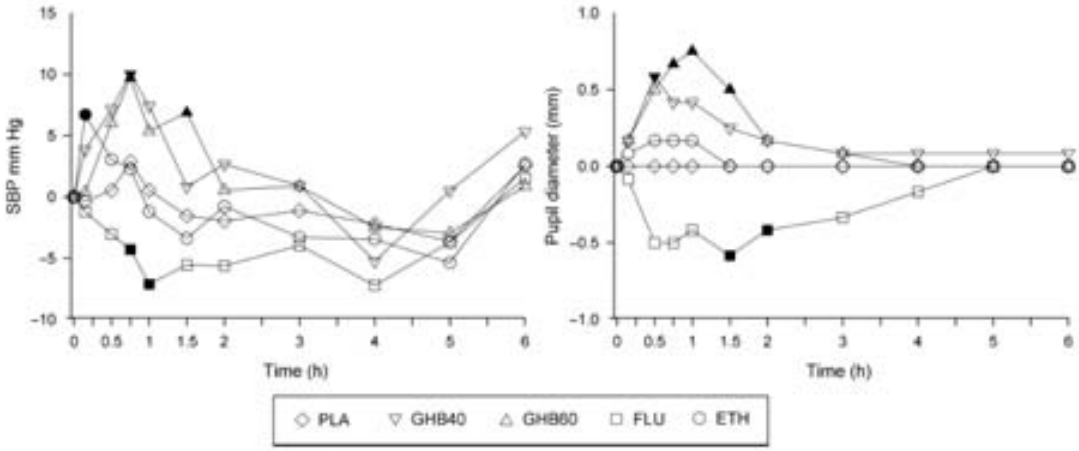

FIGURE 1. Time course of drug effects on SBP and pupil diameter (differences from baseline). Data points represent mean values from 12 subjects. Filled symbols indicate a significant difference from placebo ($P < 0.05$).

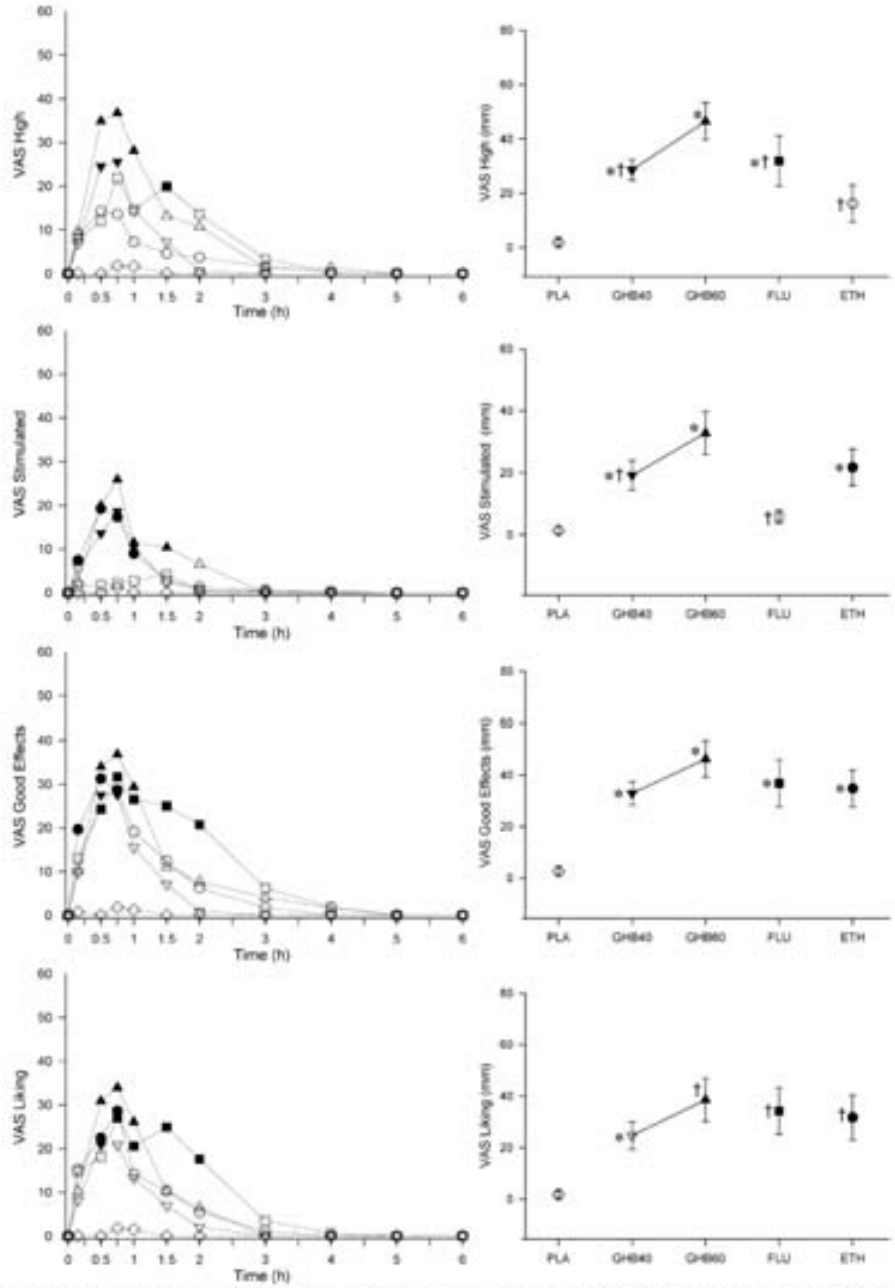

FIGURE 2. Time course of drug effects (left column) and peak drug effects (right column) on VAS "high," "stimulated," "good effects," and "liking" (differences from baseline). Data points represent mean values ± SE from 12 subjects. Filled symbols indicate a significant difference from placebo ($P < 0.05$). * and † indicate comparisons among the 4 active conditions; within the same panel, any 2 means designated with the same letter are not significantly different from each other at $P < 0.05$ (Tukey post hoc test).

column (15 m × 0.33 mm × 1 μm) (HP-INNOWax; Hewlett-Packard).

Determination of Flunitrazepam Plasma Concentrations

Plasma concentrations of flunitrazepam were determined by capillary gas chromatography with electron capture detector and flurazepam as the internal standard, using a validated method previously published.[31] Samples were extracted in solid-phase cationic exchange and hydrophobic interaction columns (Bond Elut Certify, Harbor City, Mich) at pH 6. Extracts were injected in a gas chromatographic system (HP 5890A Series II, Hewlett-Packard) fitted with an automatic injector (HP 7673A) and coupled to an electron capture detector using as emission source Ni^{63}.

Data Analysis: Statistical Methods

The peak effect in the first 3 hours, that is, the maximum absolute change from baseline values (Emax) and the 3 hours' area under the curve (AUC) of effects versus time calculated by the trapezoidal rule were determined for each variable. These transformations were analyzed by 1-way repeated-measures analysis of variance (ANOVA) with drug conditions as factor. When ANOVA results showed significant differences between treatment conditions, post hoc multiple comparisons were performed using the Tukey test for repeated measures. Time course of effects was compared using 2-way repeated-measures ANOVA with treatment condition and time (0–6 hours) as factors. Whenever treatment condition or the treatment condition × time interaction was statistically significant, multiple Tukey post hoc comparisons for repeated measures were performed at each time point using the mean square error term of the treatment condition × time interaction.

Pharmacokinetic Parameters

The following parameters were determined from GHB and flunitrazepam plasma concentrations and ethanol total blood concentrations over time: peak concentration (C_{max}), time to reach peak concentration (t_{max}), and area under the concentration-time curve from 0 to 6 hours (AUC_{0-6}). The estimated elimination half-life ($t_{1/2}$) was also calculated from GHB plasma concentrations.

The Wilcoxon test was used for statistical analysis. Differences associated with $P < 0.05$ were considered to be statistically significant. Pharmacokinetic parameters were obtained with use of specific functions of computer program (PK Functions for Microsoft Excel, Microsoft Corporation).

Pharmacokinetic/Pharmacodynamic Relationship

The within-subject correlations of different VAS scales with both plasma concentrations of GHB 60 mg/kg and flunitrazepam and blood concentrations of ethanol were calculated using a multiple regression approach.[42] Within-subject correlation indicates whether high values of 1 variable are associated with high values of another within a subject.

RESULTS

A summary of results for pharmacological effects showing statistical significant differences between treatments is presented in Table 1.

Pharmacological Effects

Physiological Effects

γ-Hydroxybutyric acid produced a slight nonsignificant increase in SBP and DBP for both doses tested. γ-Hydroxybutyric acid was capable of inducing an increase in SBP 45 minutes and 1.5 hours after administration in the time course analysis with a return to baseline at 3 hours after administration (Fig. 1). Flunitrazepam significantly decreased DBP, whereas ethanol significantly increased heart rate. No apparent changes in heart rate were observed after GHB and flunitrazepam administration. Flunitrazepam produced a significant reduction in oral temperature (−0.66°C, Emax value) that lasted for 4 hours. Both GHB doses (40–60 mg/kg) produced significant pupil diameter increases (0.67 and 0.83 mm, respectively). In contrast, flunitrazepam produced a significant reduction in pupil diameter (Fig. 1). With regard to the time course, GHB increased pupil diameter between 30 minutes and 1.5 hours after administration.

Subjective Effects

All active conditions produced changes in subjective effects as measured by questionnaires and VAS scales (Fig. 2). Overall, the subjective effects of GHB reached their maximum between 45 minutes and 1.5 hours and returned to baseline about 3 hours after drug administration.

γ-Hydroxybutyric acid, flunitrazepam, and ethanol induced pleasurable-related effects compared with placebo. The administration of GHB significantly increased all subjective measures related to euphoria and pleasurable effects (eg, VAS-high, VAS-liking, ARCI-MBG). The GHB 60-mg/kg dose produced slightly (nonsignificant) superior ratings than flunitrazepam and ethanol in VAS dealing with pleasurable effects such as "high," "good effects," "liking," and "content" (Fig. 2). Nevertheless, the higher dose of GHB also induced mild changes in VAS "bad effects," "dizziness," and "confusion," and ARCI-LSD (dysphoria), all related to unpleasant effects. Flunitrazepam also significantly increased VAS "dizziness" and "confusion," and ARCI-LSD (dysphoria). No differences were observed between ethanol and placebo in these variables. γ-Hydroxybutyric acid 60 mg/kg, flunitrazepam, and ethanol were different from placebo (and not different from each other) for the key measure of abuse potential, ARCI-MBG.

γ-Hydroxybutyric acid 60 mg/kg and flunitrazepam, but not ethanol, decreased ARCI-BG scores, a scale related to intellectual efficiency and energy, in comparison with placebo. Flunitrazepam induced more pronounced and long-lasting effects than GHB.

All active conditions produced slight stimulantlike effects (ARCI-A Scale) significantly different from placebo. Both GHB doses and ethanol also significantly increased VAS "stimulated" when compared with placebo and flunitrazepam.

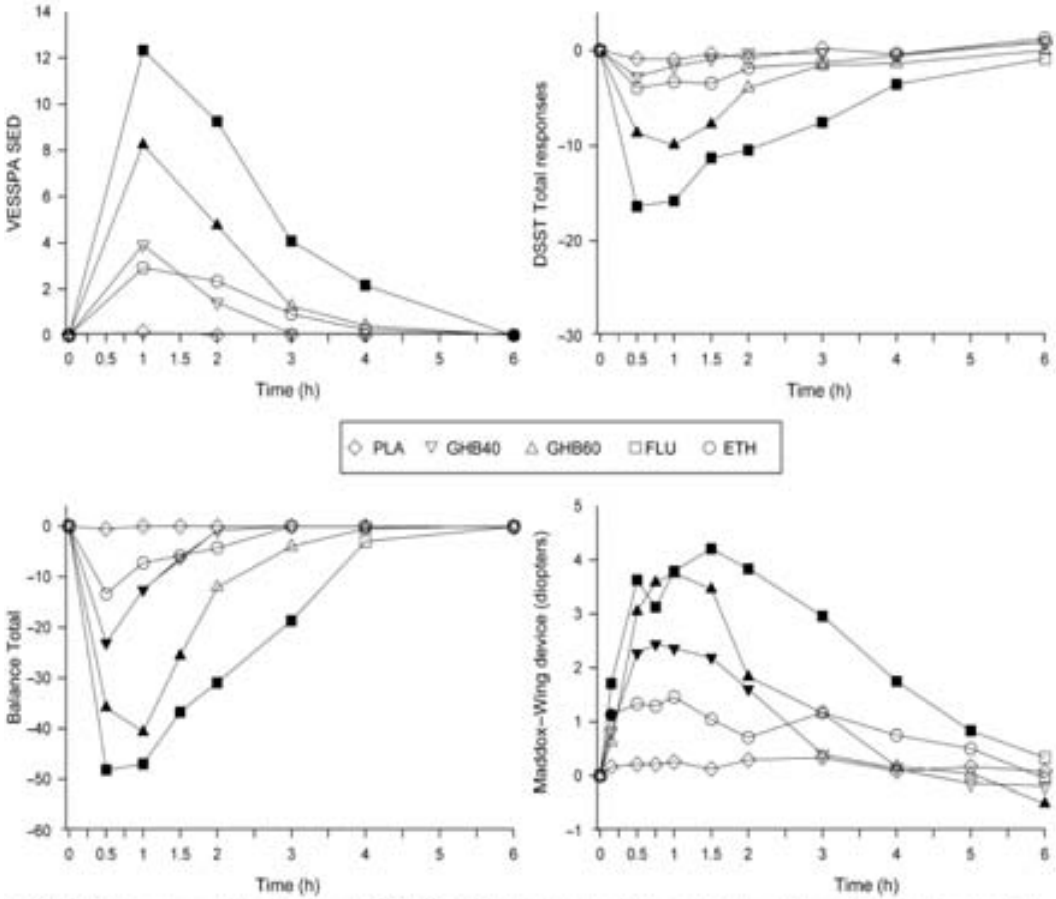

FIGURE 3. Time course of drug effects on VESSPA SED, DSST total, balance task, and Maddox-Wing (differences from baseline). Data points represent means from 12 subjects. Filled symbols indicate a significant difference from placebo ($P < 0.05$).

Ethanol was also able to significantly increase scores in VESSPA-ACT (also related to activity and energy) when compared with placebo. In the temporal course analysis, GHB stimulantlike effects followed a dose-dependent trend (but were not statistically significant), peaked at 45 minutes after administration and lasted 1.5 hours.

γ-Hydroxybutyric acid produced a mixed sedative-stimulant effect pattern. Stimulantlike effects preceded sedativelike ones. γ-Hydroxybutyric acid and flunitrazepam produced objective (assessed by observers as subjects falling asleep) and subjective sedative significant effects as reflected in VAS "drowsiness," ARCI-PCAG, and VESSPA SED scales. Regarding time course, GHB-related sedative effects peaked between 1.5 and 2 hours after administration and lasted for 3 hours. Those elicited by flunitrazepam peaked at 1 hour, lasted 4 hours, and were significantly higher than those corresponding to GHB.

Ethanol did not produce objective sedative effects, although it produced a significant drunkenness, as measured by VAS "drunkenness."

Psychomotor Performance

Results of psychomotor performance tests after administration of drug conditions are shown in Table 1 and Figure 3. γ-Hydroxybutyric acid and flunitrazepam produced a significant deterioration of psychomotor performance. In general terms, GHB effects peaked at 1 hour and lasted for 2 hours, whereas flunitrazepam effects peaked at 30 minutes and lasted for 4 hours.

In the DSST task, GHB 60 mg/kg and flunitrazepam produced a significant decrease in the number of total and correct responses compared with placebo and also to ethanol and GHB 40 mg/kg. In addition, flunitrazepam significantly increased the number of errors. γ-Hydroxybutyric acid 40 mg/kg

TABLE 2. Experimental Pharmacokinetic Parameters of GHB and Flunitrazepam in Plasma and Ethanol in Total Blood

Parameter	GHB 40 mg/kg	GHB 60 mg/kg	Flunitrazepam	Ethanol
C_{max}	111.0 ± 37.4*	166.9 ± 48.4*	14.5 ± 13.7†	994.2 ± 213.7*
t_{max} (h)‡	0.75	0.75	0.25	0.75
AUC_{0-6}	200.5 ± 62.0§	347.8 ± 105.7§	37.4 ± 9.0§	2860.8 ± 445.3§
$t_{1/2}$ (h)	0.6 ± 0.2	0.8 ± 0.5		

Values are expressed as mean ± SD.
*μg/mL, †ng/mL, ‡median, §μg/mL. h, ||ng/mL. h.

and ethanol also decreased (not significantly) the number of total and correct responses of DSST. Overall, flunitrazepam induced a significantly lower performance in the DSST task when compared with both GHB doses and ethanol.

All active conditions were associated to an impairment of the balance task, being flunitrazepam the most active treatment. γ-Hydroxybutyric acid 60 mg/kg and flunitrazepam induced a significant poorer performance than either GHB 40 mg/kg or ethanol.

In the time course of effects, the peak effect was seen at 30 minutes after administration for all active conditions. γ-Hydroxybutyric acid 60 mg/kg significantly impaired psychomotor performance from 0.5 to 1.5 hours after drug administration. The impairment produced by flunitrazepam in the DSST and in the balance task lasted longer than those induced by GHB and ethanol.

All active conditions induced a significant exophoria, as measured by the Maddox-Wing device. γ-Hydroxybutyric acid produced a dose-dependent increase in diopters. Along the time course, GHB produced a peak effect at 1 hour after administration that lasted 4 hours after administration. Flunitrazepam produced the highest degree of exophoria lasting 5 hours after administration.

In the pharmacological class identification questionnaire, the administration of GHB was considered as GHB in all choices. Ethanol was identified as placebo (1 of 12 possible identifications) and as GHB (1/12). Placebo was considered placebo. Flunitrazepam was identified as a benzodiazepine.

None of the participants required specific therapy or special care during the study, and serious adverse events were not observed. Altogether, 2 volunteers presented vomiting and 1 diaphoresis after the administration of GHB 60-mg/kg dose, whereas only 1 subject vomited after the administration of the lower dose. Three subjects also presented with mild headache after GHB 40 mg/kg, ethanol, and placebo, respectively.

Two subjects experiencing these adverse events after the administration of GHB 60-mg/kg dose translated these as mild undesirable negative effects. The first presented the following symptoms: dizziness, nausea and vomiting, horizontal nystagmus, increased somnolence, and a decreased level of alertness from 1 hour to 3 hours after drug administration. The second presented diaphoresis and felt sick to the stomach. Although they both fully improved from these symptoms, these subjects rated near zero for most of the scales and questionnaires related to abuse potential of the study during the corresponding sessions.

No hallucinations, psychotic episodes, or any other psychiatric symptoms was observed during the experimental sessions.

Concentration-Time Profiles of GHB and Flunitrazepam in Plasma

Pharmacokinetic parameters of GHB after oral doses of 40 and 60 mg/kg in plasma and those of flunitrazepam after an oral dose of 1.25 mg are presented in Table 2. Mean concentration-time curves in plasma of both GHB doses tested and those of flunitrazepam are presented in Figure 4.

γ-Hydroxybutyric acid was readily absorbed after oral administration and rapidly eliminated with a significant interindividual variability. γ-Hydroxybutyric acid concentrations peaked between 30 and 90 minutes after administration (C_{max} range, 67.4–197.3 μg/mL for 40 mg/kg and 102.2–277.7 μg/mL for 60 mg/kg). After the absorption phase, concentrations declined to mean values of 1.4 (40 mg/kg) and 2.3 (60 mg/kg) μg/mL at 6 hours. γ-Hydroxybutyric acid 60 mg/kg average C_{max} ($P < 0.0001$), AUC_{0-6} ($P < 0.0001$), and $t_{1/2}$ ($P < 0.05$) values were significantly higher than those observed for GHB 40 mg/kg, although no significant differences were found for T_{max} values. γ-Hydroxybutyric acid normalized (1 mg/kg) AUC values were higher for 60 mg/kg (5.8) when compared with the 40 mg/kg dose (5.0). Normalized C_{max} values were similar for both doses tested.

Flunitrazepam was rapidly absorbed with concentrations peaking between 15 and 90 minutes after administration (C_{max} range, 8.78–17.42 ng/mL). After the absorption phase, concentrations declined to a mean value of 3.7 ng/mL at 6 hours.

Concentration-Time Profiles of Ethanol in Total Blood

Pharmacokinetic parameters of ethanol in total blood after an oral dose of 0.7 g/kg are presented in Table 2. Mean concentration-time curves of ethanol in total blood are presented in Figure 4. Ethanol was rapidly absorbed with concentrations peaking between 15 and 90 minutes after administration (C_{max} range, 595.2–1301.0 μg/mL). After the bsorption phase, concentrations declined to a mean value of 74 μg/mL at 6 hours.

Pharmacokinetic/Pharmacodynamic Relationship

γ-Hydroxybutyric acid plasma concentrations and ethanol blood concentrations were well correlated to subjective effects related with stimulation ($r = 0.54$ for

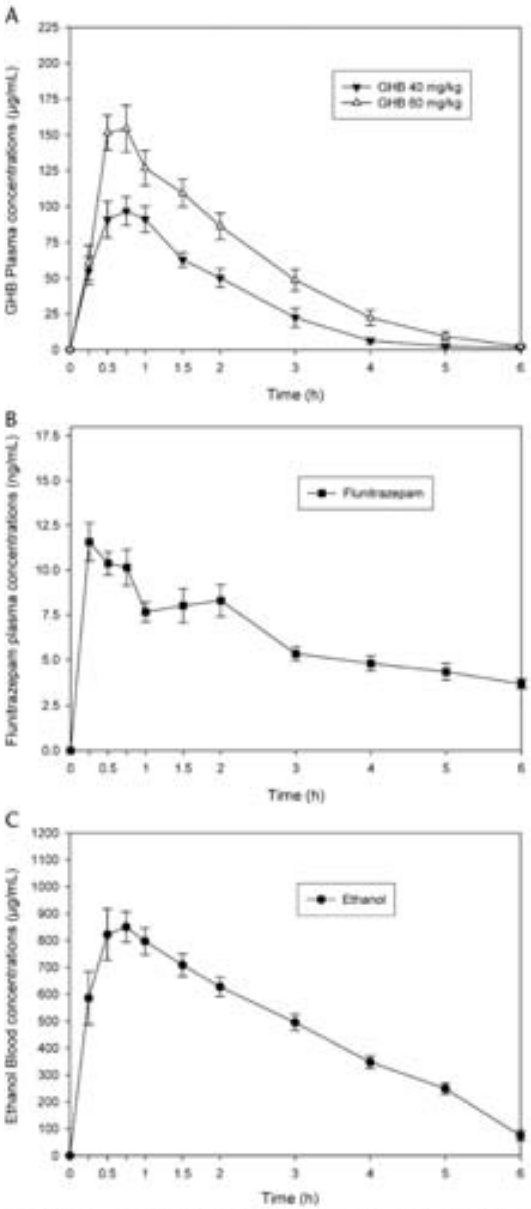

FIGURE 4. A, Time course of GHB plasma concentrations after GHB 40 and 60 mg/kg doses. B, Time course of flunitrazepam plasma concentrations after a dose of 1.25 mg. C, Time course of ethanol blood concentrations after a dose of 0.7 g/kg (data points represent means ± SE from 12 subjects).

GHB 60 mg/kg and $r = 0.51$ for ethanol, $P < 0.001$), whereas they were not correlated to sedative effects ($r = 0.36$ for GHB 60 mg/kg and $r = 0.35$ for ethanol, $P < 0.001$) (Fig. 5). On the contrary, flunitrazepam plasma concentrations are better correlated to sedativelike effects ($r = 0.52$, $P < 0.001$), whereas they are poorly correlated to stimulant effects ($r = 0.27$, $P = 0.013$).

γ-Hydroxybutyric acid plasma concentrations were also well correlated to different VAS related to abuse potential ("liking," $r = 0.61$; "good effects," $r = 0.61$; and "high," $r = 0.66$; $P < 0.0001$). Good correlations were also found for ethanol blood concentrations and different VAS scales related with abuse potential ("good effects," $r = 0.64$; VAS "liking," $r = 0.57$; $P < 0.0001$) and with VAS "drunkenness" ($r = 0.59$, $P < 0.0001$). In the case of flunitrazepam, VAS scales were poorly correlated with

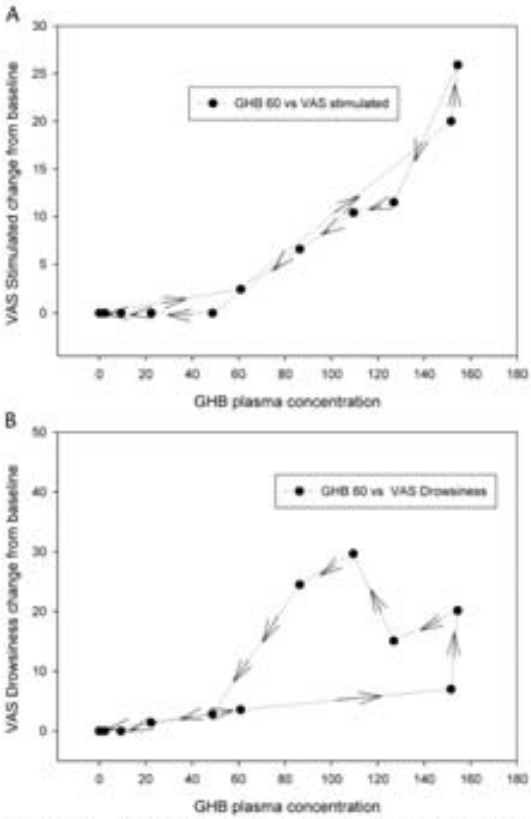

FIGURE 5. Pharmacokinetic-pharmacodynamic relationship between GHB plasma concentrations after a 60-mg/kg dose and simultaneous mean VAS "stimulated" and VAS "drowsiness" scores. Arrows indicate the direction of increasing time. Data points represent means from 12 subjects.

plasma concentrations, except for VAS "drowsiness" and "relax" ($r = 0.51$, $P < 0.0001$).

DISCUSSION

To our knowledge, this is the first controlled study comparing the profiles of sedativelike club drugs in recreational club drug users. The main finding of this study is that GHB induced euphoria, well being, pleasurable effects, and some stimulantlike effects. These effects are similar to those reported by GHB abusers[8,9] and are on the basis for its misuse at dance clubs and raves. These effects were close to those induced by flunitrazepam and ethanol, drugs also largely consumed within the club/rave culture. Although there were no significant differences, GHB tended to produce higher euphoric and pleasurable effects than those elicited by the other active treatments evaluated.

Abuse Liability and Subjective Effects

All active conditions induced positive effects related to their abuse potential with substantial similarities in subjective effects elicited. The administration of GHB significantly increased all subjective measures related to euphoria and pleasurable effects with slightly superior ratings to those observed for flunitrazepam and ethanol in some scales dealing with pleasurable effects. However, GHB produced also greater unpleasant effects than ethanol (bad effects, dizziness, or confusion). Furthermore, the highest dose of GHB produced a significant increase of unwanted side effects such as nausea and vomiting in some subjects. Nevertheless, it is important to notice that the GHB-induced adverse effects were not observed in all study participants (2/12). As stated before, participants who developed unwanted side effects rated close to zero scores in scales related to abuse potential. In contrast, participants who experienced positive drug effects had a good experience free of adverse effects. In other words, overall, we did not find within-subject coincidence of positive and negative GHB-related effects. These findings are in accordance with (1) a previous study in sedative abusers, in which under controlled conditions, substantial differences in sensitivity to the adverse effects of GHB were found,[19] (2) reports of GHB abuse outside of the laboratory setting in club drug users,[7,8] and (3) well-documented GHB intoxication and overdose cases.[43–45]

As a corollary of these findings, it can be speculated that GHB abuse potential could even be higher in users who do not usually experience bad effects.[46] On the contrary, it is possible that the unpleasant effects of GHB might limit its abuse potential in subjects who are sensitive to the adverse effects (nausea and vomiting) and, in particular, those who experience loss of consciousness secondary to its administration.[46] Even taking into account this variability, our results suggest a high abuse liability of GHB in participants with prior GHB use in the range of doses usually abused.

The profile of effects induced by flunitrazepam was consistent with the results of previous studies and supports the hypothesis that it is capable of inducing pleasurable feelings related to its abuse potential.[30,31,47] To our knowledge, this is the first controlled study providing evidence of its abuse potential in club drug users, a population claimed to be increasingly abusing this drug.[29] Flunitrazepam induced some undesirable effects also partially related to its sedative effects. This drug significantly increased dysphoria, "dizziness," and "confusion" compared with placebo. These effects might also limit its abuse potential in this population and are in line with previous reports.[30,31]

The ethanol dose (0.7 g/kg) tested was chosen to induce stimulantlike effects,[32] and this was reflected in subjective effects measured in the present study. Ethanol significantly increased VAS "liking," "content," and "good effects," and produced a mild drunkenness, also mediating significant stimulantlike effects. All of them are related to its abuse potential and similar to previous reports where analogous doses were given.[34,48–50]

To simulate GHB use at dance clubs and raves, doses were selected taking into account the results of a pilot study. The low dose (40 mg/kg) induced perceptible subjective effects, whereas the high dose (60 mg/kg) induced euphoria without significant somnolence requiring stimuli for arousal.[26] Doses of flunitrazepam and ethanol were chosen to minimize their sedative effects. Interestingly, GHB, ethanol, and flunitrazepam, although to different degrees, were able to induce slight stimulation. On the other hand, GHB and flunitrazepam also produced objective and subjective sedative effects. Flunitrazepam produced a more prolonged sedative effect in accordance with previous studies where similar doses were administered.[30,31] Ethanol did not produce objective sedative effects, although it produced significant drunkenness. This effect could be related to the dose studied and is in agreement with the mild ethanol impact on psychomotor performance seen in this study. Therefore, although with different effects, the 3 study drugs showed a mixed sedative-stimulant pattern as stimulantlike effects preceded sedativelike ones. This biphasic time profile has been previously described for ethanol and some other sedatives.[26,51,52] In the case of GHB, the stimulantlike effects peaked at 45 minutes after administration. Such profile of effects is consistent with the results from a pilot study[26] but differs from those reported in previous studies testing lower GHB doses.[23,24]

Psychomotor Performance

γ-Hydroxybutyric acid and flunitrazepam impaired psychomotor performance, including DSST and balance tasks, whereas ethanol induced only mild effects exclusively on the balance task. γ-Hydroxybutyric acid effects were dose dependent and peaked 1 hour after its administration. These effects are in agreement with the results of the pilot study[26] and also to those reported in a previous study performed in sedative drug abusers administered with similar GHB doses (4 g).[19] Effects produced by flunitrazepam were significantly more pronounced and long-lasting, in agreement with its pharmacological profile.

Ethanol was not capable of inducing a significant deterioration in psychomotor performance at the dose tested. These findings are in line with previous reports where similar doses were given.[48–50] Similar observations concerning ethanol/sedative differences were reported in a study

where ethanol and diazepam produced comparable subjective effects, but diazepam produced a greater degree of psychomotor impairment.[53]

All active conditions induced significant exophoria as measured by the Maddox-Wing device. This task is a direct measure of the extraocular musculature relaxation and an indirect marker of central sedation. These findings are in accordance with results of previous studies.[26,31,34]

Physiological Effects

Regarding physiological effects, GHB administration (both doses) produced a slight significant (time course) rise in SBP lasting for 1 to 2 hours after administration. This finding is in line with results of the pilot study.[26] Early reports pointed out the possibility of GHB central noradrenaline–mediated cardiovascular effects.[54] Recent results suggest also that GHB has sympathomimetic cardiovascular effects that could induce increases in blood pressure after its immediate administration.[55]

γ-Hydroxybutyric acid induced slight mydriasis between 30 minutes and 1 hour after administration. This finding is in accordance with the results of the pilot study and in agreement with observations made in acute intoxication cases where GHB is the only drug involved.[5,56,57] In contrast, flunitrazepam produced a significant reduction in pupil diameter and oral temperature in line with previous reports where similar doses were given.[30]

Pharmacokinetics

Given by the oral route, GHB is rapidly absorbed and eliminated (t_{max} and $t_{1/2}$ for both doses) and displays a highly variable bioavailability. Experimental pharmacokinetic parameters C_{max} and AUC (Table 2) from the present study are in agreement with the pilot study and with those obtained in healthy subjects[58] administered with 4.5 g of oxybate (equivalent to 50–60 mg/kg of GHB) and lower to those observed in severe acute intoxications.[59] γ-Hydroxybutyric acid elimination seems to be capacity-limited at the higher dose as it has been observed in some narcoleptic patients administered a fixed dose of 3 g twice nightly at 4-hour intervals.[60] At the lower GHB dose tested, this phenomenon is not observed, in agreement with previous reports.[58,61] The accumulation of GHB in the body as a result of its nonlinear disposition might have some implications in the susceptibility of some subjects to develop acute intoxications.

Ethanol also presented a highly variable bioavailability. Ethanol pharmacokinetics was in line with previous reports when similar doses were given.[34]

Flunitrazepam plasma concentrations were in agreement with previous studies where similar doses were given in terms of C_{max} and AUC values.[31] Nonetheless, we found a shorter t_{max} indicating faster absorption probably caused by its administration in a liquid form instead of the usual tablet preparations. This approach allowed us to synchronize the peak effects of flunitrazepam, GHB, and ethanol.

Pharmacokinetics/Pharmacodynamics

γ-Hydroxybutyric acid induced a biphasic time profile with an initial stimulantlike, euphoric, and pleasurable effect related to the simultaneous rise of plasma drug concentrations and ulterior sedative effect collateral to a decrease in GHB plasma concentrations (Fig. 5). Ethanol also produced stimulantlike effects related to its increase in blood concentrations, as previously published. On the contrary, flunitrazepam plasma concentrations were only well correlated to its sedativelike effects.

Regarding a possible relationship between GHB kinetics and sensitivity to undesirable effects, unusual GHB plasma concentrations were not found in subjects presenting GHB-mediated adverse effects. In addition, participants who tended to score higher in scales related to abuse potential did not display higher GHB plasma concentrations. Based on these findings and previous research, it could be argued that sensitivity to GHB effects probably relies on variations in neurotransmitters and receptors involved in GHB actions, rather than on differences in GHB plasma concentrations.[62]

Interestingly, results from this study point out that GHB is capable of inducing euphoria and pleasurable effects and inducing slight stimulantlike effects as previously reported by club attendees.[8,9,22] This population frequently refers to GHB as "liquid ecstasy" in resemblance to MDMA effects. Thus, at the doses tested, GHB produced slight increases in pupil diameter, SBP, VAS "stimulated," and ARCI-A scale, similar effects to those elicited by MDMA.[34] At higher doses, sedative effects would predominate, leading to the observed effects in acute intoxications. γ-Hydroxybutyric acid differs from other sedatives in that sedation does not progress in a dose-dependent manner.[19,26] There is a very narrow time window between being awake and presenting a level of sedation (greater than intended) that may result in a medical intervention. Cases of unintended GHB overdose can appear in club drug abusers who consume large amounts of GHB or combine it with psychostimulants[8,45,46] in pursuit of an augmented level of subjective effects.

Our study provides evidence of the abuse potential of sedativelike drugs among club drug users. γ-Hydroxybutyric acid, flunitrazepam, and ethanol display a high abuse liability in this population at the doses tested.

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REFERENCES

1. Wong GT, Gibson KM, Snead OC 3rd. From street to the brain: neurobiology of the recreational drug gamma-hydroxybutyric acid. *Trends Pharmacol Sci*. 2004;25:29–34.
2. Laborit H. Sodium 4-hydroxybutyrate. *Int J Neuropharmacol*. 1964;3:433–451.
3. Caputo F, Addolorato G, Lorenzini F, et al. Gamma-hydroxybutyric acid versus naltrexone in maintaining alcohol abstinence: an open randomized comparative study. *Drug Alcohol Depend*. 2003;70:85–91.
4. Black J, Houghton WC. Sodium oxybate improves excessive daytime sleepiness in narcolepsy. *Sleep*. 2006;29:939–946.

5. Dyer JE. Gamma-hydroxybutyrate: a health-food product producing coma and seizurelike activity. *Am J Emerg Med*. 1991;9:321–324.
6. Chin RL, Sporer KA, Cullison B, et al. Clinical course of gamma-hydroxybutyrate overdose. *Ann Emerg Med*. 1998;31:716–722.
7. Varela M, Nogue S, Oros M, et al. Gamma hydroxybutyrate use for sexual assault. *Emerg Med J*. 2004;21:255–256.
8. Miotto K, Darakjian J, Basch J, et al. Gamma-hydroxybutyric acid: patterns of use, effects and withdrawal. *Am J Addict*. 2001;10: 232–241.
9. Degenhardt L, Darke S, Dillon P. GHB use among Australians: characteristics, use patterns and associated harm. *Drug Alcohol Depend*. 2002;67:89–94.
10. Gonzalez A, Nutt DJ. Gamma hydroxy butyrate abuse and dependency. *J Psychopharmacol*. 2005;19:195–204.
11. Beardsley PM, Balster RL, Harris LS. Evaluation of the discriminative stimulus and reinforcing effects of gammahydroxybutyrate (GHB). *Psychopharmacology*. 1996;127:315–322.
12. Winter JC. The stimulus properties of gamma-hydroxybutyrate. *Psychopharmacology*. 1981;73:372–375.
13. Woolverton WL, Rowlett JK, Winger G, et al. Evaluation of the reinforcing and discriminative stimulus effects of gamma-hydroxybutyrate in rhesus monkeys. *Drug Alcohol Depend*. 1999;54:137–143.
14. Lobina C, Agabio R, Reali R, et al. Contribution of GABA-A and GABA-B receptors to the discriminative stimulus produced by gamma-hydroxybutyric acid. *Pharmacol Biochem Behav*. 1999;64:363–365.
15. Martellotta MC, Fattore L, Cossu G, et al. Rewarding properties of gamma-hydroxybutyric acid: an evaluation through place preference paradigm. *Psychopharmacology*. 1997;132:1–5.
16. Colombo G, Agabio R, Balakhevskaia N, et al. Oral self-administration of gammahydroxybutyric acid in the rat. *Eur J Pharmacol*. 1995;285: 103–107.
17. Colombo G, Agabio R, Diaz G, et al. Gamma-hydroxybutyric acid intake in ethanol- preferring sP and -nonpreferring sNP rats. *Physiol Behav*. 1998;64:197–202.
18. Martellotta MC, Cossu G, Fattore L, et al. Intravenous self-administration of gamma-hydroxybutyric acid in drug-naive mice. *Eur Neuropsychopharmacol*. 1998;8:293–296.
19. Carter LP, Richards BD, Mintzer MZ, et al. Relative abuse liability of GHB in humans: a comparison of psychomotor, subjective, and cognitive effects of supratherapeutic doses of triazolam, pentobarbital, and GHB. *Neuropsychopharmacology*. 2006;31:2537–2551.
20. NIDA InfoFacts: Rohypnol and GHB. Available at: http://www.drugabuse.gov/infofacts/RohypnolGHB.html. Accessed June 15, 2007.
21. Abanades S, Peiro AM, Farre M. Club drugs: old medicines as new party drugs. *Med Clin (Barc)*. 2004;123:305–311.
22. Parks KA, Kennedy CL. Club drugs: reasons for and consequences of use. *J Psychoactive Drugs*. 2004;36:295–302.
23. Rosen MI, Pearsall HR, Woods SW, et al. Effects of gamma-hydroxybutyric acid (GHB) in opioid-dependent patients. *J Subst Abuse Treat*. 1997;14:149–154.
24. Ferrara SD, Giorgetti R, Zancaner S, et al. Effects of single dose of gamma-hydroxybutyric acid and lorazepam on psychomotor performance and subjective feelings in healthy volunteers. *Eur J Clin Pharmacol*. 1999;54:821–827.
25. Thai D, Dyer JE, Benowitz NL, et al. Gamma-hydroxybutyrate and ethanol effects and interactions in humans. *J Clin Psychopharmacol*. 2006;26:524–529.
26. Abanades S, Farre M, Segura M, et al. Gamma-hydroxybutyrate (GHB) in humans: pharmacodynamics and pharmacokinetics. *Ann N Y Acad Sci*. 2006;1074:559–576.
27. Degenhardt L, Copeland J, Dillon P. Recent trends in the use of "club drugs": an Australian review. *Subst Use Misuse*. 2005;40:1241–1256.
28. Griffiths RR, Bigelow GE, Ator NA. Principles of initial experimental drug abuse liability assessment in humans. *Drug Alcohol Depend*. 2003;70(3 suppl):S41–S54.
29. Wu LT, Schlenger WE, Galvin DM. Concurrent use of methamphetamine, MDMA, LSD, ketamine, GHB, and flunitrazepam among American youths. *Drug Alcohol Depend*. 2006;84:102–113.
30. Farre M, Teran MT, Cami J. A comparison of the acute behavioral effects of flunitrazepam and triazolam in healthy volunteers. *Psychopharmacology (Berl)*. 1996;125:1–12.
31. Roset PN, Farre M, de la Torre R, et al. Modulation of rate of onset and intensity of drug effects reduces abuse potential in healthy males. *Drug Alcohol Depend*. 2001;64:285–298.
32. Davidson D, Hutchison K, Dagon C, et al. Assessing the stimulant effects of alcohol in humans. *Pharmacol Biochem Behav*. 2002;72: 151–156.
33. Pickworth WB, Fant RV, Bunker EB. Effects of abused drugs on papillary size and the light reflex. In: Karch SB, ed. *Drug Abuse Handbook*. Boca Raton, FL: CRC Press; 1998:266–275.
34. Hernandez-Lopez C, Farre M, Roset PN, et al. 3,4-Methylenedioxymethamphetamine (ecstasy) and alcohol interactions in humans: psychomotor performance, subjective effects, and pharmacokinetics. *J Pharmacol Exp Ther*. 2002;300:236–244.
35. Wechsler D. *The Measurement and Appraisal of Adult Intelligence*. Baltimore: Williams and Wilkins; 1958.
36. Hannington-Kiff JG. Measurement of recovery from out patient general anesthesia with a simple ocular test. *BMJ*. 1970;3:132–135.
37. Evans SM, Troisi JR II, Griffiths RR. Tandospirone and alprazolam: comparison of behavioral effects and abuse liability in humans. *J Pharmacol Exp Ther*. 1994;271:683–694.
38. Lamas X, Farré M, Llorente M, et al. Spanish version of the 49-item short version of the Addiction Research Center Inventory (ARCI). *Drug Alcohol Depend*. 1994;35:203–209.
39. Poudevida S, Farré M, Roset PN, et al. Construcción de un cuestionario para la Valoración de los Efectos Subjetivos de Sustancias con Potencial de Abuso (VESSPA): Evaluación del éxtasis. *Adicciones*. 2003;15: 115–126.
40. Abanades S, Farré M, Segura M, et al. Disposition of GHB in conventional and non-conventional biological fluids after a single drug administration: issues in methodology and drug monitoring. *Ther Drug Monit*. 2007;29:64–70.
41. Farre M, de la Torre R, Llorente M, et al. Alcohol and cocaine interactions in humans. *J Pharmacol Exp Ther*. 1993;266:1364–1373.
42. Bland JM, Altman DG. Calculating correlation coefficients with repeated observations: Part 1—correlation within subjects. *BMJ*. 1995;310:446.
43. Miro O, Nogue S, Espinosa G, et al. Trends in illicit drug emergencies: the emerging role of gamma-hydroxybutyrate. *J Toxicol Clin Toxicol*. 2002;40:129–135.
44. Abanades S, Iglesias ML, Echarte JL, et al. Gammahydroxibutyrate: a novel toxicological emergency. *Meth Find Clin Exp Pharmacol*. 2001;23:326.
45. Liechti ME, Kupferschmidt H. Gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL): analysis of overdose cases reported to the Swiss Toxicological Information Centre. *Swiss Med Wkly*. 2004;134: 534–537.
46. Degenhardt L, Darke S, Dillon P. The prevalence and correlates of gamma-hydroxybutyrate (GHB) overdose among Australian users. *Addiction*. 2003;98:199–204.
47. Mintzer MZ, Griffiths RR. An abuse liability comparison of flunitrazepam and triazolam in sedative drug abusers. *Behav Pharmacol*. 2005; 16:579–584.
48. Roache JD, Cherek DR, Bennett RH, et al. Differential effects of triazolam and ethanol on awareness, memory, and psychomotor performance. *J Clin Psychopharmacol*. 1993;13:3–15.
49. Farre M, de la Torre R, Gonzalez ML, et al. Cocaine and alcohol interactions in humans: neuroendocrine effects and cocaethylene metabolism. *Pharmacol Exp Ther*. 1997;283:164–176.
50. Walker DJ, Zacny JP. Lack of effects of ethanol pretreatment on the abuse liability of nitrous oxide in light and moderate drinkers. *Addiction*. 2001;96:1839–1845.
51. Holdstock L, de Wit H. Individual differences in the biphasic effects of ethanol. *Alcohol Clin Exp Res*. 1998;22:1903–1911.
52. Heishman SJ, Arasteh K, Stitzer ML. Comparative effects of alcohol and marijuana on mood, memory, and performance. *Pharmacol Biochem Behav*. 1997;58:93–101.
53. Schuckit MA, Greenblatt D, Gold E, et al. Reactions to ethanol and diazepam in healthy young men. *J Stud Alcohol*. 1991;52: 180–187.
54. Persson B, Henning M. Central cardiovascular effects of gamma-hydroxybutyric acid: interactions with noradrenaline, serotonin,

dopamine and acetylcholine transmission. *Acta Pharmacol Toxicol (Copenh).* 1980;47:335–346.
55. Hicks AR, Kapusta DR, Varner KJ. Mechanisms underlying the sympathomimetic cardiovascular responses elicited by gamma-hydroxybutyrate. *J Cardiovasc Pharmacol.* 2004;44:631–638.
56. Couper FJ, Thatcher JE, Logan BK. Suspected GHB overdoses in the emergency department. *J Anal Toxicol.* 2004;28:481–484.
57. Espinosa G, Miro O, Nogue S, et al. Liquid ecstasy poisoning: study of 22 cases. *Med Clin (Barc).* 2001;117:56–58.
58. Palatini P, Tedeschi L, Frison G, et al. Dose-dependent absorption and elimination of gamma-hydroxybutyric acid in healthy volunteers. *Eur J Clin Pharmacol.* 1993;45:353–356.
59. Spoerer KA, Chin RL, Dyer JE, et al. Gamma-hydroxybutyrate serum levels and clinical syndrome after severe overdose. *Ann Emerg Med.* 2003;42:3–8.
60. Scharf MB, Lai AA, Branigan B, et al. Pharmacokinetics of gammahydroxybutyrate (GHB) in narcoleptic patients. *Sleep.* 1998;21:507–514.
61. Borgen LA, Okerholm R, Morrison D, et al. The influence of gender and food on the pharmacokinetics of sodium oxybate oral solution in healthy subjects. *J Clin Pharmacol.* 2003;43:59–65.
62. Raybon JJ, Boje KM. Pharmacokinetics and pharmacodynamics of gamma-hydroxybutyric acid during tolerance in rats: effects on extracellular dopamine. *J Pharmacol Exp Ther.* 2007;320:1252–1260.

6. Discussion

The main results of the present work have been presented as independent articles in chapter 5 of this thesis including focused discussions. This chapter will provide a broader discussion of the findings and review the effects of GHB in humans specially focusing in some aspects that were not extensively discussed within the articles. The two studies performed will be expounded separately. Some specific results of the pilot study are discussed first, while all the results in common of both studies and the results of the final study will be discussed at ending.

6.1. Pilot study

The pilot study was the first evaluation in humans of GHB physiological and subjective effects after controlled administration of doses compatible with those consumed by recreational users. The disposition of GHB in conventional and non-conventional matrices in humans was also studied. The results of this study were published in two separate publications.

6.1.1. Gamma-hydroxybutyrate (GHB) in humans: pharmacodynamics and pharmacokinetics. Ann New York Acad Sci. 2006;1074:559–576.

Taking into account the contradictory previous data regarding GHB dose-related effects the main objective of the pilot study was to select appropriate doses for the final study. As a pilot pharmacology phase I study, increasing doses of GHB were given. An appropriate interval between the lowest dose with noticeable effects and doses that lead to significant somnolence was investigated. Increasing doses of sodium GHB (GHB) were given from 40 (33.1) mg/Kg to the maximum dose of 72 (60.1) mg/Kg allowed by the research protocol. GHB was well tolerated during all study sessions and no serious adverse events were noticed (only one subject vomited 1h after the administration of the 60 mg/kg dose). Even at the highest dose, increased somnolence leading to a decreased level of alertness was not observed in any of the participants. As highlighted in the introduction, this was in contrast to previous reports that GHB oral doses of 50-60 mg/kg could lead to coma (Metcalf et al. 1966). At the same time our results are in accordance with some studies in narcoleptic patients in which doses of 4.5 g (64 mg/kg for a 70kg person) were tested and no significant sedation leading to coma was observed (Borgen et al. 2004). Taking into account these results doses of 40 and 60 mg/kg of GHB were selected for the final trial.

It is important to note that the study was limited by both a dose escalation schedule design and the number of volunteers tested at each dose. In the dose escalation schedule placebo was only given once. Consequently the study did not allow for full drug-placebo comparisons. However, differentiation of GHB dose related effects was possible. For the first time in humans, the study provided knowledge about GHB induced subjective effects and alterations of psychomotor performance at doses compatibles with those being abused. The main finding of the study was that GHB induced physiological and subjective effects were dose dependent and related to GHB plasma concentrations. Interestingly, GHB induced a mixed sedative-stimulant effect pattern. Psychostimulant effects were predominant in the first hour and appeared related to GHB plasma concentrations, while sedative effects initiated more slowly and predominated in the second hour after drug administration. GHB mediated stimulant-like effects were previously suggested by GHB users in a report of GHB perceived subjective effects (Miotto et al. 2001). However, this study provided the first demonstration of GHB related stimulant effects after controlled administration. In reference to its abuse liability, GHB induced apparent dose-dependent euphoria, well being and pleasurable effects, in line with those previously reported by GHB abusers (Miotto et al. 2001; Degenhardt et al. 2002). GHB also induced slight subjective feelings of sedation. Intriguingly, sedative effects did not follow a dose dependent trend and no dose-dependent significant differences were found.

Regarding psychomotor performance, this was the first controlled study where GHB was found to impair psychomotor performance. However, as previously discussed, it is possible that the design of the study did not allow us finding significant dose dependent differences. All GHB doses impaired the DSST task, and sodium GHB 60 mg/kg and 72 mg/kg doses also induced a deterioration of the balance task.

An initial examination of the pharmacokinetics of GHB was also performed in the pilot study. Some of the results were published in an additional publication.

6.1.2. Disposition of GHB in conventional and non-conventional biological fluids after a single drug administration: issues in methodology and drug monitoring. Ther Drug Monit. 2007;29:64-70.

This study provided new insights on GHB pharmacokinetics in non-conventional matrices in humans. The disposition of GHB in plasma, oral fluid, urine and sweat in the 5 participants

receiving sodium GHB 50 mg/kg dose and the results of the validation of the method of detection of GHB in biological fluids will be discussed.

The first remarkable finding is that the method of detection of GHB in plasma, urine and oral fluid used throughout the studies was validated. The intra and inter-day precision and accuracy calculations for GHB in biological matrices under examination, satisfactorily met the internationally established acceptance criteria. No relevant variability due to the effect of storage temperature in GHB plasma concentrations was found. A partial limitation of the method is the limit of quantification of GHB, set at 0.2 μg/mL. Consequently, only estimations of the baseline GHB in plasma, urine and oral fluid could be made. However it should be acknowledged that this is a common limitation of all previously published methods of GHB detection (Brenneisen et al. 2004; LeBeau et al. 2002). Nevertheless drug consumption could be differentiated from GHB endogenous concentrations both in plasma and urine 6h post-ingestion.

GHB disposition in non-conventional biological fluids was a main objective of this preliminary work. The increasing recreational use of GHB at dance clubs and rave parties and the rise in the number of intoxications is a matter of concern. In addition, GHB is used to assist in the commission of sexual assaults (Varela et al. 2004). Detecting GHB is a serious problem, because GHB is metabolized in the body by oxidative enzymes with rapid degradation and the eventual by-products are carbon dioxide and water with no metabolite present. Further, a compounding difficulty resides in the fact that GHB is an endogenous substance present in human body with measurable baseline concentrations both in blood and urine. Hence, different authors have proposed employing cut-off concentrations (i.e. 10 μg/mL for urine samples) to identify exogenous GHB exposure (Elian 2002; LeBeau et al. 2006). Finally, several studies showed that endogenous GHB concentrations can be elevated both in ante-mortem and post-mortem biological samples due to storage conditions and enzymes responsible for biotransformation of GHB into the body (Sakurada et al. 2002). Furthermore using the best available techniques (GC–MS) GHB is detectable in humans for only a limited amount of time, specifically 8-12h in the urine and 6-8h in blood. Therefore detecting this drug in victims and in intoxication cases is a serious problem. In this context we decided to study the disposition of GHB in matrices different than plasma and urine in order to explore a possible extended detection window of GHB in these matrices.

Oral fluid concentrations of GHB were lower to those observed in plasma as indexed by C_{max} and AUC. They quickly reach baseline concentrations at 3h post-ingestion, limiting somewhat the time window for detecting its consumption in this biological matrix and therefore its usefulness

in GHB forensic toxicology. Oral fluid presented several drawbacks as a possible alternative matrix. Firstly, GHB was found in oral fluid at peak value concentrations equivalent to one third to one fourth of those found in plasma, with an even quicker return to baseline values than that observed in plasma. Secondly, the saliva plasma ratio was always lower than 1, which indicates that GHB, an acidic drug, did not diffuse to a large extent into oral fluid. These results are in line with the limited previous data of disposition of GHB in oral fluid (Brenneisen et al. 2004). The present study improved upon these methods by having subjects perform a mouthwash immediately after drug administration in order to minimize oral fluid contamination. GHB disposition in oral fluid was clearly in contrast with disposition of basic drugs, such as MDMA, that appears in saliva in concentrations notably higher than those in plasma (Navarro et al. 2001). In addition, no dose dependent differences were detected and a large inter-subject variability in saliva/plasma ratio was found. All these results do not support the notion of a relationship between dose and oral fluid concentration and show that oral fluid is not an appropriate matrix to monitor GHB use.

To our knowledge there have been no previous reports of the detection of GHB in sweat. In the present study GHB was measured in sweat in cotton wipes (which represent a punctual measure of excretion) and in sweat patches (which represent an accumulative measure of excretion). However, it appears that drug diffusion in this matrix suffers from even more limitations than those found for oral fluid. First, the baseline amounts of GHB measured in cotton wipes of subjects participating in the study showed a high variability. This was in agreement with the results of a preliminary study in twenty drug-free male healthy volunteers (completed in our laboratory) showing a very high variability in GHB endogenous basal concentrations in blank cotton wipe samples. There were more than a 100 fold (0.03-5.46 µg) inter–subject differences in sweat GHB values. Secondly, concentrations measured over time never increased more than 1-2 µg/wipe over basal values and no dose-dependent differences were found. Thirdly, no accumulation (0-6h) of GHB was found in the sweat patches, with amounts ranging between 0.9 µg to 1.3 µg. Finally, after drug intake, GHB concentrations are marginally higher than baseline values when peak concentrations are achieved in plasma. In addition, taking into account the variability in basal concentrations, concentrations of GHB in sweat higher than reported basal values cannot be attributed to its consumption. Therefore, as with oral fluid, sweat does not appear to be a suitable biological matrix for monitoring GHB use.

Regarding GHB urinary excretion, less than 2% of doses administered were recovered in the collection period. Our results were in accordance with previous reports (Kavanagh et al. 2001; Brenneisen et al. 2004). Highest recoveries were found in the 0-3h urine samples.

6.2. Final study. Relative abuse liability of gamma-hydroxybutyric acid (GHB), flunitrazepam and ethanol in 'Club Drug' users. J Clin Psychopharmacol. 2007;27:625-638.

The final study was undertaken to further evaluate GHB clinical pharmacology and its relative abuse liability in 'Club Drug' users. The rationale for the selection of healthy volunteers with previous experience with 'Club Drugs' was three-fold. First, these participants should provide low false positive rates as measured by response to placebo administration (Griffiths et al. 2003). Second, they are less likely to provide false negative results than subjects without histories of drug use (Roset et al. 2003). Third, 'Club Drug' users appeared to be increasingly abusing this drug and epidemiological investigations reported increased abuse of sedative-like drugs by this demographic (Wu et al. 2006).

The procedures and variables measured were identical to those employed in the pilot study. This helped to maintain comparability across studies and to confirm results of the pilot study. The final study was conducted as a double-blind, controlled, cross-over, and randomized according to a balanced 5 × 5 Latin-square design. An authentic complete cross-over design allowed us to fully characterize GHB effects and to perform drug to drug comparisons. The first notable finding was that similar subjective effects were elicited by all active conditions. These similarities in the profile of drug induced effects strongly support (i) the selection of ethanol and flunitrazepam as positive controls, and (ii) the rationale for the doses chosen. As stated in the introduction, onset and duration of action can affect abuse liability. The interpretation of the results of an abuse liability evaluation will be facilitated if the positive controls and the tested compound have similar onsets and durations (Griffiths et al. 2003; Roset et al. 2003). The administration of flunitrazepam in a liquid form instead of the usual tablet preparations allowed for a faster absorption (shorter t_{max}) and similar onset of action than ethanol and GHB. In addition, it allowed masking drug administration as all drugs were administered dissolved in a liquid. Together with our results, it can be argued that doses chosen and the administration procedure, allow us to correctly synchronize the effects to the different drugs used. On the other hand, it is important to notice that the effects were clearly differentiated by the participants and the drugs were readily

distinguished via the pharmacological class questionnaire. Therefore the effects of GHB were by some means similar to flunitrazepam and ethanol but qualitative and quantitative (time course of the effects) differences were found. Theses differences will be further discussed.

6.2.1. Subjective effects and abuse liability

One of the aims of the present work was to elucidate the motivations for the increasing abuse of GHB in humans. The first remarkable finding is that, as suggested by the results of the pilot study, GHB induced euphoria, well being, and pleasurable effects. All these effects are similar to those previously reported by GHB users and thought to be related to a high abuse potential (Miotto et al. 2001; Griffiths et al. 2003). The only previous study in humans regarding GHB abuse potential reported a likelihood for GHB to be abused intermediate to triazolam and pentobarbital (Carter et al. 2006). Authors based this rating for GHB mainly on 2 different criteria. First, GHB produced an intermediate effect (greater than those of triazolam but lower than pentobarbital) on most measures of likelihood of abuse (i.e. ratings of liking and reinforcing effects). Second, GHB generally produced greater unpleasant drug effects than the other active conditions and this was interpreted as a possible limit of GHB abuse potential. Nevertheless, alternative explanations could elucidate the rate of GHB abuse potential found in this study. An important drawback is that subjects had histories of sedative drug abuse but no previous GHB use. Further, they were primarily selected based on reporting liking pentobarbital during a practise session. Therefore a possible bias towards a higher abuse liability reported for pentobarbital was present. Furthermore, it was an increasing dose study where different oral doses of 2, 4, 8 and up to 18 g of GHB were given. Surprisingly no doses between 4 and 8 g were tested (equivalent respectively to 57 and 114 mg/kg for a 70 kg person) despite the observation that 30% of the participants were technically in coma after the GHB 8 g dose (and obviously too impaired to be evaluated). It is possible that doses between 4 and 8 g (equivalent to those used in our study) combined with previous GHB use could have shown greater abuse liability for GHB.

Alternatively it can also be argued that our results do not demonstrate that GHB liability for abuse is any greater than flunitrazepam or ethanol at the doses tested. However, the results suggest the opposite, that GHB abuse liability could have been underestimated in our study. First, the administration of GHB significantly increased all subjective measures related to euphoria and pleasurable effects with slightly higher ratings to those observed for flunitrazepam and ethanol. Thus, GHB clearly tended to produce higher euphoric and pleasurable effects in all variables measured than those elicited by the other active treatments. Second, the two participants who

developed unwanted GHB side effects provided ratings close to zero on scales related to abuse potential. This resulted in a decrease in the mean GHB scores in the scales related to abuse potential. Third, the ARCI questionnaire, and in particular the key measure of euphoria and abuse liability (MBG scale), was administered 30min (ethanol peak effect) and 60min (flunitrazepam peak effect) after drug administration However, it was not administered at GHB's peak effect (between 40-50min). Thus, we may not have measured the actual GHB euphoric effect at its peak; which should be acknowledged as a limitation of the study.

The overall abuse liability of a drug refers to both its likelihood of abuse and its potential for producing adverse consequences as a result of its abuse (Griffiths et al. 2003). It is possible that the unpleasant effects of GHB observed in our study might limit its abuse potential. Nevertheless it should be highlighted that GHB-induced adverse effects were only observed in two subjects (2 over 12). No within-subject coincidence of positive and negative GHB related effects was found. Based on our results and the previous study in sedative abusers, it can be argued that sensitivity to the differential effects of GHB is a key issue regarding its abuse liability. GHB would have less potential of abuse in subjects who are sensitive to the adverse effects (nausea and vomiting), in particular those who experience loss of consciousness secondary to its administration. However as a corollary of these findings it could also be speculated that GHB abuse potential could be higher in users who do not usually experience adverse effects, and even more in those who do not consider GHB overdose itself a dangerous thing (Degenhardt et al. 2003). Even taking into account the inter-individual variability described, our results suggest a high abuse liability of GHB in participants with prior GHB use in the range of doses usually abused. Thus, our results reveal potential factors related to the widespread abuse of GHB reported during the last years.

The study also provides further evidence of the abuse potential of flunitrazepam in humans. Despite being a sedative, this drug has been classified as a 'Club Drug'. Additionally 'Club Drug' users have been shown to be increasingly abusing this drug (Wu et al. 2006). Again, reasons for its use as a party drug have been puzzling. The profile of effects induced by flunitrazepam was consistent with the results of previous studies and supports the hypothesis that this benzodiazepine is capable of inducing 'high' and pleasurable feelings related to its abuse potential (Farré et al. 1996; Roset et al. 2001). Flunitrazepam also induced undesirable effects partially related to its sedative effects. However, in contrast with GHB, no high inter-individual differences in sensibility to this drug were found.

Another important point to consider is that 'Club Drug' users may seek both the euphoric and sedative effects of drugs. Interestingly GHB, ethanol and flunitrazepam, although to different degrees, were able to induce a slight stimulation. In the case of GHB, the stimulant-like effects peaked at 45min post-administration. This profile differs from those reported in previous studies testing lower GHB doses (Ferrara et al. 1999), however it is consistent with the results from the pilot study. It differs also from the results of a study in sedative abusers in which GHB administration was not related to the induction of stimulant-like effects (Carter et al. 2006). However, the limitations of these studies discussed previously prevent further comparisons with our study. Our results on the contrary fit perfectly with the fact that GHB is abused while dancing to electronic music by club/'rave' attendees (Parks and Kennedy, 2004). This population frequently refers to GHB as 'liquid ecstasy' suggesting a resemblance to MDMA effects. Keen GHB doses tested produced slight increases in pupil diameter, systolic blood pressure, VAS 'stimulated' and the ARCI-A scale, similar effects to those elicited by MDMA (Camí et al. 2000). Therefore although with differentiated effects, the three study drugs showed a mixed sedative-stimulant pattern as stimulant-like effects preceded sedative-like ones. This biphasic time profile has been previously described for ethanol and some other sedatives (Holdstock and de Wit, 1998; Heishman et al. 1997). Furthermore it has been suggested that the ability of sedative drugs to produce stimulant effects and psychomotor activation is positively correlated to their ability to induce reinforcing effects (Wise and Bozarth, 1987). In other words, the biological mechanisms underlying the induction of psychomotor activation would be homologous with the biological mechanisms that underlie the positive reinforcing effects of drugs of abuse. An explanation for the reasons why humans often choose to consume alcohol during times of celebration to enhance positive mood states associated with such events can be made based in our results and in the previous discussion. This also would apply for GHB and might also explain the underlying reasons for using GHB, flunitrazepam and ethanol (primarily sedative drugs) in Clubs and 'Raves'.

GHB also produced objective and subjective sedative effects. It was previously noted that in the pilot study GHB's sedative effects did not follow a dose dependent trend. In the final study only a non-significant trend to a dose-dependent sedation was found (except in the VESSPA SED). In the study of Carter et al. in 2006 increasing GHB doses (2–18 g/70 kg, up to 257 mg/kg) were given to sedative abusers. Following the highest dose, 71% of participants were arousable only by rough tactile or painful stimulation. Perhaps surprisingly, the authors considered that there were no serious adverse events in the study. However, this level of sedation would commonly be classified as stupor or coma depending on the degree of arousal that pain induces. At the same time, none of the volunteers tested at 4 g dose in this study were asleep. If we put together this

information with the results of our study and the reports of the well documented GHB overdose cases, GHB actual sedative effects could be accurately predicted (Miró et al. 2002; Liechti et al. 2006). Thus, GHB seems to differ from other sedatives in that sedation does not progress in a dose dependent manner. It can be hypothesised that a threshold mechanism is responsible for switching from being awake to be strongly sedated. Accordingly, in a number of cases of GHB intoxication subjects were able to recall only that they were dancing prior to overdose (Abanades et al. 2001). The results from our study could be wrongly interpreted indicating that GHB is a safe drug since 60 mg/kg doses of GHB were given and none of the participants experienced loss of consciousness. However, one subject experienced an increased somnolence and a decreased level of alertness from 1h to 3h after drug administration. Therefore, it can be proposed that after GHB doses over 60 mg/kg there is a very narrow time window between being awake or presenting a level of sedation (greater than intended), that may result in a drop in the level of consciousness.

6.2.2. Psychomotor performance

As stated in the introduction little is known about the repercussion of GHB use in psychomotor performance. A previous study did not find a significant impairment after administration of GHB 12.5 and 25 mg/kg doses (Ferrara et al. 1999). However the doses administered were lower than those being recommended in narcolepsy and significantly lower than doses being abused (Dyer 1991). We demonstrated that GHB produced a significant dose-dependent impairment of psychomotor performance as measured by DSST and balance tasks. Moreover, GHB induced a significant exophoria as measured by the Maddox Wing device. This task is a direct measure of the extraocular musculature relaxation and an indirect marker of central sedation. Interestingly, GHB effects on psychomotor performance peaked 1h after its administration. However, this effect did not parallel GHB sedative peak effects occurring 1.5h after drug administration. In a similar way in the study of Carter et al. the balance task was also slightly impaired after a dose (4 g) that did not produce significant sedation, and a decrease in the number of correct responses in the DSST was found after this dose. Hence it can be hypothesised that GHB induced deterioration of psychomotor performance is only partially related to sedation. In this line, an elegant previous study with low doses of flunitrazepam (0.6, 0.8, and 1.0 mg) in healthy volunteers demonstrated that this drug impaired subjective and objective measures of attention and psychomotor activities in the absence of classical objective effects associated with sedation/sleepiness (measured with simultaneous EEG). Authors suggested that independent mechanisms could be

responsible for this impairment (Luchesi et al. 2003). Alternatively, it can also be hypothesised that GHB changes in psychomotor performance are probably due to other intrinsic effects, rather than closely related to the effects of sedation.

Our results could also help to inform the legal debate around driving under the influence of GHB. At the doses tested GHB was capable of inducing a clear alteration of psychomotor performance significantly higher to that elicited by ethanol. In addition, ethanol peak concentrations were above the legal limits. Therefore it can be assumed that at doses usually abused, GHB alterations in psychomotor performance are sufficient to significantly impair driving. This should be acknowledged as an important issue in a possible risk reduction strategy providing information regarding GHB to actual GHB users.

6.2.3. Physiological effects

Prior to this work, GHB effects on blood pressure were unclear. Hypotension was described in about 10% of GHB intoxication cases while no mention of changes in blood pressure were reported in GHB therapeutic studies. In line with results of the pilot study, GHB administration produced a slight significant rise in systolic blood pressure lasting for 1-2h post-administration. Our results are supported by similar increases in mean systolic blood pressure observed after controlled administration of 25 mg/kg of BD (metabolized to GHB) to 8 healthy volunteers (Thai et al. 2007). $GABA_B$ mediated sympathomimetic cardiovascular effects after GHB administration has been described in rats and could be the mechanism underlying this particular effect (Hicks et al. 2004).

GHB induced also a slight mydriasis between 30min and 1h post-administration. No previous controlled studies were available to compare this finding. However it is in accordance with the results of the pilot study and in agreement with observations made in acute intoxication cases where GHB is the only drug involved (Dyer et al. 2001; Couper et al. 2004; Espinosa et al. 2001). Therefore it can be argued that in cases of pure GHB intoxication mydriasis should be expected.

6.2.4. Pharmacokinetics

The results of the study are in accordance with the extensive previous work done in humans. Given by the oral route, GHB is rapidly absorbed and eliminated, and displays a highly variable

bioavailability. GHB peak concentrations (C_{max}) and AUC from the present study were dose dependent and confirm the results of the pilot study. Our results parallel those obtained in healthy subjects (Palatini et al. 1993) administered with 4.5 g of oxybate (equivalent to 50-60 mg/kg of GHB) and lower to those observed in severe acute intoxications (Sporer et al. 2003). GHB elimination appears to be capacity-limited at the higher dose as it has been observed in some narcoleptic patients administered at a fixed dose of 3 g, twice nightly, at a 4h interval (Scharf et al. 1998). At the lower GHB dose tested, this phenomenon is not in agreement with previous reports (Palatini et al. 1993; Borgen et al. 2003). The clearance of GHB is dose dependent, with the predominant pathway (more than 90% of the dose) representing metabolism of GHB to CO_2 and H_2O through the Krebs cycle (Ferrara et al. 1992; 1996; Palatini et al. 1993; Scharf et al. 1998). Capacity-limited metabolism and/or absorption seem to be the main mechanisms underlying the nonlinear pharmacokinetics of GHB. The accumulation of GHB in the body as a result of its non-linear disposition might have implications in the susceptibility of some subjects to develop acute intoxications. Compared with elimination by metabolism, the renal clearance of GHB is a minor pathway as less than 2% of GHB is recovered in urine after its administration to humans. However, the renal clearance of GHB is significantly increased at higher GHB plasma concentrations, hence playing a more important role in the elimination of GHB after high doses or overdoses. Recently, MCT transporters have been identified as relevant in both the bioavailability and renal clearance of GHB (Morris et al. 2005; Wang and Morris, 2007).

6.2.5. Pharmacokinetics and pharmacodynamics

Measurement of plasma concentrations in clinical trials where psychoactive substances are tested is a key approach to help in the understanding of drug effects and is strength of our study. As discussed, it allows correlating drugs effects with simultaneous plasma concentrations. It can also help to explain unexpected results or inter-individual differences in adverse effects or sensitivity to drug effects. One of the aims of the present work was to asses a potential correlation of the plasma concentrations of GHB and the effects produced by this drug. As suggested by the results of the pilot study, GHB induced a biphasic time profile. Initially stimulant-like, euphoric and pleasurable effects were related to the simultaneous rise of plasma drug concentrations. The opposing sedative effect was associated with the descending curve of GHB plasma concentrations. Ethanol also produced stimulant-like effects related to its increase in blood concentrations, as previously published. This association of the stimulant and euphoric

effects to the ascending limb of plasma concentrations was previously reported for ethanol (Davidson et al. 2002).

Measurement of plasma concentrations helped us to explore a possible relationship between GHB kinetics and sensitivity to undesirable effects. Thus, unusual GHB plasma concentrations were not found in subjects presenting GHB-mediated adverse effects. In addition, participants who tended to score higher in scales related to abuse potential did not display higher GHB plasma concentrations. Based on these findings and previous research, it could be argued that sensitivity to GHB effects probably relies on variations in neurotransmitters and receptors involved in GHB actions, rather than on differences in GHB plasma concentrations (Raybon and Boje, 2007).

Conversely, the apparent high frequency of accidental GHB overdose could also be explained by the variability of volume, concentration, and identity of GHB solutions on the street. This would make it difficult for users to estimate doses for self-administration (Degenhardt et al. 2003). In this work, controlled doses of pharmaceutical probe GHB were administered on a weight-adjusted basis. We have shown that even under these conditions, substantial differences in sensitivity to GHB can exist, at least in recreational 'Club Drug' users. Taking into account the high variability in sensitivity to GHB effects described, cases of unintended GHB overdose can appear in 'Club Drug' abusers who consume large amounts of GHB, or combine it with psychostimulants (Degenhardt et al. 2003; Liechti and Kupferschmidt, 2004) in pursuit of an augmented level of subjective effects.

6.3. Potential Further Limitations

Several further potential limitations not previously addressed are discussed below.

Possibly the main potential limitation of the present work is the sample size. Therefore the present work should be regarded cautiously. Even though it permitted us to find statistically significant differences in many variables studied, it had possibly prevented us of finding or confirming some of the results. For instance, we were not able to confirm the trend of a significant increase in heart rate after GHB administration. Nonetheless, several effects found were consistent along the pilot and final study (20 volunteers in total) and should be taken as a strong evidence of the effects of GHB in humans.

A second limitation of the study stems from the range of doses tested. As discussed previously we decided to limit the higher dose to 60 mg/kg to prevent inducing a drop in the level of consciousness. This approach allowed us to explore GHB related effects in limited a range of doses with fewer safety concerns for subjects. Consequently knowledge about GHB effects over 60 mg/kg and a possible relation of GHB plasma concentrations and sedative effects leading to coma remain to be elucidated.

Third, the study was originally designed and funded only for male users and therefore female subjects were not included. This was intended to decrease the variability of the results in a human pharmacology typical trial, taking into account the limited funding for the study. No relevant pharmacokinetic sexual differences were previously published (Borgen et al. 2003) and no apparent differences were reported in cases of intoxication due to GHB. Therefore results have to be regarded cautiously applied to female GHB users. Further studies are needed to characterize the effects of GHB in female participants. The realization of studies in female subjects should be encouraged by the public administration and private sponsors by providing extra financial support for these indispensable clinical studies.

Fourth, a potential limitation emerges from testing subjects simultaneously. Anytime two or more human subjects are tested together, there is the possibility of the behaviour of one subject affecting the other (Caudill and Liscomb, 1980). Thus, the behaviour assessed in a subject in this study could be partially contaminated by the behaviour of another. One subject, for example, could be exquisitely sensitive to the stimulant effects of the drugs whereas the other is not. The stimulant-insensitive partner may have increased their activity levels because of the influence of their partner and not because of the stimulant effect of the drug. However, it can be also argued that this effect is partially avoided by randomisation of drugs and study sessions by means of a balanced 5 × 5 Latin-square design.

It should be also addressed a possible impact of the circadian rhythm over the appearance and detection of stimulant-like effects. The study was performed during the morning and this could help to find drug stimulant related effects. In the case of GHB this is in contrast to the fact that lower doses given at night are capable to induce sleep in healthy volunteers and patients.

7. Conclusions

1. At the doses tested, GHB was capable of inducing euphoria, pleasurable feelings, sedation and slight stimulant-like effects as previously reported by GHB users.

2. GHB administration also induced dose-dependent mild unpleasant effects such as dizziness, confusion and dysphoria.

3. GHB tolerability highly differed between subjects with no within-subject coincidence of positive and negative GHB related effects.

4. The acute administration of GHB increased systolic blood pressure and pupil diameter.

5. GHB administration induced a dose-dependent impairment of psychomotor performance as measured by the digit symbol substitution test and the balance task.

6. Following oral administration of GHB, measurable plasma, urine, oral fluid and sweat were observed. Oral fluid and sweat appear not to be suitable biological matrices for monitoring GHB consumption. GHB is rapidly absorbed and eliminated with high interindiviidual variability. GHB elimination appears to be capacity-limited at the higher dose studied.

7. GHB induced a biphasic time profile with an initial stimulant-like, euphoric and pleasurable effect related to the simultaneous rise of plasma drug concentrations and ulterior sedative effect collateral to a decrease in GHB plasma concentrations.

8. GHB plasma concentrations were well correlated to subjective effects related with stimulation and its abuse potential whereas they were not to sedative effects.

9. Ethanol and flunitrazepam effects seen in the study were consistent with the results of previous studies. Ethanol induced its prototypical effects and flunitrazepam produced marked sedation.

10. The three study drugs, GHB, flunitrazepam and ethanol, although known sedate-like drugs, they all induced a mixed sedative-stimulant pattern

11. GHB, flunitrazepam and ethanol display a high abuse liability in recreational ‘Club Drug’ users at the doses tested.

1. *La administración de GHB, produjo euforia, sensaciones placenteras, sedación y suaves efectos estimulantes, tal y como había sido descrito por los usuarios de la sustancia.*

2. *La administración de GHB produjo asimismo efectos indeseables dependientes de la dosis, como mareo, confusión y disforia.*

3. *La tolerabilidad del GHB difirió en gran medida entre los diferentes sujetos, sin coincidencia intra-sujeto de efectos positivos y negativos.*

4. *La administración de GHB incrementó la presión arterial sistólica y el diámetro pupilar.*

5. *La administración de GHB indujo un deterioro del rendimiento psicomotor dosis-dependiente (evaluado por la tarea de sustitución de símbolos por dígitos y del balance).*

6. *Tras su administración oral, se halló GHB a diferentes concentraciones en plasma, orina, fluido oral y sudor. No obstante, tanto el fluido oral como el sudor no parecen convenientes para monitorizar el consumo de GHB. El GHB se absorbe y se elimina de forma rápida, existiendo una gran variabilidad interindividual. A la dosis más alta estudiada, la capacidad de eliminación de GHB parece estar limitada.*

7. *El perfil de efectos inducidos por el GHB fue de tipo bifásico, inicialmente de tipo estimulante-euforizante y relacionado con el incremento simultáneo de las concentraciones plasmáticas, seguido de un efecto de tipo sedante no relacionado con la cinética.*

8. *Se apreció una buena correlación entre las concentraciones plasmáticas de GHB y los efectos subjetivos estimulantes y relacionados con el potencial de abuso.*

9. *Los efectos producidos por etanol y flunitrazepam fueron consistentes con los resultados de estudios previos. Etanol produjo sus efectos prototípicos y flunitrazepam una marcada sedación.*

10. *GHB, flunitrazepam y etanol, indujeron un patrón de efectos mixto de tipo estimulante y sedante, a pesar de ser reconocidas sustancias de tipo sedante.*

11. *Los resultados sugieren un alto potencial de abuso de GHB, flunitrazepam y etanol en usuarios de "Club Drugs"*

8. References

Abanades S, Iglesias ML, Echarte JL, Puig Dou J, Roset PN, Farré M. Gammahydroxibutirate: A novel toxicological emergency. *Methods Find Exp Clin Pharmacol.* 2001;23:326.

Abanades S, Peiro AM, Farré M. [Club drugs: old medicines as new party drugs] [Article in Spanish] *Med Clin (Barc)*. 2004;123:305-311.

Addolorato G, Castelli E, Stefanini GF, Casella G, Caputo F, Marsigli L, et al. An open multicentric study evaluating 4-hydroxybutyric acid sodium salt in the medium-term treatment of 179 alcohol dependent subjects. *Alcohol Alcohol*. 1996;31:341-345.

Addolorato G, Capristo E, Gessa GL, Caputo F, Stefanini GF, Gasbarrini G. Long-term administration of GHB does not affect muscular mass in alcoholics. *Life Sci*. 1999;65:191-196.

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR). 4th Ed, Text revision. Washington DC (US): American Psychiatric Association; 1994.

Anglin D, Spears KL, Hutson HR. Flunitrazepam and its involvement in date or acquaintance rape. *Acad Emerg Med*. 1997;4:323-326.

Bareggi SR, Ferini-Strambi L, Pirola R, Smirne S. Impairment of memory and plasma flunitrazepam levels. *Psychopharmacology (Berl)*. 1998;140:157-163.

Barnas C, Rossmann M, Roessler H, Riemer Y, Fleischhacker WW. Benzodiazepines and other psychotropic drugs abused by patients in a methadone maintenance program: familiarity and preference. *J Clin Psychopharmacol*. 1992;12:397-402.

Beardsley PM, Balster RL, Harris LS. Evaluation of the discriminative stimulus and reinforcing effects of gammahydroxybutyrate (GHB). *Psychopharmacol*. 1996;127:315–322.

Benschop A, Rabes M, Korp D. Pill Testing Ecstasy and Prevention. A scientific evaluation in three European cities. Amsterdam: Rozenberg Publishers; 2002.

Black J, Houghton WC. Sodium oxybate improves excessive daytime sleepiness in narcolepsy. *Sleep*. 2006;29:939-946.

Blumenfeld M, Suntay RG, Harmel MH. Sodium gammahydroxybutyric acid: a new anaesthetic adjuvant. *Anesth Analg.* 1962;41:721-726.

Bond A, Seijas D, Dawling S, Lader M. Systemic absorption and abuse liability of snorted flunitrazepam. *Addiction*. 1994;89:821-830.

Borgen L, Lane E, Lai A. Sodium Oxybate: A study of dose proportionality in healthy humans subjects [abstract]. *J Clin Pharmacol*. 2000;40:1045

Borgen LA, Okerholm R, Morrison D, Lai A. The influence of gender and food on the pharmacokinetics of sodium oxybate oral solution in healthy subjects. *J Clin Pharmacol*. 2003;43:59-65.

Borgen LA, Okerholm RA, Lai A, Scharf MB. The pharmacokinetics of sodium oxybate oral solution following acute and chronic administration to narcoleptic patients. *J Clin Pharmacol*. 2004;44:253-257.

Boxenbaum HG, Posmanter HN, Macasieb T, Geitner KA, Weinfeld RE, Moore

JD, et al. Pharmacokinetics of flunitrazepam following single- and multiple-dose oral administration to healthy human subjects. *J Pharmacokinet Biopharm*. 1978;6:283-293.

Brady KT, Lydiard RB, Brady JV. Assessing abuse liability in clinical trials. Drug Alcohol Depend. 2003;70(3 Suppl):S87-95.

Braestrup C, Squires RF. Pharmacological characterization of benzodiazepine receptors in the brain. *Eur J Pharmacol.* 1978;48:263-270.

Brenneisen R, Elsohly MA, Murphy TP, Passarelli J, Russmann S, Salamone SJ, et al. Pharmacokinetics and excretion of gamma-hydroxybutyrate (GHB) in healthy subjects. *J Anal Toxicol.* 2004;28:625–630.

Caldicott DG, Chow FY, Burns BJ, Felgate PD, Byard RW. Fatalities associated with the use of gamma-hydroxybutyrate and its analogues in Australasia. *Med J Aust*. 2004;181:310-313.

Camí J, Farré M, Mas M, Roset PN, Poudevida S, Mas A, et al. Human pharmacology of 3,4-methylenedioxymethamphetamine ("ecstasy"): psychomotor performance and subjective effects. *J Clin Psychopharmacol*. 2000;20:455-466.

Cano JP, Sumirtapura YC. Comparative pharmacokinetics of diazepam and flunitrazepam. *Ann Anesthesiol Fr.* 1981;22:175–179.

Caputo F, Addolorato G, Lorenzini F, Domenicali M, Greco G, del Re A, et al. Gamma-hydroxybutyric acid versus naltrexone in maintaining alcohol abstinence: an open randomized comparative study. *Drug Alcohol Depend*. 2003;70:85-91.

Carai MA, Colombo G, Gessa GL. Protection by the GABAB receptor antagonist, SCH 50911, of gamma-hydroxybutyric acid-induced mortality in mice. *Eur J Pharmacol*. 2004;503:77-80.

Carai MA, Colombo G, Gessa GL. Resuscitative effect of a gamma-aminobutyric acid B receptor antagonist on gamma-hydroxybutyric acid mortality in mice. *Ann Emerg Med.* 2005;45:614-619.

Carter LP, Richards BD, Mintzer MZ, Griffith RR. Relative abuse liability of GHB in humans: A comparison of psychomotor, subjective, and cognitive effects of supratherapeutic doses of triazolam, pentobarbital, and GHB. *Neuropsychopharmacology*. 2006;31:2537-2551.

Carter LP, Wu H, Chen W, Matthews MM, Mehta AK, Hernandez RJ, et al. Novel gamma-hydroxybutyric acid (GHB) analogs share some, but not all, of the behavioral effects of GHB and GABAB receptor agonists. *J Pharmacol Exp Ther*. 2005; 313:1314-1322.

Caudill BD, Liscomb TR. Modeling influences on alcoholics' rates of alcohol consumption. *J Appl Behav Anal*. 1980;13:355– 365.

Chin RL, Sporer KA, Cullison B, Dyer JE, Wu TD. Clinical course of gamma-hydroxybutyrate overdose. *Ann Emerg Med.* 1998;31:716-722.

Colfax GN, Mansergh G, Guzman R, Vittinghoff E, Marks G, Rader M, et al. Drug use and sexual risk behavior among gay and bisexual men who attend circuit parties: a venue-based comparison. *J Acquir Immune Defic Syndr*. 2001;28:373-379.

Colombo G, Agabio R, Balaklievskaia N, Diaz G, Lobina C, Reali R, et al. Oral self-administration of gamma-hydroxybutyric acid in the rat. *Eur J Pharmacol*. 1995;285:103–107.

Colombo G, Agabio R, Diaz G, Fà M, Lobina C, Reali R, et al. Gamma-hydroxybutyric acid intake in ethanol- preferring sP and -nonpreferring sNP rats. *Physiol Behav*. 1998;64:197–202.

Couper FJ, Thatcher JE, Logan BK. Suspected GHB overdoses in the emergency department. *J Anal Toxicol*. 2004;28:481-4.

Cruz HG, Ivanova T, Lunn ML, Stoel M, Slesinger PA, Lüscher C. Bi-directional effects of GABA(B) receptor agonists on the mesolimbic dopamine system. *Nat Neurosci*. 2004;7:153–159.

Darke SG, Ross JE, Hall WD. Benzodiazepine use among injecting heroin users. *Med J Aust*. 1995;162:645-647.

Davidson D, Hutchison K, Dagon C, Swift R. Assessing the stimulant effects of alcohol in humans. *Pharmacol Biochem Behav*. 2002;72:151-156.

Degenhardt L, Darke S, Dillon P. GHB use among Australians: characteristics, use patterns and associated harm. *Drug Alcohol Depend*. 2002;67:89-94.

Degenhardt L, Darke S, Dillon P. The prevalence and correlates of gamma-hydroxybutyrate (GHB) overdose among Australian users. *Addiction*. 2003 ;9:199-204.

Drasbek KR, Christensen J, Jensen K. Gamma-hydroxybutyrate--a drug of abuse. *Acta Neurol Scand*. 2006;114:145-156.

Druid H, Holmgren P, Ahlner J. Flunitrazepam: an evaluation of use, abuse and toxicity. *Forensic Sci Int.* 2001; 22: 136–141.

Dyer JE. Gamma-Hydroxybutyrate: a health-food product producing coma and seizurelike activity. *Am J Emerg Med*. 1991;9:321-324.

Dyer JE, Reed JH. Álcali burns from illicit manufacture of GHB. *J Toxicol Clin Toxicol*. 1997;35:553.

Dyer JE, Roth B, Hyma BA. Gamma-hydroxybutyrate withdrawal syndrome. *Ann Emerg Med.* 2001; 37:147-153.

Eckardt MJ, File SE, Gessa GL, Grant KA, Guerri C, Hoffman PL, et al. Effects of moderate alcohol consumption on the central nervous system. *Alcohol Clin Exp Res*. 1998;22:998-1040.

Elian AA. Determination of endogenous gamma-hydroxybutyric acid (GHB) levels in antemortem urine and blood. *Forensic Sci Int*. 2002;128:120–122.

Elliott SP. Nonfatal instances of intoxication with gamma-hydroxybutyrate in the United Kingdom. *Ther Drug Monit*. 2004;26:432-440.

ElSohly MA, Salamone SJ. Prevalence of drugs used in cases of alleged sexual assault. *J Anal Toxicol*. 1999;23:141-146.

Espinosa G, Miro O, Nogue S, To-Figuera J, Sánchez M, Coll-Vinent B. [Liquid ecstasy poisoning: study of 22 cases] [Article in Spanish]. *Med Clin (Barc)*. 2001;117:56-58.

European monitoring Centre for Drugs and Drug Addiction. Annual report 2004: the state of the drugs problem in the European Union and Norway. [monograph on the Internet]. Luxembourg: Office for Official Publications of the European

Communities; 2004. [Cited 2007 Sep 16]. Available from: http://ar2004.emcdda.europa.eu/download/ar2004-en.pdf.

Farré M, Teran MT, Cami J. A comparison of the acute behavioral effects of flunitrazepam and triazolam in healthy volunteers. *Psychopharmacology (Berl)*. 1996;125:1-12.

Farré M, Terán MT, Roset PN, Mas M, Torrens M, Camí J. Abuse liability of flunitrazepam among methadone-maintained patients. *Psychopharmacology (Berl)*. 1998;140:486-495.

Farré M, Abanades S. Farmacología del poliabuso de drogas (Pharmacology of drug polyabuse). In: Pérez de los Cobos J, Valderrama JC, Cervera G, Rubio G, editors. Tratado SET de Trastornos Adictivos. Madrid: Editorial Médica Panamericana, S.A; 2006.p. 10-13.

Ferrara SD, Zotti S, Tedeschi L, Frison G, Castagna F, Gallimberti L, et al. Pharmacokinetics of gamma-hydroxybutyric acid in alcohol dependent patients after single and repeated oral doses. *Br J Clin Pharmacol.* 1992;34:231-235.

Ferrara SD, Tedeschi L, Frison G, Orlando R, Mazzo M, Zordan R, Padrini R, Palatini P. Effect of moderate or severe liver dysfunction on the pharmacokinetics of gamma-hydroxybutyric acid. *Eur J Clin Pharmacol*. 1996;50:305-310.

Ferrara SD, Giorgetti R, Zancaner S, Orlando R, Tagliabracci A, Cavarzeran F, et al. Effects of single dose of gamma-hydroxybutyric acid and lorazepam on psychomotor performance and subjective feelings in healthy volunteers. *Eur J Clin Pharmacol*. 1999;54:821-827.

Gerra G, Caccavari R, Fontanesi B, Marcato A, Fertonani Affini G, Maestri D, et al. Flumazenil effects on growth hormone response to gamma-hydroxybutyric acid. *Int Clin Psychopharmacol*. 1994;9:211-215.

Gibson KM, Gupta M, Pearl PL, Tuchman M, Vezina LG, Snead OC 3rd, et al. Significant Behavioral Disturbances in Succinic Semialdehyde Dehydrogenase (SSADH) Deficiency (Gamma-Hydroxybutyric Aciduria). *Biol Psychiatry*. 2003;54:763-768.

Gahlinger PM. Club drugs: MDMA, gamma-hydroxybutyrate (GHB), Rohypnol, and ketamine. *Am Fam Physician*. 2004;69:2619-2626.

Gobaille S, Schleef C, Hechler V, Viry S, Aunis D, Maitre M. Gamma-hydroxybutyrate increases tryptophan availability and potentiates serotonin turnover in rat brain. *Life Sci.* 2002;70:2101–2112.

Goddard L**.** Smoking and drinking among adults, General Household Survey, 2005. [monograph on the Internet]. London, UK: Office for National Statistics; 2005. [Cited 2008 Jan 1]. Available from: http://www.statistics.gov.uk/downloads/theme_compendia/GHS05/GHS2005_SmokingandDrinking_Report.pdf.

Gonzalez A, Nutt DJ. Gamma hydroxy butyrate abuse and dependency. *J Psychopharmacol*. 2005;19:195-204.

Griffiths RR, Bigelow GE, Ator NA. Principles of initial experimental drug abuse liability assessment in humans. *Drug Alcohol Depend*. 2003;70(3 Suppl):S41-54

Grove-White IG, Kelman GR. Effect of methohexitone, diazepam and sodium 4-hydroxybutyrate on short-

term memory. *Br J Anaesth*. 1971;43:113-116.

Hajak G, Rodenbeck A, Bandelow B, Friedrichs S, Huether G, Rüther E. Nocturnal plasma melatonin levels after flunitrazepam administration in healthy subjects. *Eur Neuropsychopharmacol*. 1996;6:149-153.

Halkitis PN, Palamar JJ. GHB use among gay and bisexual men. *Addict Behav*. 2006;31:2135-2139.

Harrington RD, Woodward JA, Hooton TM, Horn JR. Life-threatening interactions between HIV-1 protease inhibitors and the illicit drugs MDMA and gamma-hydroxybutyrate. *Arch Intern Med*. 1999; 159:2221–2224

Heishman SJ, Arasteh K, Stitzer ML. Comparative effects of alcohol and marijuana on mood, memory, and performance. *Pharmacol Biochem Behav*. 1997;58:93-101.

Helrich M, Mcaslan TC, Skolnik S, Bessman SP. Correlation of blood levels of 4-Hydroxybutyrate with state of consciousness. *Anesthesiology*. 1964;25:771-775.

Hernandez-Lopez C, Farre M, Roset PN, et al. 3,4-Methylenedioxymethamphetamine (ecstasy) and alcohol interactions in humans: psychomotor performance, subjective effects, and pharmacokinetics. *J Pharmacol Exp Ther*. 2002;300:236-244.

Hicks AR, Kapusta DR, Varner KJ. Mechanisms Underlying the Sympathomimetic Cardiovascular Responses Elicited by [gamma]-Hydroxybutyrate. *Journal of Cardiovascular Pharmacology*. 2004;44:631-638.

Hoes MJ, Vree TB, Guelen PJ. Gamma-hydroxybutyric acid as hypnotic. Clinical and pharmacokinetic evaluation of gamma-hydroxybutyric acid as hypnotic in man. *Encephale*. 1980;6:93-99.

Hoffman-La Roche F**.** [homepage on the Internet] Roche History. F.Hoffmann-La Roche Ltd.; c1996-2008 [updated 2008 January 21; cited 2008 February 1]. Available from http://www.roche.com/home/company/com_hist.htm.

Holford GH. Clinical Pharmacokinetics of ethanol. *Clinical Pharmacokinetics*. 1987;13:273-292.

Holdstock L, de Wit H. Individual differences in the biphasic effects of ethanol. *Alcohol Clin Exp Res*. 1998;22:1903–1911.

Ingels M, Rangan C, Bellezzo J, Clark RF. Coma and respiratory depression following the ingestion of GHB and its precursors: three cases. *J Emerg Med*. 2000;19:47-50.

Johnston LD, O'Malley PM, Bachman JG, Schulenberg JE. Overall, illicit drug use by American teens continues gradual decline in 2007. [monograph on the Internet ohttp://www.monitoringthefuture.org/data/07data.html/.

Kavanagh PV, Kenny P, Feely J. The urinary excretion of gammahydroxybutyric acid in man. *J Pharm Pharmacol*. 2001;53:399–402.

Kleinschmidt S, Schellhase C, Mertzlufft. Continuous sedation during spinal anaesthesia: gamma-hydroxybutyrate vs. propofol. *Eur J Anaesthesiol*. 1999;16:23-30.

Kril JJ, Halliday GM. Brain shrinkage in alcoholics: a decade on and what

have we learned?. *Prog Neurobiol*. 1999;58:381-387.

Laborit H. Sodium 4-Hydroxibutyrate. *Int J Neuropharmacol*. 1964;3:433-451.

Labouèbe G, Lomazzi M, Cruz HG, Creton C, Luján R, Li M, et al. RGS2 modulates coupling between GABAB receptors and GIRK channels in dopamine neurons of the ventral tegmental area. *Nat Neurosci*. 2007;10:1559-1568.

Lader M. Anxiolytic drugs: dependence, addiction and abuse. *Eur Neuropsychopharmacol*. 1994;4:85-91.

Lapierre O, Montplaisir J, Lamarre M, Bedard MA. The effect of gamma - hydroxybutyrate on nocturnal and diurnal sleep of normal subjects: further considerations on REM sleep-triggering mechanisms. *Sleep*. 1990;13:24-30.

LeBeau MA, Christenson RH, Levine B, Darwin WD, Huestis MA. Intra- and interindividual variations in urinary concentrations of endogenous gamma-hydroxybutyrate. *J Anal Toxicol.* 2002 ;26:340-346**.**

LeBeau MA, Montgomery MA, Morris-Kukoski C, Schaff JE, Deakin A, Levine B. A comprehensive study on the variations in urinary concentrations of endogenous gamma-hydroxybutyrate (GHB). *J Anal Toxicol*. 2006;30:98–105.

Leppäluoto J, Vuolteenaho O, Arjamaa O, Ruskoaho H. Plasma immunoreactive atrial natriuretic peptide and vasopressin after ethanol intake in man. *Acta Physiol Scand*. 1992;144:121-127.

Li J, Stokes SA, Woeckener A. A tale of novel intoxication: seven cases of gamma-hydroxybutyric acid overdose. *Ann Emerg Med.* 1998;31:723-728.

Li Q, Kuhn CM, Wilson WA, Lewis DV. Effects of gamma hydroxybutyric acid on inhibition and excitation in rat neocortex. *Neuroscience*. 2007;150:82-92.

Lieber CS. Alcohol and the liver: Metabolism of alcohol and its role in hepatic and extrahepatic diseases. *Mt. Sinai J. Med*. 2000;67:84-94.

Liechti ME, Kupferschmidt H. Gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL): analysis of overdose cases reported to the Swiss Toxicological Information Centre. *Swiss Med Wkly*. 2004;134:534-537.

Liechti ME, Kunz I, Greminger P, Speich R, Kupferschmidt H. Clinical features of gamma-hydroxybutyrate and gamma-butyrolactone toxicity and concomitant drug and alcohol use. *Drug Alcohol Depend*. 2006;81:323-326.

Lora-Tamayo C, Tena T, Rodriguez A, Sancho JR, Molina E. Intoxication due to 1,4-butanediol. *Forensic Sci Int.* 2003;133:256-259.

Lobina C, Agabio R, Reali R, Gessa GL, Colombo G. Contribution of GABA-A and GABA-B receptors to the discriminative stimulus produced by gamma-hydroxybutyric acid. *Pharmacol Biochem Behav*. 1999;64:363–365.

Lucchesi LM, Pompéia S, Manzano GM, Kohn AF, Galduroz JC, Bueno OF, Tufik S. Flunitrazepam-induced changes in neurophysiological, behavioural, and subjective measures used to assess sedation. *Prog Neuropsychopharmacol Biol Psychiatry*. 2003;27:525-533.

Maitre M. The gamma-hydroxybutyrate signalling system in brain: organization and functional implications. *Prog Neurobiol.* 1997; 51:337-361.

Mansbach RS, Feltner DE, Gold LH, Schnoll SH. Incorporating the assessment of abuse liability into the drug discovery and development process. *Drug Alcohol Depend*. 2003;70(3 Suppl):S73-85.

Martellotta MC, Fattore L, Cossu G, Fratta W. Rewarding properties of gamma-hydroxybutyric acid: an evaluation through place preference paradigm. *Psychopharmacol*. 1997;132:1–5.

Martellotta MC, Cossu G, Fattore L, Gessa GL, Fratta W. Intravenous self-administration of gamma-hydroxybutyric acid in drug-naive mice. *Eur Neuropsychopharm*. 1998;8:293–296.

Mason PE, Kerns WP. Gamma hydroxybutyric acid (GHB) intoxication. *Acad Emerg Med*. 2002;9:730-739.

Mattila MJ, Palva E, Seppälä T, Ostrovskaya RU. Actions and interactions with alcohol of drugs on psychomotor skills: comparison of diazepam and gamma-hydroxybutyric acid. *Arch Int Pharmacodyn Ther*. 1978;234:236-246.

Mattila MA, Larni HM. Flunitrazepam: a review of its pharmacological properties and therapeutic use. *Drugs*. 1980;20:353-374.

Mattison AM, Ross MW, Wolfson T, Franklin D. Circuit party attendance, club drug use, and unsafe sex in gay men. *J Subst Abuse*. 2001;13:119-126.

Mayo-Smith MF. Pharmacological management of alcohol withdrawal. A meta-analysis and evidence-based practice guideline. American Society of Addiction working group on pharmacological management of alcohol withdrawal. *JAMA* 1997;278:144–151.

McCambridge J, Winstock A, Hunt N, Mitcheson L. 5-Year trends in use of hallucinogens and other adjunct drugs among UK dance drug users. *Eur Addict Res*. 2007;13:57-64.

McIntosh C, Chick J. Alcohol and the nervous system. *J Neurol Neurosurg Psychiatry*. 2004;75 S3:S16-21.

Metcalf DR, Emde RN, Stripe JT. An EEG-behavioral study of sodium hydroxybutyrate in humans. *Electroencephalogr Clin Neurophysiol.* 1966;20:506-512.

Michael H, Harrison M. Best evidence topic report: endotracheal intubation in gamma-hydroxybutyric acid intoxication and overdose. *Emerg Med J*. 2005;22:43.

Miotto K, Darakjian J, Basch J, Murray S, Zogg J, Rawson R. Gamma-hydroxybutyric acid: patterns of use, effects and withdrawal. *Am J Addict*. 2001;10:232-241.

Ministerio de Sanidad y Consumo. Encuesta Domiciliaria sobre Alcohol y Drogas en España 2005-2006-análisis y principales resultados. [monograph on the Internet]. Madrid: Delegación del Gobierno para el Plan Nacional sobre Drogas; 2006 [cited 2008 Feb 2]. Available from: http://www.pnsd.msc.es/Categoria2/observa/pdf/Domiciliaria2005-2006.pdf.

Mintzer MZ, Griffiths RR. Flunitrazepam and triazolam: a comparison of behavioral effects and abuse liability. *Drug Alcohol Depend*. 1998;53:49-66.

Mintzer MZ, Griffiths RR. An abuse liability comparison of flunitrazepam and triazolam in sedative drug abusers. *Behav Pharmacol*. 2005;16:579-584.

Miró O, Nogué S, Espinosa G, To-Figueras J, Sanchez M. Trends in illicit drug emergencies: the emerging role of gamma-hydroxybutyrate. *J Toxicol Clin Toxicol*. 2002;40:129-135.

Morris ME, Hu K, Wang Q. Renal clearance of gamma-hydroxybutyric acid in rats: increasing renal elimination as a detoxification strategy. *J Pharmacol Exp Ther*. 2005;313:1194-1202.

Nava F, Carta G, Bortolato M, Gessa GL. Gamma-Hydroxybutyric acid and baclofen decrease extracellular acetylcholine levels in the hippocampus via GABA(B) receptors. *Eur J Pharmacol*. 2001;430:261–263.

Navaratnam V, Foong K. Opiate dependence--the role of benzodiazepines. *Curr Med Res Opin*. 1990;11:620-630.

Navarro M, Pichini S, Farré M, Ortuño J, Roset PN, Segura J, et al. Usefulness of saliva for measurement of 3,4-methylenedioxymethamphetamine and its metabolites: correlation with plasma drug concentrations and effect of salivary pH. *Clin Chem*. 2001;47:1788–1795.

Nelson T, Kaufman E, Kline J, Sokoloff L. The extraneural distribution of gamma-hydroxybutyrate. *J Neurochem*. 1981;37:1345-1348.

Nicholson KL, Balster RL. GHB: a new and novel drug of abuse. *Drug Alcohol Depend*. 2001;63:1-22.

Nimmerrichter AA, Walter H, Gutierrez-Lobos KE, Lesch OM. Double-blind controlled trial of gamma-hydroxybutyrate and clomethiazole in the treatment of alcohol withdrawal. *Alcohol Alcohol*. 2002;37:67-73.

The National Institute on Drug Abuse (NIDA) [homepage on the Internet]. InfoFacts: Rohypnol and GHB. [updated 2008 Jan 2; cited 2007 Jun 15]. Available from: http://www.drugabuse.gov/infofacts/RohypnolGHB.html

O'Connell T, Kaye L, Plosay JJ 3rd. Gamma-hydroxybutyrate (GHB): a newer drug of abuse. *Am Fam Physician*. 2000;62:2478-2483.

Ottani A, Saltini S, Bartiromo M, Zaffe D, Renzo Botticelli A, Ferrari A, Bertolini A, Genedani S. Effect of gamma-hydroxybutyrate in two rat models of focal cerebral damage. *Brain Res*. 2003 ;986:181-190.

Palamar J, Halkitis P. A qualitative analysis of GHB use among gay men: Reasons for use despite potential adverse outcomes. *International Journal of Drug Policy*. 2006;17:23-28.

Palatini P, Tedeschi L, Frison G, Padrini R, Zordan R, Orlando R, Gallimberti L, Gessa GL, Ferrara SD. Dose-dependent absorption and elimination of g-hydroxybutyric acid in healthy volunteers. *Eur J Clin Pharmacol.* 1993;45:353-356.

Palmer RB. Gamma-butyrolactone and 1,4-butanediol: abused analogues of gamma-hydroxybutyrate. *Toxicol Rev.* 2004;23:21-31.

Parks KA, Kennedy CL. Club drugs: reasons for and consequences of use. *J Psychoactive Drugs*. 2004;36:295-302.

Perez E, Chu J, Bania T. Seven days of gamma-hydroxybutyrate (GHB) use produces severe withdrawal. *Ann Emerg Med*. 2006;48:219-220.

Quéva C, Bremner-Danielsen M, Edlund A, Ekstrand AJ, Elg S, Erickson S, Johansson T, Lehmann A, Mattsson JP. Effects of GABA agonists on body temperature regulation in GABAB(1))-/- mice. *Br J Pharmacol*. 2003;140:315–322.

Raybon JJ, Boje KM. Pharmacokinetics and pharmacodynamics of gamma-hydroxybutyric acid during tolerance in rats: effects on extracellular dopamine. *J Pharmacol Exp Ther*. 2007;320:1252-1260.

Ricaurte GA, McCann UD. Recognition and management of complications of new recreational drug use. *Lancet*. 2005;365:2137-2145.

Rhodium. GHB Synthesis FAQ. [monograph on the Internet]. Grass Valley CA: Erowid; 1999 [cited 2007 Sep 29]. Available from: http://www.erowid.org/chemicals/ghb/ghb_faq2.shtml

Robinson DM, Keating GM. Sodium oxybate: a review of its use in the management of narcolepsy. *CNS Drugs*. 2007;21:337-354.

Roldán J, Frauca C, Dueñas A. [Alcohol intoxication] [Article in Spanish] *An Sist Sanit Navar*. 2003;26 Suppl 1:S129-139.

Rosen MI, Pearsall HR, Woods SW, Kosten TR. Effects of gamma-hydroxybutyric acid (GHB) in opioid-dependent patients. *J Subst Abuse Treat*. 1997;14:149-154.

Roset PN, Farré M, de la Torre R, Mas M, Menoyo E, Hernández C, et al. Modulation of rate of onset and intensity of drug effects reduces abuse potential in healthy males. *Drug Alcohol Depend*. 2001;64:285-298.

Roset PN, Farré M, Poudevida S, Abanades, S. Camí J. Evaluación experimental del potencial de abuso en humanos (Abuse liability experimental assessment in humans). In: Aspectos básicos y clínicos sobre la neurobiología de la adicción. Monografías Dr. Antonio Esteve; 2003. p. 99-108.

Roth RH, Giarman NJ. Conversion in vivo of gamma-aminobutyric to gamma-hydroxybutyric acid in the rat. *Biochem Pharmacol*. 1969;18:247-250.

Rubin BA, Giarman NJ. The therapy of experimental influenza in mice antibiotic lactones and related compounds. *Yale J Biol Med*. 1947;19:1017-1022.

Sakurada K, Kobayashi M, Iwase H, Yoshino M, Mukoyama H, Takatori T, et al. Production of gammahydroxybutyric acid in postmortem liver increases with time after death. *Toxicol Lett.* 2002;129:207–217.

San L, Tato J, Torrens M, Castillo C, Farré M, Camí J. Flunitrazepam consumption among heroin addicts admitted for inpatient detoxification. *Drug Alcohol Depend*. 1993;32:281-286.

Scharf MB, Lai AA, Branigan B, Stover R, Berkowitz DB. Pharmacokinetics of -hydroxybutyrate (GHB) in narcoleptic patients. *Sleep*. 1998;21:507-514.

Scharf MB, Baumann M, Berkowitz DV. The effects of sodium oxybate on clinical symptoms and sleep patterns in patients with fibromyalgia. *J Rheumatol*. 2003;30:1070-1074.

Schuckit MA, Greenblatt D, Gold E, Irwin M. Reactions to ethanol and diazepam in healthy young men. *J Stud Alcohol*. 1991;52:180-7.

Sevak RJ, France CP, Koek W. Neuroleptic-like effects of gamma-hydroxybutyrate: interactions with haloperidol and dizocilpine. *Eur J Pharmacol*. 2004;483:289-293.

Simmons MM, Cupp MJ. Use and abuse of flunitrazepam. *Ann Pharmacother*. 1998;32:117–119.

Snead OC 3rd, Morley BJ. Ontogeny of gamma-hydroxybutyric acid: I. Regional concentration in developing rat, monkey and human brain. *Brain Res*. 1981;227:579-589.

Snead OC 3rd, Liu CC. Gamma-hydroxybutyric acid binding sites in rat and human brain synaptosomal membranes. *Biochem Pharmacol*. 1984;33:2587-2590.

Snead OC 3rd, Gibson KM. Gamma-hydroxybutyric acid. *N Engl J Med*. 2005;352:2721-2732.

Solway J, Sadove MS. 4-Hydroxybutyrate: a clinical study. *Anesth Analg*. 1965;44:532-539.

Sporer KA, Chin RL, Dyer JE, Lamb R. Gamma-hydroxybutyrate serum levels and clinical syndrome after severe overdose. *Ann Emerg Med*. 2003;42:3-8.

Tarabar AF, Nelson LS. The gamma-hydroxybutyrate withdrawal syndrome. *Toxicol Rev*. 2004;23:45-49.

Teter CJ, Guthrie SK. A comprehensive review of MDMA and GHB: two common club drugs. *Pharmacotherapy*. 2001;21:1486-1513.

Thai D, Dyer JE, Benowitz NL, Haller CA. Gamma-hydroxybutyrate and ethanol effects and interactions in humans. *J Clin Psychopharmacol*. 2006;26:524-529.

Thai D, Dyer JE, Jacob P, Haller CA. Clinical pharmacology of 1,4-butanediol and gamma-hydroxybutyrate after oral 1,4-butanediol administration to healthy volunteers. *Clin Pharmacol Ther*. 2007;81:178-184.

Thomas G, Bonner S, Gascoigne A. Coma induced by abuse of gamma-hydroxybutyrate (GBH or liquid ecstasy): a case report. *BMJ*. 1997;314:35-36.

Timby N, Eriksson A, Bostrom K. Gamma-hydroxybutyrate associated deaths. *Am J Med*. 2000;108:518-519.

Traub SJ, Nelson LS, Hoffman RS. Physostigmine as a treatment for gamma-hydroxybutyrate toxicity: a review. *J Toxicol Clin Toxicol*. 2002;40:781-787.

US Xyrem Multicenter Study Group. A randomized, double blind, placebo-controlled multicenter trial comparing the effects of three doses of orally administered sodium oxybate with placebo for the treatment of narcolepsy. *Sleep*. 2002;25:42-49.

Uys JD, Niesink RJ. Pharmacological aspects of the combined use of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) and gamma-hydroxybutyric acid (GHB): a review of the literature. *Drug Alcohol Rev*. 2005;24:359-368.

Van Cauter E, Plat L, Scharf MB, Leproult R, Cespedes S, L'Hermite-Baleriaux M. Simultaneous stimulation of slow-wave sleep and growth hormone secretion by g-hydroxy-butyrate in normal young men. *J Clin Invest*. 1997;100:745-753.

Varela M, Nogué S, Orós M, Miró O. Gamma hydroxybutirate use for

sexual assault. *Emerg Med J*. 2004;21:255-256.

Various authors. 10th Special Report to the U.S. Congress on Alcohol and Health. [monograph on the Internet]. Washington DC: US: Department of Health and Human Services; 2000. [cited 2007 Oct 8]. Available from: http://pubs.niaaa.nih.gov/publications/10report/intro.pdf.

Vickers MD. G-Hydroxybutyric acid. *Int Anesthesiol Clin*. 1969;7:75-89.

Virtue RW, Lund LO, Beckwitt HJ, Vogel JH. Cardiovascular reactions to -hydroxybutyrate in man. *Can Anaesth Soc J*. 1966;13:119-123.

Volpi R, Chiodera P, Caffarra P, Scaglioni A, Malvezzi L, Saginario A, et al. Muscarinic cholinergic mediation of the GH response to gamma-hydroxybutyric acid: neuroendocrine evidence in normal and parkinsonian subjects. *Psychoneuroendocrinology*. 2000;25:179-185.

Vree TB, Damsma J, van den Bogert AG, van der Kleijn E. Pharmacokinetics of 4-hydroxybutyric acid in man, rhesus monkey and dog. *Anaesthesiol Intensiv med*. 1978;110:21-39.

Walker DJ, Zacny JP. Lack of effects of ethanol pretreatment on the abuse liability of nitrous oxide in light and moderate drinkers. *Addiction*. 2001;96:1839-1845.

Wang Q, Morris ME. The role of monocarboxylate transporter 2 and 4 in the transport of gamma-hydroxybutyric acid in mammalian cells. *Drug Metab Dispos*. 2007;35:1393-1399.

Weir E. Raves: a review of the culture, the drugs and the prevention of harm. *CMAJ.* 2000;162:1843-1848.

Winstock AR, Griffiths P, Stewart D. Drugs and the dance music scene: a survey of current drug use patterns among a sample of dance music enthusiasts in the UK. *Drug Alcohol Depend*. 2001;64:9-17.

Winter JC. The stimulus properties of gamma-hydroxybutyrate. *Psychopharmacol*. 1981;73:372–375.

Wise RA, Bozarth MA. A psychomotor stimulant theory of addiction. *Psychol Rev*. 1987;94:469-492.

Wong CG, Chan KF, Gibson KM, Snead OC. Gamma-hydroxybutyric acid: neurobiology and toxicology of a recreational drug. *Toxicol Rev*. 2004;23:3-20.

Woods JH, Winger G. Abuse liability of flunitrazepam. *J Clin Psychopharmacol*. 1997; 17 (3 Suppl 2):1S–57S.

Woolverton WL, Rowlett JK, Winger G, Woods JH, Gerak LR, France CP. Evaluation of the reinforcing and discriminative stimulus effects of gamma-hydroxybutyrate in rhesus monkeys. *Drug Alcohol Depend*. 1999:54;137–143.

Wu LT, Schlenger WE, Galvin DM. Concurrent use of methamphetamine, MDMA, LSD, ketamine, GHB, and flunitrazepam among American youths. *Drug Alcohol Depend*. 2006;84:102-113.

Xyrem (sodium oxybate) [Package insert]. Palo Alto, CA: Jazz Pharmaceuticals;, 2005.

Yamada Y, Yamamoto J, Fujiki A, Hishikawa Y, Kaneko Z. Effect of butyrolactone and gamma- hydroxybutyrate on the EEG and sleep cycle in man. *Electroencephalogr Clin Neurophysiol*. 1967;22:558-562.

Zvosec DL, Smith SW. Agitation is common in gamma-hydroxybutyrate toxicity. *Am J Emerg Med*. 2005;23:316-320.

Zvosec DL, Smith SW, McCutcheon JR, Spillane J, Hall BJ, Peacock EA. Adverse events, including death, associated with the use of 1,4-butanediol. *N Engl J Med.* 2001; 344:87-94.

9. Abbreviations

3-HF: 3-hydroxyflunitrazepam

7-AF: 7-aminoflunitrazepam

ADH: alcohol dehydrogenase

ALDH: aldehyde dehydrogenase

AMP: adenosine monophosphate

cAMP: cyclic adenosine monophosphate

AMPA: α-amino-3-hydroxy-5-methylisoxazole-4- propionic acid receptor

AP: allo-pregnanolone

AV: atrioventricular

ARCI: addiction research center inventory

AUC: area under the curve

BBB: blood brain Barrier

BD: 1,4-butanediol

Ca2+: Calcium ions

Cmax: peak plasma concentration

CNS: central nervous system

CT: computed tomography

CYP: cytocrome p-450

DBP: diastolic blood pressure

DMF: N-desmethylflunitrazepam

DSST: dygit symbol substitution test

ECG: electrocardiogram

EEG: electroencephalogram

FDA: U.S. Food and Drug Administration

GABA: gamma-aminobutyric acid

$GABA_A$: gamma-aminobutyric acid receptor type A

$GABA_B$: gamma-aminobutyric acid receptor type B

GBL: γ-butyrolactone

GC-MS: gas chromatography–mass spectrometry

GCS: glasgow coma scale

GH: growth hormone

GHB: gamma-hydroxybutyrate

GHBR: gamma-hydroxybutyrate receptor

GHV: gamma-hydroxyvalerate

GIRK: g-protein-coupled inwardly rectifying potassium channel

GVL: gamma-valerolactone

I.P: intraperitoneal

I.V: intravenous

Kd: dissociation constant

KO: knock-out

MAP kinase: mitogen-activated protein kinase

MCT: monocarboxylate transporter

MDMA: methylendioxymetamphetamine

MR: magnetic resonance

Na GHB: sodium GHB

NAD+: nicotinamide adenine dinucleotide

NADP+: nicotinamide adenine dinucleotide phosphate

NMDA: n-methyl-D-aspartic acid

NREM: non-rapid eye movement

OH: hydroxyl group

PCP: phencyclidine

PD: pharmacodynamics

PK: pharmacokinetics

pKa: cologarithm of the equilibrium constant for the dissociation of a weak acid

PO: *per os*

REM: rapid eye movement

RGS: regulator of G protein signalling

SBP: systolic blood pressure

SSA: succinic semialdehyde

SSADH: succinic semialdehyde dehydrogenase

SSR: succinic semialdehyde reductase

$t_{1/2}$: elimination half-life

t_{max}: time to peak plasma concentration

THDOC: allo-tetrahydrodeoxy corticosterone

VAS: visual analogue scale

V_D: volume of distribution

VESSPA: evaluation of the subjective effects of substances with potential of abuse

VTA: ventral tegmental area

10. Appendix

Abanades S, Peiró AM, Farré M. Club drugs: old medicines as new party drugs. Med Clin (Barc). 2004;123:305-311.

Club drugs: los viejos fármacos son las nuevas drogas de la fiesta

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Artículo 94.925

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En los últimos años se ha utilizado el término *club drugs* para definir a un grupo heterogéneo de sustancias químicas que se consumen con ánimo recreativo y que está en permanente evolución. Estas sustancias han sido utilizadas por gran cantidad de usuarios, primero en la cultura *rave* y posteriormente por la denominada cultura de club. Estas culturas son movimientos caracterizados por la búsqueda de amplificación de sensaciones, mediante la combinación de música electrónica, baile maratoniano y consumo de sustancias.

Tras unos años en los que predominaba el consumo de anfetaminas de diseño y derivados en estos colectivos, parece que aumenta el uso de otro tipo de sustancias de efectos fundamentalmente alucinógenos.

En esta revisión se discuten la farmacología, consumo recreacional, efectos adversos y la intoxicación por 4 de estas sustancias: la ketamina, el dextrometorfano, el óxido nitroso y el gammahidroxibutirato («éxtasis» líquido). Estas sustancias tienen en común ser a la vez drogas de abuso y fármacos o medicamentos con indicaciones concretas en terapéutica, y un aumento importante de su consumo en los últimos años. Además se realiza una descripción de las culturas *rave* y de club.

Palabras clave: Club drugs. Ketamina. Dextrometorfano. Óxido nitroso. Gammahidroxibutirato. GHB. *Rave*.

Club drugs: old medicines as new party drugs

During the last few years the term club drugs has been used for defining an heterogeneous group of chemical substances in permanent evolution, that are consumed for recreational purposes. These substances have been extensively used, firstly by the Rave culture and later by the so called Club culture. These movements are characterized by the search of amplified sensations, by means of the combination of electronic music, marathon dancing and substance abuse.

After years with a predominating consumption of designer amphetamines in these groups, it seems that the use of another type of substances is increasing, fundamentally drugs with hallucinogenic effects. This review focus in four of these substances; ketamine, dextromethorphan, nitrous oxide and gamma-hydroxybutyric acid (GHB, liquid ecstasy), and includes a discussion of their pharmacology, recreational use, adverse effects and patient management.

These drugs are, at he same time, drugs of abuse and medicines with concrete indications in therapeutics, with an important increase of their consumption in the last few years. The Rave and Club cultures are also described.

Key words: Club drugs. Ketamine. Dextromethorphan. Nitrous Oxide. Gamma-hydroxybutyric acid. GHB. Rave.

En los últimos años se ha utilizado el término *club drugs* para definir a un grupo heterogéneo de sustancias químicas consumidas con ánimo recreativo[1]. En este grupo se suele incluir a la 3,4-metilendioximetanfetamina (MDMA o «éxtasis») y sus derivados, el gammahidroxibutirato (GHB, «éxtasis líquido»), la metanfetamina *(speed)*, la ketamina, el flunitrazepam, el dextrometorfano, el óxido nitroso, la lisergida (LSD) o los hongos tipo *Psylocibe*. En realidad abarca a múltiples sustancias de diferente índole, que frecuentemente se consumen de forma simultánea en lo que se denominaba la cultura *rave*[2]. Esta se caracteriza por fiestas clandestinas, música electrónica y uso de drogas de abuso, con una filosofía en consonancia con el fenómeno hippy de la década de los sesenta, pero con el nivel de sofisticación de la nueva era. El eslogan «PLUR» *(peace, love, unity and respect)* ha sido utilizado para definir los valores de este movimiento, nutrido en buena parte de personas integradas plenamente en la sociedad, que nada tienen que ver con el estereotipo de «drogadicto» de décadas pasadas[3].

Para intentar entender el auge del consumo de estas sustancias es necesario conocer este fenómeno sociocultural. En España, al igual que en otros países, este fenómeno se ha transformado en parte en lo que se denomina la «cultura de club», o coloquialmente, «la fiesta». Si bien durante muchos años el consumo de estas sustancias lo realizaban grupos reducidos de personas en ceremonias o fiestas de culto (fenómeno *rave*), la prohibición de estas fiestas clandestinas y el gran negocio, tanto legal como ilegal, que supone esta forma de ocio trasladó el fenómeno a las pistas de baile de las discotecas o clubs de todo el mundo (cultura de club)[4]. Este fenómeno se caracteriza por la búsqueda de amplificación de sensaciones, de un estado trascendente de euforia, mediante la combinación de música electrónica, baile maratoniano y consumo de sustancias[5]. Durante las sesiones de baile, que se pueden prolongar a lo largo de todo un fin de semana, se consumen de forma simultánea o encadenada diversas sustancias buscando sus propiedades euforizantes o alucinógenas. Hoy día es muy difícil encontrar consumidores puros de una sustancia, y son múltiples los estudios epidemiológicos que demuestran la frecuente asociación de varias de ellas[6-8]. Si bien la MDMA o sus derivados han sido objeto de numerosas investigaciones y publicaciones, existen otras sustancias englobadas dentro de este fenómeno de club de las que se dispone de un nivel menor de conocimiento, pero que han experimentado un auge tanto en su consumo como en el número de casos atendidos en los servicios de urgencias de los hospitales.

Esta revisión se centrará en el estudio de 4 de estas sustancias: la ketamina, el dextrometorfano, el óxido nitroso y el GHB. Los 3 primeros producen durante la intoxicación los llamados efectos disociativos, es decir, un estado de analgesia profunda y amnesia con conservación de la conciencia y de los reflejos protectores. Además, aparecen efectos psicomiméticos intensos con sensación de separación entre cuerpo y mente (en inglés, *out of body experience*). Es decir, se produce una disociación entre el sujeto y el entorno que recuerda a un estado catatónico. Los ojos permanecen abiertos mientras el sujeto se mantiene consciente pero aislado del mundo externo por una integración anómala de los estímulos. Es capaz, de esta manera, de experimentar ensoñaciones y «viajes» o *trips*, que son, a la vez, el objetivo bus-

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cado por los usuarios durante la intoxicación y los efectos adversos más temidos cuando se utilizan en la clínica diaria[4,9]. Por último se revisará el GHB, sustancia que ha pasado inadvertida durante décadas y que ha registrado uno de los incrementos más espectaculares en su consumo en los últimos años, lo que ha generado un aumento importante de casos de intoxicación en los servicios de urgencias de nuestro país[10].

Estas 4 sustancias, además de consumirse en ocasiones de forma simultánea o encadenada por esta cultura de club durante las sesiones de baile, se emplean en otro tipo de ambientes y situaciones, con otras finalidades que veremos más adelante. Además, tal como se recoge en el título de esta revisión, tienen en común el ser a la vez drogas de abuso y fármacos o medicamentos con indicaciones concretas en terapéutica. Ya Paracelso decía que la dosis hacía el veneno. Así, serán la dosis, la vía de administración, la frecuencia y, sobre todo, el objetivo de la administración lo que diferenciará la frágil frontera entre droga de abuso y medicamento.

Ketamina

La ketamina es un anestésico general comercializado en nuestro país tanto para su uso en humanos como en animales. Se introdujo en el mercado durante la década de los sesenta como una alternativa de menor toxicidad a la fenciclidina. Durante su uso clínico se observó que se asociaba, al igual que la fenciclidina, a ensoñaciones desagradables, delirios, alucinaciones y sensaciones de despersonalización durante el despertar de la anestesia (reacciones de emergencia)[9]. Su uso clínico ha quedado restringido a situaciones como la anestesia en el choque hemodinámico, la sedación intramuscular de pacientes no cooperantes (como la población pediátrica), distintos procedimientos de corta duración en cuidados intensivos o intervenciones extremadamente dolorosas como los cambios de apósitos en los quemados[11]. En el Tercer Mundo se sigue utilizando como anestésico dado su buen perfil de seguridad. También se emplea en el tratamiento del dolor crónico[12]. Además, es un modelo experimental de psicosis inducida al producir algunos de los típicos síntomas positivos y negativos de la esquizofrenia[13-15].

Farmacología

La ketamina es un antagonista no competitivo del receptor glutamatérgico del tipo N-metil-D-aspartato (NMDA). Estructuralmente es una arilciclohexilamina en estrecha relación con la fenciclidina. Se presenta comercialmente como una mezcla racémica al 50% de 2 isómeros ópticos o enantiómeros, la S(+)-ketamina y la R(–)-ketamina, la primera de las cuales es 4 veces más potente que la segunda. En estudios de tomografía por emisión de positrones se ha visto que la magnitud de la unión de la S(+)-ketamina a sus receptores específicos es directamente proporcional a los efectos psicodislépticos que produce[16]. Además de su acción como antagonista del receptor NMDA, interactúa también con receptores opioides kappa y, en menor medida, receptores colinérgicos[17]. También estimula la liberación de noradrenalina, dopamina y serotonina, e inhibe la recaptación de las 3 monoaminas antes citadas por acción sobre los transportadores de membrana[18]. Estas acciones justifican en parte la estimulación simpática que se produce tras su administración y su capacidad de producir abuso y dependencia, con liberación de dopamina en el núcleo *accumbens* al igual que otras drogas de abuso[19].

La ketamina tiene una buena disponibilidad tras inyección intramuscular y después de su inhalación intranasal. Se absorbe peor por vía oral al sufrir un importante fenómeno de primer paso. Presenta un volumen de distribución de 3 l/kg, se une a las proteínas plasmáticas en un 50%, se metaboliza en el hígado y su principal metabolito, la norketamina, es mucho menos activo. Tiene una semivida de eliminación ($t_{1/2}$) de 2-3 h, con una excreción de hasta el 70% por vía renal[20].

Es un anestésico eficaz con propiedades analgésicas muy importantes. A dosis bajas produce estimulación simpática con aumento de la frecuencia cardíaca, el gasto cardíaco y la presión arterial. A las dosis habituales no tiene efecto depresor sobre la ventilación[9].

Consumo recreacional

La utilización de la ketamina como droga de abuso comienza en la década de los ochenta en EE.UU. Llega a Europa posteriormente y a España, como muchas otras drogas, a través de la isla de Ibiza, donde en los años noventa se inicia su consumo en las salas de bailes o clubs junto a la MDMA, GHB y resto de *club drugs*. A la ketamina se la denomina *K, keta, vit K, kit-kat, cat valium, super K* o *special K*. Se introdujo en el mercado en forma de comprimidos y los primeros consumos fueron accidentales en usuarios que pensaban que en realidad se trataba de MDMA, lo que generó efectos desagradables no esperados y cierta mala fama[4]. Hoy día, el consumo más importante se da asociado al resto de *club drugs* durante las sesiones de baile en las discotecas. Se presenta como polvo blanco similar a la cocaína y se administra por vía nasal. Esto ha sido clave para el aumento de su uso en los últimos años, ya que ha llevado a que un gran número de usuarios la haya probado pensando que consumían cocaína o metanfetamina *(speed)* durante las sesiones de baile[21]. Además existe un consumo más controlado por parte de los que podrían denominarse «buscadores de nuevas sensaciones» o «psiconautas», que la utilizan en un ambiente relajado buscando sus propiedades alucinógenas, normalmente por vía intramuscular[22]. En una encuesta del año 1999 en el Reino Unido, con datos posiblemente extrapolables a nuestro medio, entre los seguidores de la música tecno el 25% la había probado alguna vez, por encima del GHB o de la heroína[23].

La ketamina del mercado ilegal procede de la distribución tanto para uso médico en humanos como, de forma preferente, para uso en veterinaria, donde se utiliza en cirugía menor en animales como perros y gatos. Este uso veterinario ha hecho que a menudo se la denomine «anestésico o droga para caballos»[24]. La ketamina se suele extraer del contenido de las ampollas del producto comercial. Mediante calentamiento se evapora el líquido (en el argot, «se cocina») y el sobrante del secado se procesa para presentarla como polvo blanco apto para su uso recreacional. No todos los preparados comerciales contienen el mismo tipo de excipientes, lo que puede implicar diferentes efectos[22].

La dosis depende de la vía de administración y los efectos son dependientes de la dosis. Comienzan 2-4 min después de una inyección intramuscular, 5-10 min tras su consumo intranasal y 10-30 min después de su ingesta oral. La duración de sus efectos varía también con la vía de administración. El efecto más importante es la sensación de disociación del cuerpo, con pérdida de la coordinación y del equilibrio. A dosis bajas produce un cierto bienestar que se acompaña de la sensación de estar flotando. Incrementos de la dosis se siguen de cambios en la corporalidad y las percepciones, con sensación de fragmentación y desprendimiento del cuerpo o la percepción de que el organismo está compuesto de mate-

riales extraños como madera o metal. A menudo se habla de varios estadios en la intoxicación por esta sustancia, en los que se vive una auténtica experiencia alucinatoria que puede desembocar en la sensación de separase del organismo, en verse mientras la mente flota en el espacio *(out of body experiences)* o en entrar en lo que se denomina el «*K- hole*» y conducir a las llamadas «experiencias cercanas a la muerte». Este fenómeno lo han explicado en numerosas ocasiones personas que han estado a punto de morir o han despertado de un estado comatoso. Describen la percepción de que la conciencia ha abandonado por completo el cuerpo en un viaje a través de un túnel en el que se tiene la sensación de salir a la luz, con gran claridad de pensamiento y visiones de figuras místicas o religiosas. Se ha intentado explicar este fenómeno, en el que un gran estrés celular desencadenaría una corriente excitatoria de glutamato que podría provocar muerte neuronal. El organismo se defendería mediante el bloqueo de los receptores NMDA mediante antagonistas endógenos. El bloqueo de estos receptores durante estas situaciones sería causal de estas peculiares visiones, como ocurre tras la administración de ketamina[25].

Efectos adversos e intoxicación

La dosis máxima que se utiliza en indicaciones médicas es de unos 13 mg/kg en inyección intramuscular, mientras que las dosis psicodélicas de ketamina rara vez sobrepasan los 2 mg/kg por vía intramuscular. Se podría decir que a estas dosis es una sustancia relativamente segura, aunque se han publicado casos de intoxicación grave. La ketamina produce frecuentemente disociación del entorno, dificultad para el lenguaje, visión borrosa, insomnio y descoordinación motora en usuarios habituales de la sustancia[26]. Además de producir deterioro cognitivo durante la intoxicación, algunos estudios demuestran déficit de atención, de memoria y *flashbacks* hasta 3 días después del consumo[27,28]. A pesar de estos efectos displicentes, algunas personas desarrollan comportamientos compulsivos que pueden desembocar en períodos de gran consumo e indiferencia respecto al entorno. Así, cuando se consume de forma repetida durante un período prolongado, sus efectos recuerdan más a los de la cocaína, heroína o alcohol, con una disminución de los efectos psicodélicos a medida que se desarrolla un importante fenómeno de tolerancia[4].

El caso tipo de intoxicación es el de un joven que presenta taquicardia, alteración de la conciencia, discurso desorganizado, alucinaciones y nistagmo. Con las medidas de soporte habituales se resuelve la mayoría de los casos. Si existe agitación es conveniente disminuir la estimulación sensorial y ubicar al paciente en un ambiente tranquilo, y se puede utilizar benzodiacepinas de acción corta. Es recomendable el aporte de fluidos intravenosos hasta descartar analíticamente la posible rabdomiólisis derivada del consumo y dejar al paciente en observación hasta la normalización clínica[29].

Dextrometorfano

Es un fármaco antitusígeno que lleva más de 30 años comercializado y que no requiere receta para su dispensación. Está contenido en muchas especialidades farmacéuticas como principio activo único o en combinación para el tratamiento de los síntomas del resfriado o la gripe. A las dosis recomendadas como antitusígeno (30 mg cada 6-8 h en adultos) es un medicamento eficaz y seguro. El dextrometorfano tiene la ventaja con respecto a la codeína de producir menos efectos adversos gastrointestinales y menor potencial de abuso. En algunos países se utiliza como coadyuvante de la morfina en el tratamiento del dolor[30].

Farmacología

No se conoce de forma cierta el mecanismo de su acción antitusiva, pero se sabe que no actúa sobre receptores opioides porque la naloxona no revierte sus efectos. El dextrometorfano está desprovisto de los típicos efectos de los opiáceos, como miosis, estreñimiento o depresión respiratoria. Es un antagonista del receptor NMDA, pero en este caso es su metabolito dextrorfano el que tiene un efecto más importante y posiblemente el responsable de la mayoría de los efectos de carácter disociativo[31].

Tras su administración oral se absorbe fácilmente y se metaboliza a través del citocromo P450 2D6 a dextrorfano, que es la molécula activa farmacológicamente. Esta transformación lo ha hecho de referencia para fenotipificar a los individuos como metabolizadores rápidos y lentos de esta isoenzima[32-34]. Se conoce que existe un 5-10% de metabolizadores lentos en la población caucásica, con una deficiencia de metabolitos, y alrededor de un 5% de metabolizadores ultrarrápidos en quienes existiría un gran incremento de los metabolitos. Los metabolizadores lentos no presentan efecto antitusígeno. La semivida de eliminación es de 1,4-3,9 h para el dextrometorfano y algo mayor para su principal metabolito, el dextrorfano[35,36], y presentan una eliminación fundamentalmente renal.

Consumo recreacional

El consumo como droga de abuso se inicia en los años 1960-1970 y su uso se ha ido extendiendo[37]. En nuestro medio su consumo fuera de la prescripción médica se ha asociado a grupos de ideología *punki* en las décadas de los setenta y ochenta y ha pasado casi inadvertido durante años. En la actualidad este consumo recreacional se expande entre los llamados buscadores de nuevas sensaciones, que aprovechan sus potentes efectos alucinógenos a dosis altas para explorar nuevas dimensiones o «viajar» (psiconáutica). Su popularidad ha ido en aumento gracias a su fácil obtención, a sus rápidos efectos y a una fama de sustancia «segura», a pesar de que en ocasiones se consume en preparaciones donde aparece asociada a otros principios activos que pueden incrementar su toxicidad de forma importante. En Internet existen diversos sitios web con información para la extracción, preparado y uso de la sustancia como droga de abuso, con listas de medicamentos desde donde obtenerlo, principalmente jarabes contra la tos[38].

Se consume directamente desde los jarabes antitusígenos buscando sus potentes efectos alucinógenos, que se parecen a los producidos por la ketamina. La dosis alucinógena por vía oral es de al menos 300 mg, pero se han recomendado incluso dosis mucho mayores (de 1.500 a 2.400 mg). Los consumidores recreacionales, al igual que con la ketamina, refieren la existencia de una serie de «mesetas» durante las cuales se pasa por varias fases en las que progresivamente van apareciendo los efectos psicodélicos o alucinógenos que pueden ser tan intensos como los de la LSD.

Efectos adversos e intoxicación

A las dosis recomendadas prácticamente no tiene efectos adversos. En caso de intoxicación (deliberada o no), pueden aparecer inestabilidad cefálica, astenia, náuseas y vómitos y ataxia. También se han descrito euforia, nistagmo, midriasis o incluso coma, en preparados en combinación con otras sustancias[39,40], y casos de psicosis, distonía y síndrome serotoninérgico[41,42]. En caso de intoxicación se recomiendan medidas de soporte y puede estar indicada la determinación de concentraciones plasmáticas de paracetamol o de

antihistamínicos si se sospecha el consumo de la sustancia en preparados de combinaciones de varias sustancias. La mayoría de los casos se resuelven sin secuelas tras unas horas de medidas de soporte y observación[43]. Recientemente se ha descrito la muerte de varias personas tras el probable consumo de falsos comprimidos de MDMA que, en realidad, contenían altas dosis de dextrometorfano[44].

Óxido nitroso

El óxido nitroso (N_2O) es un gas incoloro que se ha utilizado durante años como coadyuvante para la sedación y para el mantenimiento de la anestesia. Fue descubierto a finales del siglo XVIII por el sacerdote y científico inglés J. Priestly. Sin embargo, fue el dentista norteamericano Horacio Wells quien constató por primera vez sus propiedades analgésicas tras observar cómo un individuo, tras el consumo de la sustancia y en pleno ataque de risa, sufría un importante traumatismo sin mostrar ninguna señal de dolor. En los años siguientes, la producción de euforia, sensación de bienestar y de risa, junto a la rapidez con la que se revierten sus efectos, hizo que se utilizara en espectáculos itinerantes alrededor del mundo, conocido como «gas de la risa» o «gas hilarante»[45]. Posteriormente se demostraron científicamente sus propiedades como anestésico y se ha empleado durante años fundamentalmente en odontología.

Farmacología

No se conoce exactamente su mecanismo de acción anestésico y analgésico; por este motivo se ha descrito en ocasiones como inespecífico. No parece que posea acción sobre receptores del ácido gammaaminobutírico (GABA-A). Recientemente se ha demostrado que es un antagonista del receptor NMDA y anestésico disociativo, al igual que la ketamina y el dextrometorfano[46]. También parece interaccionar con el sistema opioide con activación micra e inhibición kappa[47].
El principal efecto clínico que produce es la depresión del nivel de conciencia, a partir normalmente de concentraciones superiores al 40% de oxígeno. A concentraciones del 5% prácticamente no produce efectos subjetivos. Al incrementar a concentraciones del 10-20% comienza a aparecer cierto grado de excitación e hilaridad. Al 30-40% produce inestabilidad cefálica, dificultad para la concentración, pérdida de memoria, descoordinación motora y sensación de bienestar y euforia, que son los efectos buscados por los usuarios durante la intoxicación[48,49]. A dosis más altas induce sedación y anestesia, y puede producir alucinaciones visuales y auditivas. En anestesia se utiliza en concentraciones superiores al 40% pero sin superar el 70% para evitar la hipoxia por nitrógeno. El óxido nitroso se elimina fundamentalmente por vía pulmonar y renal y sus efectos se revierten en pocos minutos. Además de su uso como anestésico inhalado, se ha probado para el tratamiento de la dependencia de opioides y alcohol con resultados controvertidos.

Consumo recreacional

Durante el siglo XX se fue popularizando como droga de abuso, y hay algunas comunicaciones de abuso de esta sustancia principalmente entre odontólogos y anestesistas[50]. Sin embargo, diversos estudios realizados para tratar de caracterizar su potencial de abuso en humanos a dosis subanestésicas han dado lugar a resultados dispares. Parece que la sustancia produce sensaciones placenteras y de refuerzo tan sólo entre usuarios de otras drogas de abuso[51], principalmente el cannabis[52], y, según algunos autores, la capacidad de generar abuso estaría muy limitada en la población general y se daría principalmente entre estudiantes universitarios que utilizan la sustancia de forma esporádica[53]. Recientemente se ha publicado que, entre una serie de estudiantes universitarios neozelandeses, un 12% lo había usado recreacionalmente alguna vez, y un 1% lo consumía al menos mensualmente[54]. Parece, por lo tanto, que hasta ahora predomina un consumo esporádico de forma compulsiva sobre todo entre estudiantes de ciencias médicas y que no origina grandes problemas.
Para su consumo recreacional se presenta en forma de cartuchos metálicos cilíndricos llamados Wippets® o *poppers* conectados normalmente a un globo que funciona como reservorio desde donde se inhala el producto. Por lo general se inhala una mezcla del 65% de oxígeno y del 35% de óxido nitroso. Su acción se inicia a los pocos segundos tras una única administración, se prolonga unos 30 min y sus efectos desaparecen rápidamente si no se realizan nuevas inhalaciones.

Efectos adversos e intoxicación

Los efectos adversos suelen aparecer a dosis altas y son, fundamentalmente, náuseas, vómitos, depresión respiratoria, hipoxia y apneas. Se han descrito casos de hipertermia maligna y *delirium*[55]. En consumidores crónicos se produce una oxidación de la cianocobalamina (vitamina B_{12}) que puede ocasionar anemia megaloblástica, leucopenia y, secundariamente, una mielopatía subaguda e incluso neuropatía crónica[56,57].

Gammahidroxibutirato («éxtasis» líquido)

Al GHB también se le llama ácido gammahidroxibutírico u oxibato sódico. Erróneamente se lo denomina «éxtasis líquido» cuando en realidad es una sustancia sin ninguna relación con la MDMA o «éxtasis». Fue desarrollado en la década de los sesenta como derivado del GABA, frente al que presenta como ventaja el hecho de que atraviesa la barrera hematoencefálica. La primera indicación médica fue como anestésico, campo en el que se usó durante algunos años y que persiste en algunos países. Fue cayendo en desuso tras la publicación de algunos estudios en animales en los que se lo relacionaba con la aparición de actividad proconvulsiva[58]. Su venta estuvo permitida hasta 1991 en EE.UU. como suplemento dietético, usado principalmente por los llamados «culturistas», que buscaban los supuestos efectos anabolizantes derivados de la estimulación de la secreción de la hormona del crecimiento[59]. Fue retirado del mercado por la aparición de numerosos casos de intoxicación[60]. En la actualidad está comercializado como especialidad farmacéutica en varios países europeos: en Austria y Alemania como anestésico y en Italia para el tratamiento de la deshabituación alcohólica. En el año 2002, la Food and Drug Administration lo aprobó para el tratamiento de las crisis cataplejícas de la narcolepsia[61].

Farmacología

El GHB es un ácido graso de cadena corta de estructura similar al GABA que cumple criterios como neurotransmisor en el sistema nervioso central. Se encuentra en el tejido neuronal de los mamíferos con funciones fisiológicas aún no completamente dilucidadas[62].
Se sintetiza en el tejido neuronal por la succinato semialdehído reductasa a partir de succinato semialdehído (SSA). Éste proviene a su vez de la transformación del GABA por la

GABA aminotransferasa. A la inversa, puede metabolizarse de nuevo a succinato semialdehído por la GHB deshidrogenasa. El succinato semialdehído puede pasar posteriormente al ciclo de Krebs tras la transformación por la succinato semialdehído deshidrogenasa (SSADH) a succinato, o volver a convertirse en GABA (fig. 1)[63]. En casos de deficiencia de SSADH en humanos, las concentraciones de GHB y GABA están elevadas de forma patológica. La deficiencia de SSADH es un raro trastorno hereditario autosómico recesivo del metabolismo que cursa clínicamente con hipotonía, ataxia, convulsiones, retraso del lenguaje, problemas de comportamiento (hiperactividad y agresividad) y aciduria gammahidroxibutírica[64]. El GHB además puede sintetizarse en el tejido periférico, desde 2 precursores, la butirolactona y el butanediol.

El mecanismo de acción es complejo y comprende varias vías. Según las últimas evidencias, parece que el GHB actúa de forma fisiológica (GHB endógeno) a través del receptor de GHB (acoplado a proteína G). Cuando se administra de forma exógena (GHB exógeno), actúa además sobre otros receptores, fundamentalmente los de tipo GABA-B[65,66]. Esta interacción con el principal sistema inhibidor cerebral GABA tiene importantes implicaciones durante la intoxicación por la sustancia y es responsable de las manifestaciones centrales (principalmente la depresión del nivel de conciencia). Además, también interacciona con vías dopaminérgicas, donde parece que tiene un efecto global inhibidor[67], y los sistemas opioide y colinérgico.

Se absorbe rápidamente tras su administración por vía oral, alcanza un pico plasmático a los 20-45 min (t_{max}) y presenta una semivida de eliminación plasmática ($t_{1/2}$) de 20-50 min. Parece presentar una cinética no lineal a dosis altas que podría estar implicada en la prolongación de los efectos en caso de intoxicación[68].

Los principales efectos farmacológicos son la sedación y la hipnosis, produciendo sueño fisiológico con conservación del sueño profundo. Es además un débil analgésico, ansiolítico, estimulante de la secreción de la hormona del crecimiento y se dice que estimulante sexual posiblemente a través de mecanismos de desinhibición[69,70].

Consumo recreacional

Como droga de abuso, tal como se aventuró de forma muy acertada hace algunos años en nuestro medio[71], su consumo y el número de casos de intoxicación en los últimos años han crecido de forma exponencial. En la ciudad de Barcelona se ha producido un gran aumento del número de visitas a los servicios de urgencias hospitalarios, y durante algunos meses el GHB es la droga de abuso con mayor número de episodios[72].

Se presenta para su consumo en unos frascos de cristal o «botes, potes o biberones» (fig. 2) desde donde se bebe directamente o se mezcla con otras bebidas. La butirolactona y el butanediol se transforman en el organismo en GHB. El GHB se usa principalmente por su capacidad para producir euforia, desinhibición y sensación de bienestar. Al igual que pasa con otros sedantes como el alcohol, al aumentar la dosis estos efectos dejan paso a las típicas manifestaciones secundarias a la depresión del sistema nervioso central.

Efectos adversos e intoxicación

Se han descrito numerosos casos de intoxicación por esta sustancia[73,74], principalmente por disminución del nivel de conciencia, y podríamos estar sólo ante la punta del iceberg. El conocimiento por parte de los usuarios de la reversión espontánea del coma en la mayoría de las ocasiones hace que cada vez sean menos los casos de intoxicación que son llevados a los servicios de urgencias.

Entre sus efectos indeseables destacan somnolencia, amnesia, hipnosis, náuseas y vómitos, bradicardia, depresión respiratoria y apnea, ataxia y nistagmo, y movimientos musculares clónicos[75]. El GHB puede producir dependencia y síndrome de abstinencia tras administración prolongada[76]. Se ha comunicado su uso como droga facilitadora de agresiones sexuales o *rape drug*, al producir hipnosis y amnesia anterógrada[77].

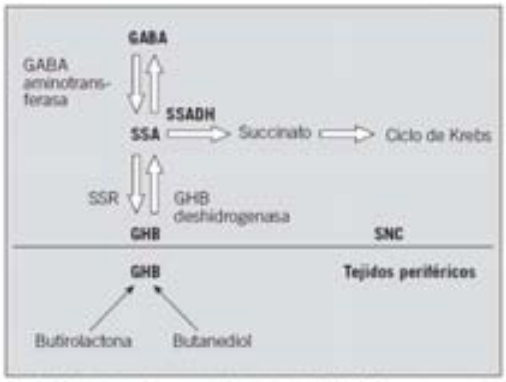

Fig. 1. Metabolismo del gammahidroxibutirato (GHB). SSA: succinato semialdehído; SSR: succinato semialdehído reductasa; SSADH: succinato semialdehído deshidrogenasa; GABA: ácido gammaaminobutírico; SNC: sistema nervioso central.

Fig. 2. Forma de presentación típica del gammahidroxibutirato (GHB, «biberón»).

La intoxicación por GHB se caracteriza fundamentalmente por la disminución del nivel de conciencia en un paciente joven, que se recupera espontáneamente en 1-2 h desde el ingreso en el hospital y en el que generalmente se encuentra consumo concomitante de otras drogas de abuso como la MDMA, cocaína, alcohol y cannabis. En la mayoría de las ocasiones bastan medidas de soporte, como posición de seguridad y la aspiración de la vía aérea[79,80]. Cuando el paciente presenta bradipnea y disminución de la saturación arterial de oxígeno, puede ser necesaria la intubación endotraqueal. Sin existir un antagonista específico, la fisostigmina (inhibidor reversible de la acetilcolinesterasa) se ha usado con cierto éxito para revertir el coma durante la intoxicación. Si bien no existen evidencias científicas, basadas en ensayos clínicos controlados, para justificar su uso de forma sistemática[81], su empleo en estos pacientes seleccionados que presentan disminución del nivel de conciencia y bradipnea puede evitar la intubación endotraqueal.

Conclusión

El consumo de drogas de abuso está en continuo cambio y evolución. Tras unos años en los que predominaban las anfetaminas de diseño y derivados, parece que aumenta el uso de otro tipo de sustancias de efectos fundamentalmente alucinógenos. La cultura de club ha acogido en nuestro país la explosión de sustancias muy heterogéneas, pero que han usado miles de personas con un mismo fin: el de divertirse[82]. En general, la sustancia utilizada de forma más generalizada es la MDMA, que reúne en sí misma la actividad anfetamínica necesaria para poder bailar durante horas y ciertos efectos «entactógenos» o de acercamiento a los demás y de «sentirse a gusto», que le confieren un lugar predominante en las pistas de baile. Otras drogas se han unido fundamentalmente para ayudar al usuario a permanecer más horas despierto (metanfetamina, cocaína) o para proporcionar más euforia o modular los efectos (GHB). Las drogas de tipo disociativo, si bien aparecen en la escena del club, se relacionan más con un usuario que busca nuevas sensaciones a través de la experimentación, por lo general personas a menudo muy bien informadas sobre estas sustancias (obtención, posología, efectos adversos) a través fundamentalmente de Internet. Es posible que la mejor manera de acercarse a estos colectivos sea a través de información veraz y contrastada, sin caer en dogmas ni moralinas, y a través de programas de reducción de riesgos como los desarrollados por algunas organizaciones en nuestro país (www.energycontrol.org) que, reconociendo un consumo de sustancias por parte de los usuarios, tratan de aportar información para disminuir los riesgos asociados, al consumo[83].

REFERENCIAS BIBLIOGRÁFICAS

1. Drug Enforcement Administration. An overview of club drugs. Drug Intelligence Brief. 2000. Disponible en: http://www.usdoj.gov/dea/pubs/intel/20005intelbrief.pdf
2. Lenton S, Boys A, Norcross K. Raves, drugs and experience: drug use by a sample of people who attend raves in Western Australia. Addiction 1997;92:1327-37.
3. Weir E. Raves: a review of the culture, the drugs and the prevention of harm. CMAJ 2000;162:1843-8.
4. Jansen KL. A review of the nonmedical use of ketamine: use, users and consequences. J Psychoactive Drugs 2000;32:419-33.
5. Abanades S, Farre M. Drogas de diseño [carta]. Med Clin (Barc) 2003; 121:38.
6. Riley SC, James C, Gregory D, Dingle H, Cadger M. Patterns of recreational drug use at dance events in Edinburgh, Scotland. Addiction 2001; 96:1035-47.
7. Winstock AR, Griffiths P, Stewart D. Drugs and the dance music scene: a survey of current drug use patterns among a sample of dance music enthusiasts in the UK. Drug Alcohol Depend 2001;64:9-17.
8. Tossmann P, Boldt S, Tensil MD. The use of drugs within the techno party scene in European metropolitan cities. Eur Addict Res 2001;7:2-23.
9. Rebono Morales JA, González Miranda F. Ketamina. Rev Esp Anestesiol Reanim 1999;46:111-22.
10. Espinosa G, Miró O, Nogué S, To-Figuera J, Sánchez M, Coll-Vinent B. Intoxicación por éxtasis líquido: estudio de 22 casos. Med Clin (Barc) 2001;117:56-8.
11. Kohrs R, Durieux ME. Ketamine: teaching an old drug new tricks. Anesth Analg 1998;87:1186-93.
12. Hocking G, Cousins MJ. Ketamine in chronic pain management: an evidence-based review. Anesth Analg 2003;97:1730-9.
13. Lahti AC, Weiler MA, Tamara Michaelidis BA, Parwani A, Tamminga CA. Effects of ketamine in normal and schizophrenic volunteers. Neuropsychopharmacology 2001;25:455-67.
14. Kegeles LS, Abi-Dargham A, Zea-Ponce Y, Rodenhiser-Hill J, Mann JJ, Van Heertum RL, et al. Modulation of amphetamine-induced striatal dopamine release by ketamine in humans: implications for schizophrenia. Biol Psychiatry 2000;48:627-40.
15. Vollenweider FX, Vontobel P, Oye I, Hell D, Leenders KL. Effects of (S)-ketamine on striatal dopamine: a [11C]raclopride PET study of a model psychosis in humans. J Psychiatr Res 2000;34:35-43.
16. Hartvig P, Valtysson J, Lindner KJ, Kristensen J, Karlsten R, Gustafsson LL, et al. Central nervous system effects of subdissociative doses of (S)-ketamine are related to plasma and brain concentrations measured with positron emission tomography in healthy volunteers. Clin Pharmacol Ther 1995;58:165-73.
17. Hustveit O, Maurset A, Oye I. Interaction of the chiral forms of ketamine with opioid, phencyclidine, sigma and muscarinic receptors. Pharmacol Toxicol 1995;77:355-9.
18. Tso MM, Blutchford KL, Callado LF, McLaughlin DP, Stamford JA. Stereoselective effects of ketamine on dopamine, serotonin and noradrenaline release and uptake in rat brain slices. Neurochem Int 2004;44:1-7.
19. Smith GS, Schloesser R, Brodie JD, Dewey SL, Logan J, Vitkun SA, et al. Glutamate modulation of dopamine measured in vivo with positron emission tomography (PET) and 11C-raclopride in normal human subjects. Neuropsychopharmacology 1998;18:18-25.
20. White PF, Way WL, Trevor AJ. Ketamine its pharmacology and therapeutic uses. Anesthesiology 1982;56:119-36.
21. Dalgarno PJ, Shewan D. Illicit use of ketamine in Scotland. J Psychoactive Drugs 1996;28:191-9.
22. Hidalgo E. Revisión del uso recreacional de ketamina. Adicciones 2002; 14:177-189.
23. Winstock AR, Griffiths P, Stewart D. Drugs and the dance music scene: a survey of current drug use patterns among a sample of dance music enthusiasts in the UK. Drug Alcohol Depend 2001;64:9-17.
24. Department of Justice. Placement of ketamine into schedule III scheduling actions. Drug Enforcement Administration [DEA-183F] 21 CFR Part 1308. 1999 [consultado 15/03/2004]. Disponible en: http://www.deadiversion.usdoj.gov/fed_regs/sched_actions/1999/fr0713.htm
25. Jansen KL, Theron L. Ketamina: nuevas observaciones sobre su consumo, consumadores y efectos. Adicciones 2003;15:135-66.
26. Dillon P, Copeland J, Jansen J. Patterns of use and harms associated with non-medical ketamine use. Drug Alcohol Depend 2003;69:23-8.
27. Curran HV, Morgan C. Cognitive, dissociative and psychotogenic effects of ketamine in recreational users on the night of drug use and 3 days later. Addiction 2000;95:575-90.
28. Curran HV, Monaghan L. In and out of the K-hole: a comparison of the acute and residual effects of ketamine in frequent and infrequent ketamine users. Addiction 2001;96:749-60.
29. Weiner AL, Vieira L, McKay CA, Bayer MJ. Ketamine abusers presenting to the emergency department: a case series. J Emerg Med 2000;18: 447-51.
30. Caruso FS. MorphiDex pharmacokinetic studies and single-dose analgesic efficacy studies in patients with postoperative pain. J Pain Symptom Manage 2000;19(1 Suppl):S15-6S.
31. Nicholson KL, Hayes BA, Balster RL. Evaluation of the reinforcing properties and phencyclidine-like discriminative stimulus effects of dextromethorphan and dextrorphan in rats and rhesus monkeys. Psychopharmacology (Berl) 1999;146:49-59.
32. Krontbach T. Bufuralol, dextromethorphan, and debrisoquine as prototype substrates for human P4501D6. Methods Enzymol 1991;206:509-17.
33. Hildebrand M, Seifert W, Reichenberger A. Determination of dextromethorphan metabolizer phenotype in healthy volunteers. Eur J Clin Pharmacol 1989;36:315-8.
34. Schmid B, Bircher J, Preisig R, Kupfer A. Polymorphic dextromethorphan metabolism: co-segregation of oxidative O-demethylation with debrisoquin hydroxylation. Clin Pharmacol Ther 1985;38:618-24.
35. Silvasti M, Karttunen P, Tukiainen H, Kokkonen P, Hanninen U, Nykanen S. Pharmacokinetics of dextromethorphan and dextrorphan: a single dose comparison of three preparations in human volunteers. Int J Clin Pharmacol Ther Toxicol 1987;9:493-7.
36. Hollander D, Pradas J, Kaplan R, McLeod HL, Evans WE, Munsat TL. High-dose dextromethorphan in amyotrophic lateral sclerosis: phase I safety and pharmacokinetic studies. Ann Neurol 1994;36:920-4.

37. Helfer J, Kim OM. Psychoactive abuse potential of Robitussin-DM. Am J Psychiatry 1990;47:672-3.
38. The Third Plateau: Guide to extracting DXM from cough medicines. [consultado 15/03/2004]. Disponible en: http://www.third-plateau.org/knowledgebase/extract.shtml
39. Banerji S, Anderson IB. Abuse of Coricidin HBP cough & cold tablets: episodes recorded by a poison center. Am J Health Syst Pharm 2001; 58:1811-4.
40. Roberge RJ, Hirani KH, Rowland PL III, Berkeley R, Krenzelok EP. Dextromethorphan- and pseudoephedrine-induced agitated psychosis and ataxia: case report. J Emerg Med 1999;17:285-8.
41. Price LH, Lebel J. Dextromethorphan-induced psychosis [carta]. Am J Psychiatry 2000;157:304.
42. Graudins A, Fern RP. Acute dystonia in a child associated with therapeutic ingestion of a dextromethorphan containing cough and cold syrup [carta]. Clin Toxicol 1996;34:351-2.
43. Bem JL, Peck R. Dextromethorphan. An overview of safety issues. Drug Saf 1992;7:190-9.
44. Boyer EW, Quang L, Woolf A, Shannon M, Magnani B. Dextromethorphan and ecstasy pills [carta]. JAMA 2001;285:409-10.
45. Shedlin M, Wallechinsky D, Salyer S. Laughing gas. Nitrous oxide. 2nd ed. Berkeley: Ronin Publishing, 1992.
46. Jevtovic-Todorovic V, Todorovic SM, Mennerick S, Powell S, Dikranian K, Benshoff N, et al. Nitrous oxide (laughing gas) is an NMDA antagonist, neuroprotectant and neurotoxin. Nat Med 1998;4:460-3.
47. Murakawa M, Adachi T, Nakao S, Seo N, Shingu K, Mori K. Activation of the cortical and medullary dopaminergic systems by nitrous oxide in rats: a possible neurochemical basis for psychotropic effects and postanesthetic nausea and vomiting. Anesth Analg 1994;78:376-81.
48. Fagan D, Paul DL, Tiplady B, Scott DB. A dose-response study of the effects of inhaled nitrous oxide on psychological performance and mood. Psychopharmacology (Berl) 1994;116:333-8.
49. Armstrong PJ, Morton C, Sinclair W, Tiplady B. Effects of nitrous oxide on psychological performance. A dose-response study using inhalation of concentrations up to 15%. Psychopharmacology (Berl) 1995;117: 486-90.
50. Rosenberg H, Orkin FK, Springstead J. Abuse of nitrous oxide. Anesth Analg 1979;58:104-6.
51. Dohrn CS, Lichtor JL, Coalson DW, Uttvlugt A, De Wit H, Zacny JP. Reinforcing effects of extended inhalation of nitrous oxide in humans. Drug Alcohol Depend 1993;31:265-80.
52. Yanik S, Thapar P, Lichtor JL, Patterson T, Zacny JP. Effects of marijuana history on the subjective, psychomotor, and reinforcing effects of nitrous oxide in humans. Drug Alcohol Depend 1994;36:227-36.
53. Gillman MA. Nitrous oxide abuse in perspective. Clin Neuropharmacol 1992;15:297-306.
54. Ng J, O'Grady G, Pettit T, Frith R. Nitrous oxide use in first-year students at Auckland University. Lancet 2003;361:1349-50.
55. Sterman AB, Coyle PK. Subacute toxic delirium following nitrous oxide abuse. Arch Neurol1983;40:446-7.
56. Flippo TS, Holder WD Jr. Neurologic degeneration associated with nitrousoxide anesthesia in patients with vitamin B_{12} deficiency. Arch Surg 1993;128:1391-5.
57. Beltramello A, Puppini G, Cerini R, El-Dalati G, Manfredi M, Roncolato G, et al. Subacute combined degeneration of the spinal cord after nitrous oxide anaesthesia: role of magnetic resonance imaging. J Neurol Neurosurg Psychiatry 1998;64:563-4.
58. Godschalk M, Dzoljic MR, Bonta IL. Slow wave sleep and a state resembling absence epilepsy induced in the rat by gamma-hydroxybutyrate. Eur J Pharmacol 1977;44:105-11.
59. Chin MY, Kreutzer RA, Dyer JE. Acute poisoning from gamma-hydroxybutyrate in California. West J Med 1992;156:380-4.
60. Dyer JE. Gamma-hydroxybutyrate: a health-food product producing coma and seizurelike activity. Am J Emerg Med 1991;9:321-4.
61. U.S. Xyrem® Multicenter Study Group. A 12-month, open-label, multicenter extension trial of orally administered sodium oxybate for the treatment of narcolepsy. Sleep 2003;26:31-5.
62. Maitre M, Andriamampandry C, Kemmel V, Schmidt C, Hode Y, Hechler V, et al. Gamma-hydroxybutyric acid as a signaling molecule in brain. Alcohol 2000;20:277-83.
63. Maitre M. The gamma-hydroxybutyrate signalling system in brain: organization and functional implications. Prog Neurobiol 1997;51:337-61.
64. Gibson KM, Gupta M, Pearl PL, Tuchman M, Vezina LG, Snead OC III, et al. Significant behavioral disturbances in succinic semialdehyde dehydrogenase (SSADH) deficiency (gamma-hydroxybutyric aciduria). Biol Psychiatry 2003;54:763-8.
65. Wong CG, Gibson KM, Snead OC. From the street to the brain: neurobiology of the recreational drug gamma-hydroxybutyric acid. Trends Pharmacol Sci 2004;25:29-34.
66. Carai MA, Colombo G, Brunetti G, Melis S, Serra S, Vacca G, et al. Role of GABA(B) receptors in the sedative/hypnotic effect ofgamma-hydroxybutyric acid. Eur J Pharmacol 2001;428:315-21.
67. Wong CG, Bottiglieri T, Snead OC III. GABA, gamma-hydroxybutyric acid, and neurological disease. Ann Neurol 2003;54(Suppl 6):3-12.
68. Palatini P, Tedeschi L, Frison G, Padrini R, Zordan R, Orlando R, et al. Dose-dependent absorption and elimination of gamma-hydroxybutyric acid in healthy volunteers. Eur J Clin Pharmacol 1993;45:353-6.
69. Okun MS, Boothby LA, Bartfield RB, Doering PL. GHB: an important pharmacologic and clinical update. J Pharm Pharm Sci 2001;4:167-75.
70. Nicholson KL, Balster RL. GHB: a new and novel drug of abuse. Drug Alcohol Depend 2001;63:1-22.
71. Dueñas A. ¿Ácido gamma-hidroxibutírico o «extasis líquido»: la intoxicación que viene. Med Clin (Barc) 1998;111:458-60.
72. Miró O, Nogué S, Espinosa G, To-Figueras J, Sánchez M. Trends in illicit drug emergencies: the emerging role of gamma-hydroxybutyrate. J Toxicol Clin Toxicol 2002;40:129-35.
73. Abanades S, Iglesias ML, Echarte JL, Puig Dou J, Roset PN, Farré M. Gammahydroxibutirate: a novel toxicological emergency. Meth Find Clin Exp Pharmacol 2001;23:326.
74. Espinosa G, Miró O, Nogué S, To-Figuera J, Sánchez M, Coll-Vinent B. Intoxicación por éxtasis líquido: estudio de 22 casos. Med Clin (Barc) 2001;117:56-8.
75. Degenhardt L, Darke S, Dillon P. GHB use among Australians: characteristics, use patterns and associated harm. Drug Alcohol Depend 2002;67:89-94.
76. Galloway GP, Frederick SL, Staggers FE Jr, Gonzales M, Stalcup SA, Smith DE. Gamma-hydroxybutyrate: an emerging drug of abuse that causes physical dependence. Addiction 1997;92:89-96.
77. ElSohly MA, Salamone SJ. Prevalence of drugs used in cases of alleged sexual assault. J Anal Toxicol 1999;23:141-6.
78. Li J, Stokes SA, Woeckener A. A tale of novel intoxication: a review of the effects of gamma-hydroxybutyric acid with recommendations for management. Ann Emerg Med 1998;31:729-36.
79. Mason PE, Kerns WP II. Gamma hydroxybutyric acid (GHB) intoxication. Acad Emerg Med 2002;9:730-9.
80. Chin RL, Sporer KA, Cullison B, Dyer JE, Wu TD. Clinical course of gamma-hydroxybutyrate overdose. Ann Emerg Med 1998;31:716-22.
81. Traub SJ, Nelson LS, Hoffman RS. Physostigmine as a treatment for gamma-hydroxybutyrate toxicity: a review. J Toxicol Clin Toxicol 2002; 40:781-7.
82. Bouso JC. Qué son las drogas de síntesis. Barcelona: Integral, 2003.
83. Bellis MA, Hughes K, Lowey H. Healthy nightclubs and recreational substance use: From a harm minimisation to a healthy settings approach. Addict Behav 2002;27:1025-35.