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Targeted screening of succinic semialdehyde dehydrogenase deficiency (SSADHD) employing an enzymatic assay for γ-hydroxybutyric acid (GHB) in biofluids

C. Wernli [a,*,1], S. Finochiaro [b,c,2], C. Volken [c,2], H. Andresen-Streichert [d,2], A. Buettler [e,2], D. Gygax [e], G.S. Salomons [f,3], E.E. Jansen [f,3], G.R. Ainslie [g,4], K.R. Vogel [g,4], K.M. Gibson [g,4]

[a] University of Applied Sciences and Arts, Northwestern Switzerland School of Life Sciences, Institute for Chemistry and Bioanalytics, Switzerland
[b] Bildungszentrum Gesundheit Basel-Stadt, Münchenstein, Switzerland
[c] Zentrallabor, Kantonsspital Baselland, Bruderholz, Switzerland
[d] University Medical Center Hamburg-Eppendorf Institute of Legal Medicine, Department of Toxicology, Hamburg, Germany
[e] University of Applied Sciences and Arts Northwestern Switzerland School of Life Sciences, Institute for Chemistry and Bioanalytics Muttenz, Switzerland
[f] Metabolic Unit, Department of Clinical Chemistry, VU University Mediscal Center, Neuroscience Campus, Amsterdam, The Netherlands
[g] Division of Experimental and Systems Pharmacology, College of Pharmacy, Washington State University, Spokane, WA, USA

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ABSTRACT

Hypothesis: An enzymatic assay for quantification of γ-hydroxybutyric acid (GHB) in biofluids can be employed for targeted screening of succinic semialdehyde dehydrogenase deficiency (SSADHD) in selected populations.
Rationale: We used a two-tiered study approach, in which the first study (proof of concept) examined 7 urine samples derived from patients with SSADHD and 5 controls, and the second study (feasibility study) examined a broader sample population of patients and controls, including plasma.
Objective: Split samples of urine and plasma (anonymized) were evaluated by enzymatic assay, gas chromatography alone (proof of concept) and gas chromatography–mass spectrometry, and the results compared.
Method: Multiple detection methods have been developed to detect GHB. We evaluated an enzymatic assay which employs recombinant GHB dehydrogenase coupled to NADH production, the latter quantified on a Cobas Integra 400 Plus. Results: In our proof of concept study, we analyzed 12 urine samples (5 controls, 7 SSADHD), and in the feasibility study we evaluated 33 urine samples (23 controls, 10 SSADHD) and 31 plasma samples (14 controls, 17 SSADHD). The enzymatic assay carried out on a routine clinical chemistry analyzer was robust, revealing excellent agreement with instrumental methods in urine (GC-FID: $r = 0.997$, $p \le 0.001$; GC–MS: $r = 0.99$, $p \le 0.001$); however, the assay slightly over-estimated GHB levels in plasma, especially those in which GHB levels were low. Conversely, correlations for the enzymatic assay with comparator methods for higher plasma GHB levels were excellent (GC–MS; $r = 0.993$, $p \le 0.001$).
Conclusion: We have evaluated the capacity of this enzymatic assay to identify patients with SSADHD via quantitation of GHB. The data suggests that the enzymatic assay may be a suitable screening method to detect SSADHD in selected populations using urine. In addition, the assay can be used in basic research the elucidate the mechanism of the underlying disease or monitor GHB- levels for the evaluation of drug candidates.
Synopsis: An enzymatic assay for GHB in biofluids was evaluated as a screening method for SSADHD and found to be reliable in urine, but in need of refinement for application to plasma.

Abbreviations: CV, coefficient of variation; GABA, gamma-aminobutyric acid; GC-FID, gas chromatography-flame ionization detector; GC–MS, gas chromatography–mass spectrometry; GHB, gamma-hydroxybutyrate (also γ-hydroxybutyric acid); GHBDH, GHB-dehydrogenase; IDM, isotope dilution method; LOD, limit of detection; LLOQ, lower limit of quantification; NADH, nicotinamide adenine dinucleotide, reduced form; r, correlation coefficient (Pearson); SSADHD, succinic semialdehyde dehydrogenase deficiency.

* Corresponding author at: University of Applied Sciences and Arts Northwestern Switzerland, School of Life Sciences, Institute for Chemistry and Bioanalytics, Gruendenstrasse 40, 4132 Muttenz, Switzerland.
E-mail address: cedric.wernli@fhnw.ch (C. Wernli).

[1] Wernli C-Design of study, implementation of enzymatic assay, manuscript preparation, editing.
[2] Finochiaro S, Volken C, Andresen-Streichert H, Buettler A, Gygax D-additional laboratory analyses, manuscript preparation, editing.
[3] Salomons GS, Jansen EE-additional laboratory analyses, manuscript preparation, editing.
[4] Ainslie GR, Vogel KR, and Gibson KM-secondary design of study, procurement of patient samples, manuscript preparation, editing.

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1. Introduction

It is well established that GABA is the primary inhibitory neurotransmitter in CNS, where >1/3 of synapses employ it [1,2]. The GABA analogue, γ-hydroxybutyric acid (GHB), is present in mammalian CNS at ~1% the level of its parent compound. The exact role for GHB in CNS remains poorly defined [3]. GHB potentiates dopaminergic activity, is employed therapeutically for narcolepsy, and is abused as a recreational drug and agent to facilitate sexual assault [4–6]. The latter properties have spurred interest in numerous toxicological settings for methods enabling rapid detection of GHB in biofluids, yet such assays are challenging because of the short $t_{1/2}$ for GHB of about 30–50 min [7].

Succinic semialdehyde dehydrogenase deficiency (SSADHD) is a rare genetic disorder in the second enzyme of GABA degradation [8,9]. The phenotype encompasses nonspecific neurological features, including developmental delay, absence of formulated speech, hypotonia, and neuropsychiatric disturbances in adolescence and adulthood. The biochemical hallmark of SSADHD is accumulation of GHB in physiological fluids, including urine, plasma, and cerebrospinal fluid (CSF) [10] (Fig. 1). As well, GABA is elevated in CSF of SSADHD-patients for whom diagnostic lumbar puncture has been performed [11]. The non-specific features of this disorder, and the recent report of a man who was diagnosed with SSADHD not before the age of 63 years, suggests that SSADHD is underdiagnosed [12]. Accordingly, a rapid and high throughput assay to detect GHB in biofluids might be beneficial for screening for SSADHD in targeted populations.

In collaboration with the University of Applied Sciences and Arts of Northwestern Switzerland, Buhlmann Laboratories (Schoenenbuch, Switzerland) developed an enzymatic assay to determine GHB in serum and urine in 2011. The method was developed to detect the recreational use of GHB (e.g. intoxication), and it can be run on clinical chemistry analyzers which are generally available around the clock. This assay employs recombinant GHB dehydrogenase (GHBDH, EC 1.1.1.61), which catalyzes the oxidation of GHB to succinic semialdehyde with stoichiometric production of NADH which is quantified spectrophotometrically at 340 nm [13,14]. Here, we have evaluated the capacity of this enzymatic assay to identify patients with SSADHD via quantitation of GHB.

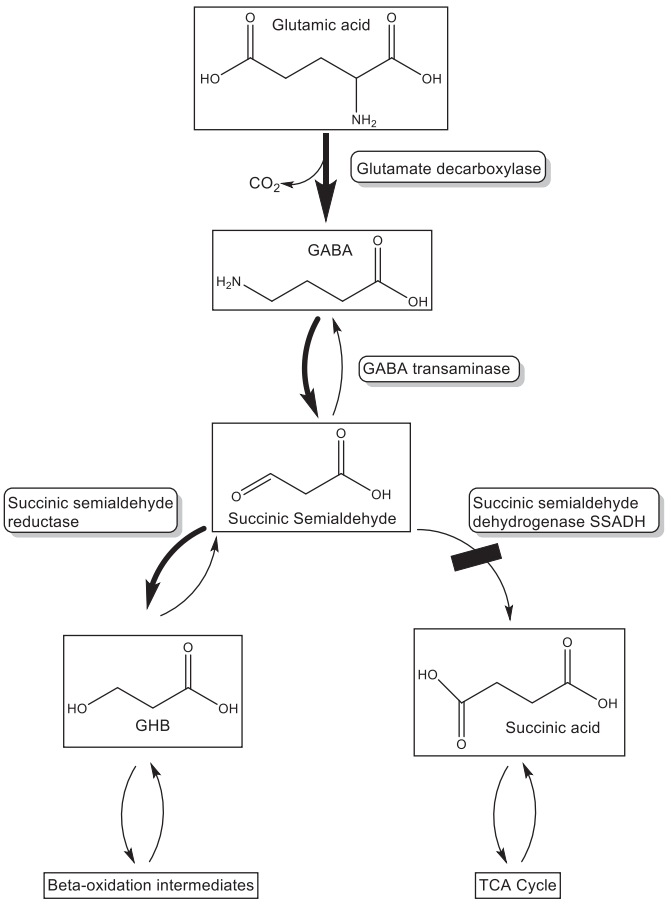

Fig. 1. Metabolic pathway of glutamic acid in SSADH-deficient patients. The black bar indicates the deficient succinic semialdehyde dehydrogenase (SSADH) and the bold arrows show the metabolic pathway of glutamic acid in SSADH-deficient patients where GHB accumulates. Modified according to Gahr et al. [24] and Pearl et al. [25].

Table 1
Imprecision of the enzymatic assay (Cobas Integra 400 plus), concentrations of the controls were: low control 12.6 mg/L, high control 68.0 mg/L.
Abbreviation: CV, coefficient of variation.

Imprecision	Low control (%)	High control (%)
Intra-assay CV (N = 10)	4.2	1.1
Inter-assay CV (N = 10)	6.8	4.1

2. Materials and methods

2.1. Biological samples

Urine and plasma from patients with SSADHD and control individuals was obtained with informed consent. Patient age range was 2–37 years. Control individuals included unrelated individuals

Table 2
Dilution linearity of the enzymatic assay on a Cobas Integra 400 plus device, determined by dilution of the urine sample from patient No. 3 with NaCl 0.9%.

Dilution of urine sample no. 3	Calculated GHB-concentration (mg/L)	Measured GHB-concentration (mg/L)	Recovery (%)
Prediluted 1:2	–	180.8	n/a
1:2	90.4	96.6	107
1:5	36.2	42.4	117
1:10	18.1	21.3	118
1:20	9.0	9.4	104
1:50	3.6	2.9	81
Mean recovery	–	–	105.4

Table 3
Detailed results of urine creatinine-normalized GHB-concentrations in all 7 SSADHD patients in urine from the first study.
Abbreviation: GC–MS, gas chromatography–mass spectrometry, GC-FID, gas chromatography- flame ionization detector.

Sample no.	Enzymatic method GHB (mmol/mol creatinine)	GC-FID GHB (mmol/mol creatinine)	GC–MS GHB (mmol/mol creatinine)
1	21.30	21.1	16.3
2	79.25	55.5	54.2
3	92.40	62.2	77.6
4	162.20	142.2	149.3
5	315.40	277.2	238.3
6	365.47	214.8	417.2
7	681.09	511.0	665.8