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Increases in serum Growth Hormone concentrations associated with GHB administration

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Abstract

The administration of gamma-hydroxybutyrate (GHB) has been reported to augment the increase in growth hormone (GH) secretion associated with the onset of sleep. The ability of GHB to stimulate GH production in the absence of sleep in both male and female volunteers was investigated as part of a GHB administration study.

Twelve healthy volunteers (6 men and 6 women) were given a small oral dose (25 mg/kg) of GHB (as Xyrem®) at 10:00 h. Basal blood samples (as serum) were taken 10 min prior to GHB administration, with additional samples taken at 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, 240, 360 and 480 min post-administration. The serum concentrations of GHB were measured by GC-MS and GH by immunometric assay.

Following GHB administration, volunteers exhibited effects consistent with mild sedation, i.e. relaxed with normal responses to verbal stimuli. Despite none being asleep, an increase in serum GH concentration occurred in 11 out of the 12 volunteers (5 women and 6 men). In these volunteers, peak GH concentrations occurred 45 – 60 min post-administration compared to a mean serum t_{max} for GHB of 23 min (sd = 5.4 min). The absolute increase in GH was similar for the men and women, averaging 3.4 ng/mL and 3.7 ng/mL respectively. The mean intra-individual increase in GH was much greater in males (29 times) compared with females (2 times), as males had (as expected) lower basal GH concentrations (mean = 0.26 ng/mL) compared to females (mean = 5.4 ng/mL). After maximising, the GH C_{max} decreased rapidly (in agreement with GHB concentrations), returning to basal concentrations at around 90 - 120 min post-administration.

GHB administration at low therapeutic concentrations results in increases in serum GH concentrations in healthy male and female volunteers in the absence of sleep onset.

Key words

Gamma-hydroxybutyrate, growth hormone, healthy volunteers, serum.

Introduction

Gamma-hydroxybutyrate (GHB) is an endogenous short-chain fatty acid which initially gained attention because of its ability to cross the blood-brain barrier, and was later used medicinally as a general anaesthetic (1, 2). Due to an unpredictable duration of action its use in anaesthesia has now ceased, but GHB (as Xyrem®) has since been licensed for the treatment of narcolepsy (associated with cataplexy) (3-5) and for alcohol withdrawal (6).

It is however the non-medicinal use of GHB which has been the subject of greater attention, both from the media and scientific community. In the last 15 years GHB (and its related analogues such as gamma-butyrolactone (GBL)) has been linked to cases of drug-facilitated sexual assault (DFSA), more commonly termed ”date-rape” (7-10). In such cases toxicological proof of GHB administration is difficult due to its endogenous nature (0.2 – 1 mg/L in urine), and rapid elimination from the body (half-life 20 – 45 min), which results in a return to baseline concentrations in urine after only 8 hours post-administration (11-18).

The recreational use of GHB has also become popular in association with the “clubbing scene”, particularly amongst homosexual men (19, 20), as a consequence of the disinhibitory effects associated with low doses (21).

The non-medicinal use however began when it was reported that GHB administration produced an increase in serum growth hormone (GH) concentrations (22). In this initial study, six healthy male volunteers were given a 2.5 g dose (around 35 mg/kg, assuming 70 kg body weight) of GHB by intravenous (iv) injection, with five volunteers falling asleep due to the associated hypnotic (soporific) effects at this dose. The mean reported GH C_{max} occurred at 60 min post-injection (32.1 ng/mL), and demonstrated a significant increase of around 20 fold from mean basal concentrations. Perhaps unsurprisingly this observation led to the use of GHB as a muscle-building supplement amongst body-builders (23).

A later study used the relationship between GHB and GH to investigate the central mechanism of action for GHB in the brain through the co-administration of several receptor agonists (24). A 1.5 g oral dose (around 21.4 mg/kg, assuming 70 kg body weight) to 10 male volunteers produced rises of around 8 – 9 ng/mL in serum GH, peaking 45 min after administration. This equated to roughly 3 times the reported control values in the study. No major side effects including sedation were observed following the administration of GHB in this instance.

Van Cauter *et al.* focused on the ability of GHB to augment the natural increase in GH serum concentrations associated with the onset of slow wave sleep (25). Eight healthy male volunteers were given doses of 2.5 g, 3.0 g or 3.5 g at 22:45 h prior to a "bedtime" of 23:00 h. Basal serum concentrations were typically less than 10 ng/mL prior to sleep, while GH C_{max} associated with the onset of sleep in the control group was around 20 ng/mL. All three doses of GHB produced augmented GH concentrations with the larger doses producing a doubling in the GH C_{max} compared with GH pulses observed in the absence of GHB administration. Neither IGF-1 nor IGFBP-3 concentrations were shown to increase following any dose. The authors suggested that GHB would only act as a GH secretagogue if sleep is induced since no increase in GH concentration was seen prior to the onset of sleep.

Several circumstances have been elucidated which are capable of decreasing or negating the increase in GH associated with GHB administration. The administration of GHB is unable to produce an increase in GH in cocaine addicts, and if co-administration with benzodiazepine or serotonin receptor antagonists (24, 26, 27).

The study reported here seeks to clarify whether the production of GH post GHB administration is solely associated with the onset of sleep. Serum collected from volunteers (6 men and 6 women), who remained awake following a low therapeutic oral dose (25 mg/kg) of GHB in the morning, was analysed to determine GH and GHB concentrations. Notably, the study reports data in female

volunteers, which has so far been under investigated. GH serum concentrations were compared with GHB concentrations and the effectiveness of GHB as a GH secretagogue is considered.

Materials and Methods

Reagents

GHB (sodium salt), GBL and trifluoroacetic acid were all purchased from Sigma-Aldrich Company Ltd, Poole, UK. Sodium hydroxide pellets, chloroform, anhydrous potassium dihydrogen phosphate and anhydrous disodium hydrogen phosphate were purchased from Fisher Scientific, Loughbourgh, UK. Deuterated(d6)-GHB (4-hydroxy-2,2,3,3,4,4-hexadeuterobutyric acid sodium salt) was supplied as 1 mg/mL in methanol by LGC Standards, Teddington, UK. All water was purified to 18 MΩ-cm, using an Elga Maxima coupled to an Elga Purelab Option - R15, Waters, UK. Xyrem® (500 mg/mL) was obtained from UCB Pharma Ltd, Berkshire, UK. Serum tubes (Becton, Dickinson Vacutainers™, 10 mL, red top, conventional closure, no additive) were purchased from MidMeds, Essex, UK.

GH assay

GH concentrations were measured using a commercially available immunoassay (Immulite 1000, growth hormone assay (LKGRH1), Siemens, Llanberis, UK). The assay is a solid phase, two-site chemiluminescent immunometric assay. As reported by the manufacturer:- The lower limit of analytical sensitivity is 0.01 ng/mL, and the reportable range is 0.05 – 40 ng/mL. The specified intra-assay precision is between 5.3 % and 6.5 % over the range 1.7 - 31 ng/mL. The specified inter-assay precision is between 5.5 % and 6.2 % over the range 3.0 - 18 ng/mL. Assay measurements were calibrated against WHO NIBSC 2nd IS 98/574.

Volunteer Recruitment

Ethical approval for the GHB administration study was obtained from our institutional research ethics committee (approval number CREC/06/07-30). Written informed consent was obtained from the volunteers (6 men and 6 women). Males had a mean age of 25 years (range 21 – 36 years) and a mean body mass index (BMI) of 23.7 kg/m^2 (21.7 – 27.1 kg/m^2). Females had a mean age of 26 years (22 – 32 years) and BMI of 23.0 kg/m^2 (19.5 – 25.9 kg/m^2). Prior to the study, volunteers were assessed to be in good health. Exclusion criteria included a history of liver disease, succinic semi-aldehyde dehydrogenase deficiency and those breast feeding. All volunteers were negative for the current use of sedatives, recreational drugs and pregnancy (females only), by analysis of a urine sample collected one week before GHB adminstration and by self reporting.

Study Design

On the day of the study, volunteers (in groups of 3) were asked to finish a light breakfast (avoiding fried foods) by 7.30 am before arriving at the secure study suite. At 10.00 am a single dose (25 mg per kg body weight) of GHB was administered (time = 0 h) in the form of the pharmaceutical preparation Xyrem® (sodium oxybate, 500 mg/L). The mean dose was 1.8 g (as the sodium salt) and ranged from 1.4 – 2.6 g (equivalent to 1.2 – 2.1 g GHB). The preparation was diluted with 60 mL water prior to adminiatration as directed by the manufacturer.

Blood samples (20 mL) where collected by cannulation for the first 4 h post-administration, and then subsequently by venepuncture. All samples were collected from the cubital vein. Blood samples were collected at -10, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, 240, 360 and 480 min for analysis relative to GHB administration (0 min). For simplicity data from -10 min representing baseline GH or GHB is plotted at 0 min in Figures 1, 2 and 3.

Sample treatment

Serum Samples: 10 mL of whole blood was allowed to clot for 30 min in the Vacutainer™ in which it was collected. Samples were then centrifuged at 1000 *g* for 10 min. The supernatant (serum) was then transferred into polypropylene tubes, which were stored at –20 °C until analysis.

GHB analysis

The developed method for the analysis of GHB in urine was validated with respect to range, linearity, accuracy, repeatability and reproducibility. Sample extraction, instrumental and data interpretation criteria are outlined in our previously published papers (14, 18).

Statistics

Statistical analysis was performed using SPSS statistics version 22. GH concentrations were checked for normality using the Shapiro-Wilks statistic, and shown to be considered normal within each time point for the range of 25 – 300 min for males and -10 to 240 min for females. A subsequent repeated measures ANOVA was performed with the within subject factor being time (relative to GHB administration), and used to identify any significant increase in GH concentrations. Following the identification of a significant rise in GH concentration, a pair-wise (Least significant difference, (LSD)) analysis was performed to identify at what time point significance occurred. Statistical analysis was performed separately on males and females, and when testing for normality GH concentrations below the LOD of the assay were excluded using a pairwise approach. For the repeated measures ANOVA, GH concentrations below the LOD were assumed to be half the LOD of the assay. Statistical significance is reported at a 95 % confidence level in all cases. A Spearman's Rank

Order Correlation (rho) was used to calculate the strength of the relationship between GHB and GH concentrations.

Results

Basal GH concentrations

As expected basal serum GH concentrations were larger in females (mean = 5.4 ng/mL, range = 1.08 to 11.2 ng/mL) compared to males (mean = 0.26 ng/mL, 0.03 ng/mL to 1.01 ng/mL). GH concentrations in females were decreasing at the time of GHB administration (Figure 1a), but no such trend could be observed in males (Figure 1b), possible due to the associated lower concentrations.

GH concentrations following GHB administration

Eleven of the twelve individuals in the study showed a marked increase in GH concentrations following GHB administration with the exception being a singular female volunteer. Figures 1a and 1b demonstrate the substantial variation that was observed in C_{max} both between the sexes and within both male and females. For males the mean serum GH C_{max} = 3.67 ng/mL (range 7.21 ng/ml to 0.12 ng/mL) and for females the mean serum GH C_{max} = 9.11 ng/mL (range 20.6 ng/ml to 4.34 ng/mL). The difference between mean basal (-10 min) concentrations and mean C_{max} in males and females was broadly similar, being 3.41 ng/mL for males and 3.75 ng/mL for females.

Mean GHB concentrations for all twelve volunteers are plotted in Figure 3 and are reported in more detail elsewhere (18). GHB serum elimination profiles were similar in male (mean C_{max} = 59.7 µg/mL) and female (mean C_{max} = 59.2 µg/mL) volunteers. The inter-individual variation was also relatively low for all 12 volunteers (mean C_{max} = 59.5 µg/mL, sd = 10.2).

Statistical analysis

GH concentrations were significantly different between time points within each sex (repeated measures ANOVA, males ($F(14, 70) = 8.32$, $p < 0.05$), females ($F(14, 70) = 6.33$, $p < 0.05$)).

Significant increases (LSD, $p = 0.05$) in GH concentration occurred in both male and female volunteers at 45 and 60 minutes in agreement with visual data inspection.

Correlation of GHB and GH data

The use of a linear model resulted in a statistically significant correlation ($p < 0.05$, $N = 180$) with an r value of 0.390 demonstrating a positive correlation between GHB and GH concentrations of medium strength following GHB administration. This data however results in a low shared variance of 15 %, therefore the predicting serum GH concentrations based on the serum GHB concentrations post GHB administration has little predictive value.

Normalised GH concentrations

In order to compensate for the large difference in the absolute GH concentrations among the volunteers, GH concentrations were normalised relative to each volunteer's C_{max} (100 %). As expected this significantly reduced the spread of data (Figure 3).

The normalised data is able to demonstrate that while absolute increases in GH concentrations in males and females had a similar mean value (3.41 ng/mL for males and 3.75 ng/mL for females) the relative increase in males is much more significant due to the lower basal GH concentrations. Female concentrations increased on average 2.0 times pre-administration basal values (range = 1.2 times to 4.0 times) while in men the mean increase was 29 times (range = 4.2 times to 68 times).

Due to the higher starting female GH concentrations, taking the pre-administration values as the sole basal concentration was likely to result in an underestimate in the relative GH

increase. Therefore, an alternative approach whereby the average GH concentration of the pre-administration sample and the 150 min sample (which was taken to represent the baseline GH concentration post the GHB induced pulse) was used to give an improved representation of the increase in relative female serum GH concentrations. While it may be considered that GH secretion would be suppressed post-stimulation due to negative feedback, only minor (if any) increases in serum GH concentrations were observed between 150 min and 480 min (Figures 1a and 1b) suggesting any inhibitory effect on endogenous GH production was minor. Using this approach female concentrations increased on average 3.0 times pre-administration basal values (2.3 times to 5.0 times) while in men the mean increase was 16 times (3.0 times to 25 times).

Discussion

It is important to consider the pulsatile nature of GH release when considering whether any observable increases in GH concentrations seen in our data are associated with the administration of GHB or representative of typical pulsatile release. Previous research has reported the mean frequency of GH pulses in 6 healthy males to be 4.3 pulses (range = 2 to 8 pulses) over a 24 hour period (28). Unlike the GH increases observed in this study natural GH pulses were shown to vary largely in their time of occurrence.

In the study presented, all 11 volunteers displaying an observable pulse of GH did so within one hour of GHB administration which therefore related to around 20 to 35 minutes post the observed GHB C_{max} (Figure 1, 2 and 3).

Our work clearly demonstrates that the administration of GHB is able to produce an observable and statistically significant increase in serum GH concentrations in both male and female volunteers, even when administered in the low therapeutic range (25 mg/kg). The variation in GH concentration was much larger than the variation in GHB concentration, suggesting a considerable inter-individual variation in the ability of GHB to stimulate GH release.

The 6 male volunteers produced a lower observed increase in GH concentration when compared with the data of Takahara *et al*. (22). Though a lower dose was given in our study (25 mg/kg) compared to this previous work (2.5 g, around 35 mg/kg), the increase was proportionally much lower with a mean C_{max} of 3.67 ng/mL rather than 32.1 ng/mL. It is however important to note that we used an oral administration, which represents the usual method of GHB administration, rather than iv route of administration used by Takahara. It should also be considered that the basal concentrations for the male volunteers were lower in

our study (mean, 0.26 ng/mL) compared with around 2 ng/mL which was reported previously. The relative increase in GH concentrations is therefore similar for both studies.

Van Cauter *et al*. gave a range of oral GHB doses (2.5 g, 3.0 g and 3.5 g) to eight healthy male volunteers. These doses are equivalent to approximately 35 mg/kg, 42 mg/kg and 50 mg/kg respectively, and therefore are all in excess of the dose administered in this study (25). As their study focused on the augmentation of GH production associated with the onset of slow-wave sleep, study volunteers were given the GHB dose prior to bedtime (23:00 h) rather than in the morning, as in the study reported herein. All three GHB doses were shown to augment the natural increase in GH associated with the onset of slow wave sleep. The lower dose produced an increase of around 10 ng/mL while the medium and higher doses produced a relative increase of around 20 ng/mL, doubling the increase seen without GHB administration. Though the authors suggest GHB only acts as a GH secretagogue if sleep is induced, our work contradicts this statement as none of the participants in our study fell asleep (generally exhibiting mild sedation or mild euphoria).

It should be noted that no increase in GH concentration was seen in volunteer 1 (female). As volunteers were confirmed to be negative for the current use of sedatives and recreational drugs based on urinary analysis (one week before GHB administration) and self reporting, we can draw no firm conclusions to explain the non response in this volunteer.

Despite the observed increases in absolute GH concentrations being small (up to around 10 ng/mL) the relative increase in GH concentrations particularly in males was significant, with GH concentrations on average 29 times greater than basal concentrations Figure 3. Such increases may still be relevant from both a therapeutic and sports doping perspective. The increases published here for this small GHB dose are around 15 % of that seen from a 0.15 U/kg subcutaneous dose of somatropin (29). Given that the work of Van Cauter suggests

that larger doses will be capable of producing an even greater response, the potential for GHB abuse in sport is noteworthy (25). Though current research in this area is limited, the long term administration of GHB to alcoholics did not affect muscle mass or waist to hip ratio, but this may be a consequence of the GH release associated with GHB administration being suppressed in alcoholics (30). Further work on healthy individuals is therefore required to ascertain the potential effects of long-term GHB use in healthy individuals.

CONCLUSION

In conclusion, our work demonstrates that low oral doses of GHB are able to produce significant increases in GH concentrations in both male and female volunteers. Unlike previous work and our data reveals increases in serum growth hormone concentrations are observed in the absence of sleep (25).

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Legends to Figures

Figure 1. Serum GH concentrations in 6 female volunteers (a) and 6 males (b) following a 25 mg/kg dose of GHB.

Figure 2. Mean GHB concentration from all 12 volunteers (6 men and 6 women). The error bar represents the standard deviation.

Figure 3. Mean of individually normalised serum GH concentrations (solid line) in 5 females (a) and 6 males (b) following a 25 mg/kg dose of GHB. The error bar representing the standard deviation. The profile of the single female volunteer who did not show an increase in GH concentrations is plotted using a dotted line in (a).

Figures

Figure 1

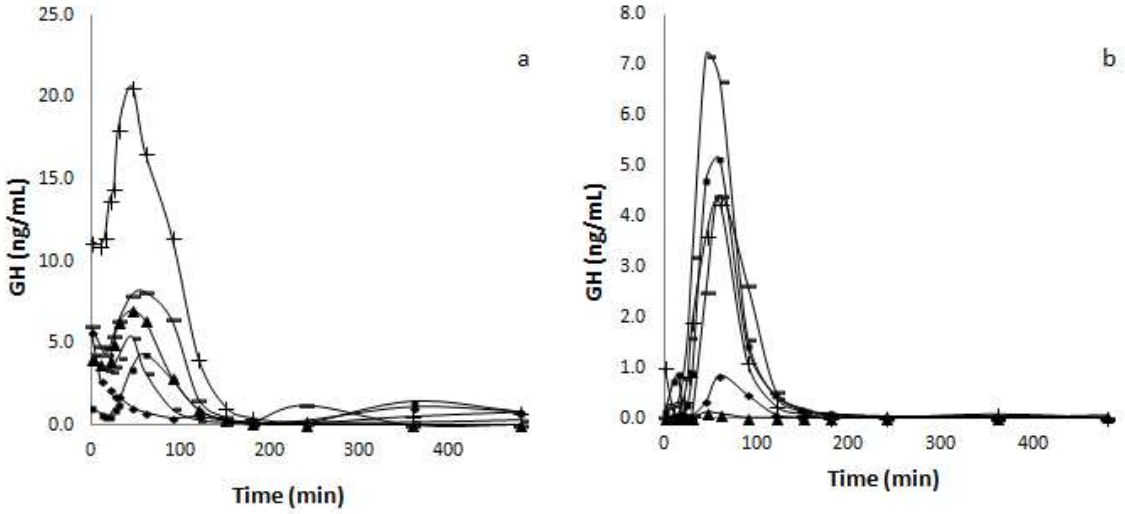

Figure 2

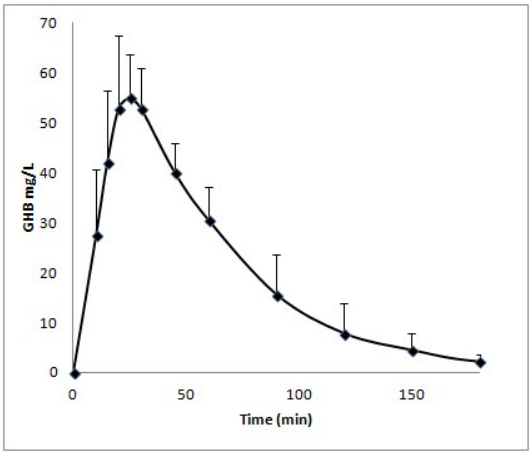

Figure 3