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SHORT COMMUNICATION

Gamma-aminobutyric acid (GABA) increases in vitro germ-tube formation and phospholipase B1 mRNA expression in *Candida albicans*

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Abstract *Candida albicans* is a commensal yeast in humans that disseminates in immunocompromised persons. Its spreading is modulated by melanin, hormones, or some neurotransmitters, among other factors. The neurotransmitter gamma-aminobutyric acid (GABA) is used by bacteria, plants, and fungi as a carbon and nitrogen source. In this article, the in vitro effect of different doses of GABA on germ-tube formation and expression of phospholipase B1 (PLB1) mRNA in two *Candida albicans* strains was investigated. Results demonstrated that GABA increases both germ-tube formation and PLB1 mRNA expression in the two *Candida strains* in a dose-dependent manner, which suggests that GABA promotes the growth of *C. albicans*.

Keywords Germ tubes · Neurotransmitter · RT-PCR · Virulence factors · Yeasts

Candida albicans (Selmecki et al. 2010) is a commensal yeast that can produce opportunistic infections in immunocompromised humans. *Candida albicans* oral, intestinal, and genital infections have also been associated with an increased expression of diverse virulence factors, such as phospholipase B1 (PLB1), adhesion molecules, melanin, and hyphal growth (Calderone and Fonzi 2001).

Virulence, morphogenesis, and growth rate in fungi can be modulated by exogenous factors such as hormones and neurotransmitters. For instance, exposure to female steroid hormones increases the resistance of *C. albicans* to antifungal drugs (Larsen et al. 2006). The neurotransmitter serotonin attenuates in vitro *C. albicans* virulence (Mayr et al. 2005) whereas dopamine inhibits its respiratory growth and survival (Macreadie et al. 2010). In other fungi, the adenosine neurotransmitter stimulates the mycelial growth of *Suillus luteus* (Zhang et al. 2010), and gamma-aminobutyric acid (GABA) increases in vitro growth of *Cladosporium fulvum* (Solomon and Oliver 2002).

GABA, an amino acid ubiquitously distributed in nature, has the fundamental role of inhibiting physiological neurotransmission in the mammalian brain (Owens and Kriegstein 2002). GABA modulates ion transport and stress response in plants (Kinnersley and Lin 2000) and can be utilized as both a nitrogen and a carbon source by filamentous fungi, yeasts, and bacteria (Ramos et al. 1985; Chevrot et al. 2006). In this article, the effect of GABA on the in vitro expression of both PLB1 mRNA and germ-tube formation in two strains of *C. albicans* was investigated. Our results demonstrate that GABA has a stimulating effect on both the formation of germ tubes and expression of the PLB1 transcript in a dose-dependent manner, suggesting that GABA promotes the expression of *C. albicans* virulence factors.

C. albicans ATCC 90028 and CA-121 were used in this study. The ATCC 90028 strain was purchased from the American Type Culture Collection (Manassas, VA, USA), and the clinical isolate CA-121 was obtained from an oral candidiasis case. Both strains were identified by their

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morphological characteristics, chlamydoconidia, and germ-tube induction, biochemical tests (automated Vitek system; BioMérieux, St. Louis, MO, USA), and a multiplex polymerase chain reaction (PCR) assay (Luo and Mitchell 2002). Stock *Candida* strains were cultured on Sabouraud dextrose agar for 24 h at 25°C to obtain exponential cultures. For assessing PLB1 mRNA expression, five flasks containing Sabouraud dextrose broth were inoculated separately with 1×10^6 yeasts/ml from each exponential culture. To four of five flasks, 1, 5, 10, or 20 mM GABA (Sigma-Aldrich, St. Louis, MO, USA) concentrations were added, respectively. The fifth flask did not contain GABA. All the flasks were incubated at 37°C for 48 h at 200 rpm with constant shaking. Yeasts were recovered, and the total RNA was extracted according to Collart and Oliviero (2001). Total RNA (1 μg) was mixed with 1× PCR buffer, 5 mM $MgCl_2$, 1 U/μl RNAsa inhibitor, 1 mM dNTPs, 2.5 μM oligo dT (Applied Biosystems, Foster City, CA, USA), and 2 U/ml SuperScript II RT (Invitrogen, Carlsbad, CA, USA) in a final volume of 20 μl. The reverse transcription assay (Reiss et al. 2003) was performed at 65°C for 5 min, 37°C for 50 min, and 70°C for 15 min. Five microliters of each cDNA were used for the PCR reaction using 2 mM $MgCl_2$, 1 mM dNTPs, 1 U AmpliTaq DNA polymerase (Applied Biosystems), and the primer set for the PLB1 gene (Mukherjee et al. 2003) at 94°C for 2 min, 33 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 3 min, and 72°C for 10 min as a final extension; a β-actin primer set (Okeke et al. 2001) was used as a housekeeping gene at the following conditions: 94°C for 5 min, 30 cycles of 94°C for 1 min, 62°C for 30 s, 72°C for 30 s, and 72°C for 10 min as a final extension. PCR products were analyzed in a 1.5% agarose gel stained with ethidium bromide, using the EDAS 290 Kodak System, the Digital Science 1D Program version 3.0 (Kodak, Rochester, NY, USA), and the 100-bp DNA ladder (Fermentas, Glen Burnie, MD, USA). For the germ-tube formation assay, two sets of six vials containing 1.0 ml human serum were inoculated with 5×10^4 yeast cells/ml from the ATCC90028 and the CA-121 strains separately, and different concentrations (1, 5, 10, or 20 mM) of GABA were added to four vials of each set. The fifth vial had no GABA, and to the sixth was added 0.25 μg/ml amphotericin B (Squibb, Billings, MT, USA) to inhibit germ-tube formation. All vials were incubated at 37°C for 3 h. Cells with germ tubes/1,000 yeast cells were counted on a Neubauer chamber under light microscopy. The effect of GABA concentration on germ-tube formation in each strain was assessed by comparing the number of yeasts having a germ tube in cultures that received GABA with those cultures without GABA. Differences among groups were analyzed by the one-way analysis of variance (ANOVA) test and Bonferroni's test with the GraphPad Prism Program version 5.0 (San Diego, CA, USA). Statistical significance was set at $P < 0.05$. Results showed that a concentration-dependent increase of PLB1 mRNA expression was induced by adding GABA to the culture medium in *C. albicans* ATCC 90028 strain and CA-121 strain (Fig. 1). However, the increment was statistically significant ($P < 0.01$) only at 10 mM GABA in the ATCC 90028 strain (116.8 ± 18.9 ng) regarding the yeast cultures without GABA (69.7 ± 16.7 ng). No differences of PLB1 mRNA expression were found between strains after adding GABA in *C. albicans* cultures. On the other hand, the mean β-actin mRNA expression in both *C. albicans* strains was stable and homogeneous. No difference of β-actin mRNA levels was found between the ATCC 90028 strain (120.1 ± 10.2 ng) and the CA-121 strain (125.4 ± 9.2 ng) after adding GABA in the yeast cultures. In addition, GABA also had a concentration-dependent stimulating effect on the yeast hyphae transformation in *C. albicans* cultures (Fig. 2). The increment of germ-tube formation was observed at all GABA concentrations used in both strains, although it was higher in the CA-121 strain than the ATCC 90028 strain. This effect was higher at 5 and 10 mM GABA in the CA-121 strain (287 ± 26 and 393 ± 29 cells with a germ tube/1,000 yeast cells) than in the ATCC 90028 strain (212 ± 27 and 253 ± 30 cells with a germ tube/1,000 yeast cells), respectively (see Fig. 2). At 10 and 20 mM GABA, no statistically significant difference of germ-tube formation was observed when comparing the two strains. Different factors have been associated with the dissemination of fungal infections in the skin, mucous membranes and the central nervous system, such as the depression of innate immune mechanisms, changes in environmental conditions, and an increment of fungal virulence factors (Dotis and Roilides 2007; Gabler et al. 2008). Of particular importance is the unbalanced production of neurotransmitters, cytokines, neurosteroids, and melanin, which can increase the pathogenicity of infectious agents through signaling mechanisms that are not yet completely clear (d'Ostiani et al. 2000; Morris-Jones et al. 2005). Furthermore, fungal invasivity can also be increased by nutrients such as glucose, which promotes in vitro oxidative stress resistance in *C. albicans* (Rodaki et al. 2009). In immunocompromised individuals, a carbohydrate-rich diet and alcohol consumption can induce oral and intestinal candidiasis (Akpan and Morgan 2002). Mammalian sera and various amino acids, such as glutamine and arginine, induce in vitro germ-tube formation in *C. albicans*. However, GABA has not yet been studied as an factor of growth enhancement in this yeast.

Our in vitro results showed that GABA increases the expression of virulence factors in *C. albicans* because both its germ-tube formation and PLB1 mRNA were upregulated when the yeast was cultured with the amino acid. This effect

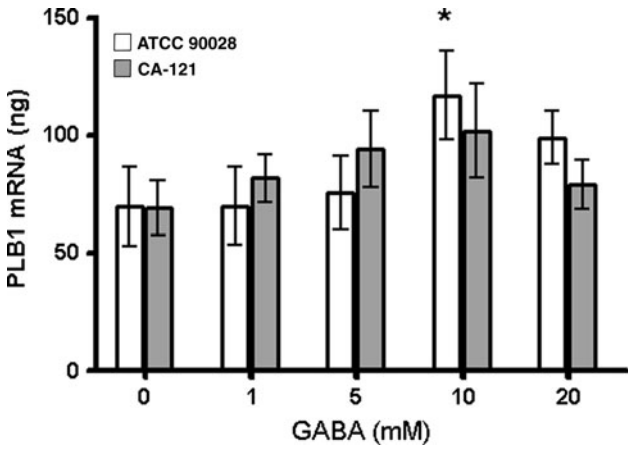

Fig. 1 Effect of gamma-aminobutyric acid (GABA) on *Candida albicans* phospholipase B1 (PLB1) mRNA. Two *C. albicans* strains were cultured in Sabouraud dextrose media to which were added different amounts of GABA for 48 h at 37°C and 200 rpm with constant shaking. Total mRNA was extracted and reverse transcribed using specific primers sets for the PLB1. PLB1 mRNA levels in *Candida* cultures (*white bars*, ATCC 90028 strain; *grey bars*, CA-121 strain). $*P < 0.001$. Values are means ± standard deviation. Results are representative of four independent experiments

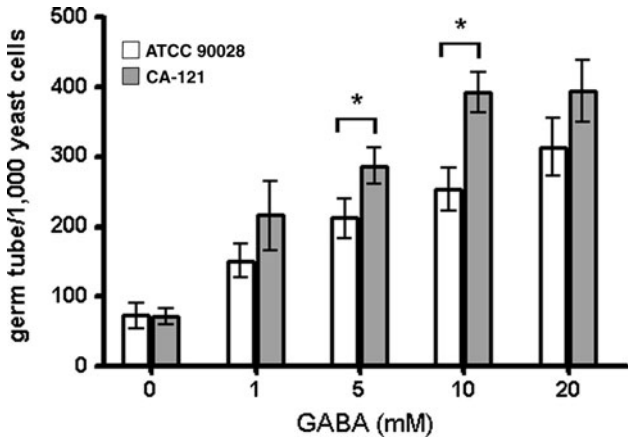

Fig. 2 Germ-tube formation in *Candida albicans* cultured with GABA. Two *C. albicans* (5×10^4 yeasts/ml) strains were cultured in human serum and different GABA concentrations at 37°C for 3 h. Germ tubes/1,000 yeast cells were counted in serum cultures with and without GABA (*white bars*, ATCC 90028 strain; *grey bars*, CA-121 strain). Values are means ± standard deviation. $*P < 0.001$ compared with untreated yeast cells. Results are representative of six independent experiments

was shown in a dose-dependent manner when different GABA concentrations were added to *C. albicans* cultures. In addition, when this fungus was cultured in human serum or Sabouraud medium that did not receive GABA, it showed both the lowest amount of yeast having germ tubes and the lowest amount of PBL1 mRNA of all cultures. Our findings show that GABA can increase the in vitro expression of virulence factors in *C. albicans* and suggest that they might enhance its in vivo spreading. On the other hand, *Candida* invasivity might be enhanced in vivo at least through two mechanisms simultaneously developed during host–pathogen interaction. The former is the GABA stimulatory effect on both yeast hyphae transformation and PLB1 expression, which are described in the present article. The latter is the GABA-mediated inhibitory effect on both the production of proinflammatory cytokines (Reyes-García et al. 2007) and phagocytosis (Lubick et al. 2007). Furthermore, *Candida* expresses a polyamine transporter, which passes GABA through its plasma membrane (McNemar et al. 2001), and diseases as well as therapeutic prescription of GABA or its analogues (Buvanendran et al. 2010) may also augment the concentration of this neurotransmitter in tissues. All these mechanisms may disrupt the host's antifungal immunity and enhance pathogen dissemination in susceptible individuals. Although GABA can be now accepted as another nutrient that increases the in vitro growth of *C. albicans*, additional studies are needed to unveil the signaling pathways by which this neurotransmitter induces PBL1 expression and the formation of germ tubes in this yeast, as well as the role that GABA might have in promoting in vivo *C. albicans* growth.

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