SHORT COMMUNICATION

Gamma-hydroxybutyrate accumulates in green tea and soybean sprouts in response to oxygen deficiency

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[1]*Department of Plant Agriculture, Bovey Bldg, University of Guelph, Guelph, Ontario, Canada N1G 2W1 (email: bshelp@uoguelph.ca);* [2]*Brock University, Department of Biological Sciences, St Catharines, Ontario, Canada L2S 3A1.* *Received 8 May 2003, 9 June 2003.*

Allan, W. L., Peiris, C., Bown, A. W. and Shelp, B. J. 2003. **Gamma-hydroxybutyrate accumulates in green tea and soybean sprouts in response to oxygen deficiency.** Can. J. Plant Sci. **83**: 951–953. Gamma-hydroxybutyrate (GHB) is an illicit drug, which may be derived in plants from a stress-inducible metabolite known as gamma-aminobutyrate. Here, oxygen deficiency caused the accumulation of GHB in green tea and soybean sprouts (76 and 155 nmol g^{-1} fresh weight, respectively). These findings demonstrate that GHB levels are elevated in severely modified atmospheres, but they pose no risk to human health. Further work is required to assess the extent of this phenomenon.

Key words: Gamma-hydroxybutyrate, gamma-aminobutyrate, oxygen deficiency, tea, bean sprouts

Allan, W. L., Peiris, C., Bown, A. W. et Shelp, B. J. 2003. **La carence en oxygène entraîne l'accumulation de gamma-hydroxybutyrate dans le thé vert et les pousses de soja**. Can. J. Plant Sci. **83**: 951–953. Le gamma-hydroxybutyrate (GHB) est une drogue illicite qu'on peut extraire des plantes qui synthétisent du gamma-aminobutyrate, métabolite induit par le stress. Dans cette étude, les auteurs ont remarqué qu'une carence en oxygène entraîne l'accumulation de GHB dans les pousses de thé et de soja (76 et 155 nmol par gramme de poids frais, respectivement). Cette découverte indique que la concentration de GHB augmente quand l'atmosphère est très modifiée, sans toutefois que la santé humaine en soit menacée. Il faudrait entreprendre d'autres recherches pour mieux évaluer l'ampleur du phénomène.

Mots clés: Gamma-hydroxybutyrate, gamma-aminobutyrate, carence en oxygène, thé, germes de soja

Gamma-hydroxybutyrate is an illicit drug that has recently attracted considerable public and scientific attention. It is being used recreationally for its euphoric properties, as well as purported beneficial effects on muscular development, weight loss and aging (Miotto et al. 2001).

Gamma-hydroxybutyrate is a short-chain fatty acid that closely resembles the inhibitory neurotransmitter gamma-aminobutyrate (GABA) (Mamelak 1989; Miotto et al. 2001). It is an endogenous constituent of the mammalian nervous system, where it is believed to act as a neurotransmitter or neuromodulator. There is also circumstantial evidence from animal studies that suggests endogenous GHB concentrations rise under oxygen deficiency. When administered, GHB crosses the blood-brain barrier with ease, penetrates the brain, and produces, depending upon the dose, a number of diverse neuropharmacological and neurophysiological effects. Gamma-hydroxybutyrate has been clinically used for applications in resuscitation, anesthesia and addiction therapy.

In developed nations, the quality of fresh market fruit and vegetables during postharvest storage and transport is extended by the use of conditions such as oxygen deficiency to suppress respiration. Fresh or minimally processed produce is often packaged in closed containers (e.g., tomatoes, soybean sprouts) or wrap (e.g., cucumbers), which restricts gas exchange. The tea industry in Japan and Korea uses low oxygen treatment of fresh leaves to produce a green tea with elevated GABA levels (i.e., "Gabaron" tea), which is marketed as an agent to control high blood pressure (Tsushida and Murai 1987; Chang et al. 1992). The role of GABA in plants is uncertain; however, it is well known that stress initiates a signal transduction pathway in which increased cytosolic Ca^{2+} stimulates Ca^{2+}/calmodulin-dependent activity of the anabolic enzyme, glutamate decarboxylase (Shelp et al. 1999). Gamma-aminobutyrate can be catabolized via GABA transaminase to alanine or glutamate and succinic semialdehyde, which in turn, is oxidized via an NAD-dependent succinic semialdehyde dehydrogenase to succinate. However, under oxygen deficiency succinic semialdehyde dehydrogenase activity is probably restricted by increases in reducing potential and adenylate energy charge. Research on animal systems indicates the existence of an alternative pathway for succinic semialdehyde catabolism to GHB that involves the enzyme GHB dehydrogenase (Andriamampandry et al. 1998; Schaller et al. 1999).

A liquid chromatography/negative ion–electrospray-mass spectrometric (LC-MS) method was developed to conveniently detect and quantify GHB in crude plant extracts

Abbreviations: **GABA**, gamma-aminobutyrate; **GHB**, gamma-hydroxybutyrate; **LC-MS**, liquid chromatography/negative ion–electrospray-mass spectrometric

Table 1. Impact of oxygen deficiency on the concentrations of gamma-hydroxybutyrate and related metabolites in green tea[z]

Treatment	Glutamate (μmol g^{-1} DW)[y]	Gamma-aminobutyrate (μmol g^{-1} DW)	Alanine (μmol g^{-1} DW)	Gamma-hydroxybutyrate (nmol g^{-1} DW)
Control	13.5 ± 0.2	14.7 ± 1.4	11.6 ± 1.0	273 ± 37
Oxygen deficiency	2.6 ± 0.2	26.3 ± 3.0	17.2 ± 1.8	759 ± 29

[z]Data represent the mean ± standard error of triplicate extractions of dried tea leaves.
[y]DW indicates dry weight.

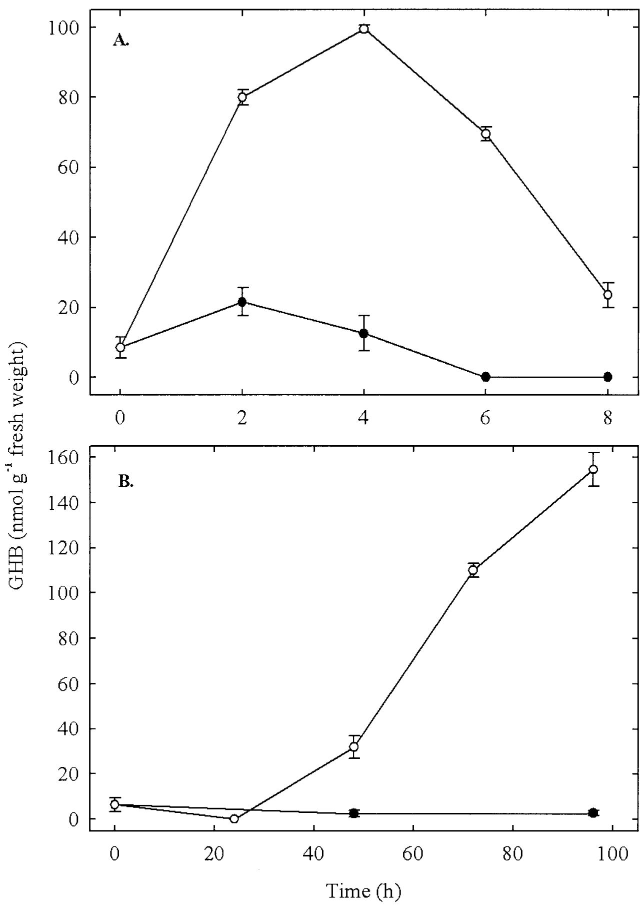

Fig 1. Impact of oxygen deficiency on gamma-hydroxybutyrate concentrations in soybean sprouts. Experimental containers (open symbols) were flushed with nitrogen (A) or air (B) and sealed, whereas control containers (closed symbols) were left open to the air atmosphere. Data represent the mean ± standard error of triplicate extractions of the pooled sample from each container.

(Allan et al. 2003). In these experiments, leaves of greenhouse-grown tobacco were shown to contain 2–31 nmol GHB g^{-1} fresh weight. Here, the LC-MS method, and previously described analytical methods (Micallef et al. 1989), were used to determine the concentrations of GHB and related amino acids in green tea (*Comellia sinesis*) received from the Korea Food Research Institute. The GHB concentration in control leaves was 273 nmol g^{-1} dry weight (Table 1). Assuming that 1 g fresh weight contains approximately 90% H_2O, this would be equivalent to 30 nmol g^{-1} fresh weight, which is similar in magnitude to the concentrations found in tobacco (Allan et al. 2003). Treatment of fresh shoots with nitrogen gas increased the GHB concentration in dried leaves twofold, and increased the concentrations of GABA and alanine, and decreased the concentration of glutamate, as reported previously (Tsushida and Murai 1987; Chang et al. 1992). These trends are consistent with the idea that GHB in plants, as in animals (Mamelak 1989), can be derived directly from succinic semialdehyde.

We also investigated the response of soybean (*Glycine max* L. 'Bobcat') sprouts to two modified atmospheres: (1) rapidly imposed as a consequence of nitrogen flushing, and (2) slowly imposed, as a consequence of respiratory depletion of oxygen in a sealed impermeable container. Seeds were germinated under moist conditions in a tissue-culture chamber (Model TC19, Enconair Ecological Chambers Inc., Winnipeg, MB, Canada) set on a light:dark cycle 8:16 h and 30:20°C. Lamps provided a photon flux density of 70 μmol m^{-2} s^{-1} at shelf level. After 4 d, 20 seedlings each were transferred to 350-mL Lock & Lock airtight containers (Atlantic Promotions Inc., Longueuil, QC, Canada). Experimental containers were flushed with nitrogen or air and sealed, whereas control containers were left open to the air atmosphere. At various times thereafter, the seedlings from one container were immediately frozen in liquid nitrogen, and the sprouts only retained for GHB analysis. In a nitrogen atmosphere, GHB concentrations increased dramatically, with a maximum occurring within 4 h. Although this maximum was followed by a decline, the GHB concentration remained considerably higher than that in control sprouts even after 8 h (Fig.1A). In an air atmosphere, the GHB concentration was unchanged for about the first 40 h, but increased in a linear fashion over the next 56 h (Fig. 1B). The oxygen concentrations in the sealed containers were not determined; however, both modified atmospheres resulted in GHB accumulation (maxima of 95 and 155 nmol g^{-1} fresh weight), indicating that the oxygen supply was, or became deficient over the time course of the experiments (Drew 1997)

This preliminary study indicated that GHB is present in green tea and soybean sprouts, as well as tobacco (Allan et al. 2003), and that its concentration increases considerably in response to oxygen deficiency. Given a GHB concentration of 155 nmol g^{-1} fresh weight, a 50-g serving of sprouts would be equivalent to only about 8 μmol GHB, a value considerably less than the 100, 200 and 500 μmol kg^{-1} (7–35 mmol for a typical 70 kg man) doses required, respectively, to induce myorelaxation, sleep and anesthetic effects (Miotto et al. 2001). This finding suggests that the potential elevation of GHB levels in horticultural produce by modified and controlled atmosphere technologies poses no risk to human health.

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