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Brief communication

Effects of drinking and smoking on endogenous levels of urinary γ-hydroxybutyric acid, a preliminary study

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Abstract

The aim of this study was to determine if the endogenous levels of γ-hydroxybutyric acid (GHB) in urine were affected by drinking and smoking. Urine samples were obtained from 20 healthy volunteers (15 males, 21–45 years; 5 females, 22–24 years). This population included four average drinkers (males), 4 average smokers (males), and 12 nonsmokers/nondrinkers (seven males and five females). Urinary levels of GHB were measured by gas chromatography. No gender differences were observed in the urinary levels of endogenous GHB. The urinary levels of GHB in males were 0.52 ± 0.37 μg/ml in smokers, 0.28 ± 0.21 μg/ml in nonsmokers/nondrinkers, and 0.23 ± 0.04 μg/ml in drinkers. Urinary GHB levels were measured three times a day for 5 consecutive days in a male from each group. Large intra-individual differences were observed over the 5-day period in a smoker and a nonsmoker/nondrinker. No significant changes in daily endogenous GHB levels were observed in a drinker during the period. Our preliminary results suggest that stimulatory effects of nicotine on the central nervous system (CNS) may result in an increase in nocturnal formation of GHB and the depressive effects of ethanol on the CNS may not affect, even may inhibit, nocturnal production of GHB.

Keywords: Endogenous γ-hydroxybutyric acid (GHB); Urinary concentration; Smoking; Drinking; Gas chromatography

1. Introduction

γ-Hydroxybutyric acid (GHB) is a recreational drug that has been used illegally in North America and Europe and is also popular as a date rape drug. Although criminal cases associated with GHB are rare in Japan, our Narcotic Drugs and Psychotropic Substances Control Act has regulated GHB since October 2001.

GHB is also an endogenous substance that acts as a depressive neurotransmitter [1,2]. GHB has its own presynaptic receptors [3] and it is an agonist of $GABA_B$ receptors [4–8]. While some researchers have reported negligible endogenous GHB concentrations in blood and urine (below 1 μg/ml) [9–12], others have reported levels of 2–3 μg/ml in blood and of 5–6 μg/ml in urine [13,14]. LeBeau et al. [14] reported large intra- and inter-individual differences in urinary concentrations of endogenous GHB. However, other factors, such as meals and diabetes mellitus, probably have no effect on urinary levels of GHB [15,16].

Snead et al. [17] reported that GHB concentrations in cerebrospinal fluid were significantly higher in children with seizures (0.429 ± 0.051 μg/ml) than in controls (0.120 ± 0.012 μg/ml). The highest levels were observed in children with myoclonic seizures (0.878 ± 0.095 μg/ml). These results suggest that abnormal conditions in the central nervous system (CNS) may affect endogenous GHB levels in body fluids.

In this study, we compared urinary GHB levels between drinkers, smokers and nonsmokers/nondrinkers.

2. Materials and methods

2.1. Chemicals

Sodium salt of GHB (Sigma Chemical, St Louis, MO, USA) and α-methylene-γ-butyrolactone (AMGBL, Aldrich

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Chemical, Milwaukee, WI, USA) were purchased. Other common chemicals used were of analytical-reagent grade.

2.2. Urine samples

Urine samples were obtained in the afternoon from 20 healthy Japanese volunteers (15 males, 21–45 year-old; and 5 females, 22–24 year-old), who had not used GHB, after obtaining informed consent. Male volunteers consisted of four average drinkers, four average smokers and seven nonsmokers/nondrinkers. All females were nonsmokers/-nondrinkers. A representative male from each group was tested for endogenous GHB in urine three times a day (8:00, 12:00 and 20:00) for 5 consecutive days. Urine samples were stored without preservatives at −35 °C until analysis.

2.3. Analysis

A Shimadzu gas chromatograph (GC-14B, Kyoto, Japan) equipped with a DB 624 column (30 m×0.545 mm i.d., 3-μm film thickness, J&W Scientific, Folsom, CA, USA) and a flame ionization detector was used to measure GHB. The temperature of the injection port and detector was 150 °C. The column temperature was 50 °C with 3-min hold, and increased at a rate of 20 °C/min up to 150 °C with 2-min hold. Nitrogen was used as carrier gas at a flow pressure of 100 kPa.

One milliliter of urine was mixed with 1 ml of distilled water and 0.1 ml of 1 mg/ml AMGBL (internal standard) in acidified water. Concentrated sulfuric acid (0.3 ml) was slowly added to the mixture and mixed on a vortex mixer to transform GHB to its cyclized form. The acidified mixture was cooled for about 15 min to room temperature and extracted by vigorous shaking with 6 ml of dichloromethane for 15 min using a mechanical shaker. The upper aqueous phase was removed by aspiration and discarded. The lower organic phase was transferred to a new disposable test tube, 1 ml of 1 N NaOH was added and the mixture was vigorously shaken for 15 min using the mechanical shaker to change cyclized GHB back to GHB. The upper aqueous phase was transferred to a new disposable test tube, mixed with 0.4 ml of concentrated sulfuric acid to transform GHB back to its cyclized form and cooled to room temperature for about 15 min. The mixture, which had a specific gravity larger than that of dichloromethane at this stage, was re-extracted with 6 ml of dichloromethane for 1 min on the vortex mixer. The upper organic phase was transferred to a new disposable test tube, dehydrated with 0.5 g of anhydrous sodium sulfate and evaporated to approximately 20 μl at 35 °C under a gentle stream of nitrogen. A 1-μl aliquot of the extract was injected into the gas chromatograph.

2.4. Statistical analysis

The Student's *t*-test was used to compare the urinary GHB concentrations between males and females, and the one-way analysis of variance (ANOVA) among drinkers, smokers and nonsmokers/nondrinkers. Differences at $P < 0.05$ were considered to be significant.

3. Results and discussion

GHB was measured as its cyclized form. Cyclized GHB and AMGBL appeared at 7.9 and 8.6 min, respectively, on gas chromatogram, and no interfering peaks were present. The limit of detection was calculated to be approximately 0.06 μg/ml. The sensitivity of this method was better with a lower limit of quantitation of 0.18 μg/ml as GHB than that of previous headspace GC (0.5 μg/ml [12]). Recoveries of GHB from urine and distilled water were approximately 40%. Calibration curves showed good linearity in the range of 0.18–87.5 μg/ml ($r^2 = 0.999$) and the slopes were quite similar between urine and distilled water. We used a calibration curve prepared with standard GHB in distilled water for quantitation of urinary endogenous GHB concentrations. Within-day and between-day precision, which were determined in the range of 0.88–87.5 μg/ml, were also excellent with CVs of 1.9–3.4 and 0.7–4.8%, respectively. Our method was so sensitive and precise that it detected endogenous GHB in body fluids of living person.

In our twenty volunteers, some showed urine GHB levels below the lower limit of quantitation (0.18 μg/ml), but above the limit of detection (0.06 μg/ml). These results were used in the data analysis to more accurately detect excretion patterns of low levels of endogenous GHB in urine.

No gender differences were observed in urinary levels of GHB between male (0.28 ± 0.21 μg/ml, $n = 7$) and female (0.29 ± 0.12 μg/ml, $n = 5$) nonsmokers/nondrinkers. In contrast, LeBeau et al. [14] reported that urinary endogenous GHB levels in African Americans were significantly higher in males (1.59 ± 1.42 μg/ml, $n = 5$) than in females (0.31 ± 0.25 μg/ml, $n = 3$). The GHB levels in their females were similar to our Japanese male and female nonsmokers/nondrinkers. Since, Elliott [15] reported that diet did not affect excretion patterns of endogenous GHB, there might be a genetic basis for difference in production of endogenous GHB between ethnic groups.

Males were divided into groups of drinkers, smokers and nonsmokers/nondrinkers. The urinary level of endogenous GHB tended to be higher in smokers (0.52 ± 0.37 μg/ml, $n = 4$) than in nonsmokers/nondrinkers (0.28 ± 0.21 μg/ml, $n = 7$) and drinkers (0.23 ± 0.04 μg/ml) although no statistical significance was attained (Fig. 1). Large intra-individual differences in urinary endogenous GHB levels were observed over a 5-day period in a smoker and a nonsmoker/nondrinker, but relatively constant levels of urinary endogenous GHB were measured in a drinker during the period (Fig. 2).