Galanin Message-Associated Peptide Suppresses Growth and the Budded-to-Hyphal-Form Transition of *Candida albicans*

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The expression of the mRNA encoding galanin message-associated peptide (GMAP) in human keratinocytes is upregulated by lipopolysaccharides and exposure to *Candida albicans*. GMAP has growth-inhibiting activity against *C. albicans* and inhibits the budded-to-hyphal-form transition, establishing GMAP as a possible new component of the innate immune system.

The commensal organism *Candida albicans* resides on epithelial surfaces and is the major fungal pathogen in humans (11). In immunocompromised individuals, infections caused by *C. albicans* constitute a serious clinical problem (6). The pathogenicity of *C. albicans* is due to its different growth forms: the highly proliferative yeast (budded) form and the hyphal form supporting invasion into the host. The budded-to-hyphal-form transition is an important virulence factor of *C. albicans* (8). Antimicrobial peptides (AMPs) participate in the innate immune response by providing a rapid first line of defense against pathogens. The fact that neuropeptides, like AMPs, are in general amphipathic molecules may explain the recent findings that several neuropeptides display antimicrobial activity (for a review, see reference 2).

Galanin, a 29-amino-acid neuropeptide (16), is processed from a 123-amino-acid precursor molecule, pre-pro galanin (ppGAL), which contains a signal peptide, the mature galanin peptide, and a carboxy-terminal 59-amino-acid galanin message-associated peptide (GMAP) (Fig. 1A). Galanin has been shown to be widely distributed in the central and peripheral nervous systems where it elicits diverse biological responses by binding to three known galanin receptors (10, 17, 18). For GMAP, no major physiological functions and receptors have been identified.

Recently, ppGAL mRNA was detected in the epidermis, hair follicles, sweat glands, and around the blood vessels of human skin (7). In vitro receptor autoradiography detected galanin-binding sites around blood vessels and sweat glands of the dermis but not in the epidermis (7), indicating a non-receptor-mediated function of ppGAL in this outermost barrier of the body.

A mix containing three human synthetic peptides—galanin, GMAP (1-41), and GMAP (44-59) (all 40 μg/ml)—caused no significant growth inhibition of *Staphylococcus aureus*, *Escherichia coli*, or * Corynebacterium jeikeium* as determined by the BacTiter-Glow microbial viability assay (Promega; data not shown). By contrast, the same galanin-GMAP mix caused a significant reduction in the growth of *C. albicans* K2 (1 × 10^5 to 3 × 10^5 CFU/ml; Sabouraud dextrose broth, 16 h at 30°C; BacTiter-Glow assay). Treatment with single-peptide GMAP (1-41) (20 μg/ml), but not galanin (20 μg/ml), resulted in a significant growth reduction (48.7%) of *C. albicans* K2 (Fig. 1B) as well as the laboratory strains *C. albicans* SC 5314 and CBS 5983 and two clinical *C. albicans* isolates from human skin. We were able to narrow the antimicrobial core sequence to GMAP (16-41) (Fig. 1). Further N-terminal and C-terminal truncations led to loss of antimicrobial activity. Porcine and human GMAP (16-41) had the same effect, indicating that the antifungal activity of GMAP is conserved across species. The antifungal activity of GMAP (16-41) occurs at a concentration of 12 μg/ml. However, GMAP is not as potent as the control peptide magainin I, which reduced the growth of *C. albicans* by more than 90%. Higher concentrations of GMAP (16-41), up to 50 μg/ml, did not increase its growth-inhibiting effect.

In accordance with the literature, adrenocorticotropic hormone (ACTH) (18-39) did not affect the growth of *C. albicans* (Fig. 1B) (3). Examination of cultures of *C. albicans* in RPMI medium [0.3 g/liter L-glutamine, 0.165 M 3-(Nmorpholino)propanesulfonic acid pH 7.0; 37°C] after 24 h showed a budded-to-hyphal transition (Fig. 2A and C). For better microscopic examination of hyphal structures, some representative cultures were stained with calcofluor white (5) (Fig. 2C and D). Treatment of these cultures with GMAP (1-41) (4 μM) or GMAP (16-41) (4 μM, human and porcine) resulted in a significant inhibition of this yeast-to-hyphal transformation (Fig. 2). This inhibition was still visible, albeit weaker, with 2 μM GMAP (1-41) or (16-41) but was lost with 1 μM concentrations of the peptides. The concentration of GMAP necessary to affect the growth features of *C. albicans* is in the range of other neuropeptides with antimicrobial activity (1 pM to 100 μM) (2). To our knowledge only the neuropeptide α-MSH has been reported to have an inhibitory effect on germ tube formation of *C. albicans* (3). That report suggested that the candidacidal effect of α-melanocyte-stimulating hormone (α-MSH) is mediated through induction of cyclic adenosine monophosphate, most likely via binding to a membrane receptor of *C. albicans*.

Since ppGAL mRNA was previously detected in human
keratinocytes, the regulation of ppGAL upon treatment of human primary cultured keratinocytes (19) with lipopolysaccharide (LPS) or living C. albicans was investigated. After isolation of the RNA (Tri-Reagent; Molecular Research Centre) and cDNA synthesis (Superscript II reverse transcriptase; Invitrogen), real-time PCR on the iCycler iQ real-time detection system using IQ SYBR Green Supermix (Bio-Rad) was carried out. Induction of intercellular adhesion molecule 1 (ICAM-1) was used as a positive control. Treatment of keratinocytes with LPS induced the ppGAL expression (3.4-fold).
Cocultivation of primary human keratinocytes with *C. albicans* SC 5314 for 6 h induced ppGAL expression 1.8-fold (Fig. 3). Several properties of ppGAL are shared by other AMPs (1), suggesting that ppGAL is a natural AMP. These common properties include the following: a high level of expression in the epidermis (7), which, for example, parallels the expression of the antimicrobial neuropeptide α-MSH (14); sites of potential microbial entry in the skin, such as follicular structures and sweat glands, express ppGAL (7); and posttranslational processing of the precursor molecule is required for a functional peptide. Moreover, the ability of LPS to induce AMP gene expression has been demonstrated for several antimicrobial neuropeptides, including adrenomedullin, α-MSH, and proenkephalin (12, 13, 15).

During the last decades, microbial resistance against antibiotics has emerged as a serious problem in the treatment of infection (4). In the search for new antimicrobial compounds, AMPs and their synthetic analogues seem to be a promising source (9). Synthetic derivatives of GMAP, chemically stable and resistant to enzymatic degradation, could form the basis for novel therapies. A potential advantage of using GMAP analogues as therapeutics for *C. albicans* infection is that, as an endogenous peptide, GMAP might be a nontoxic alternative to other commonly used agents. The mechanism of action of GMAP is different from those of common antifungal substances like fluconazole, which are cytotoxic to the pathogen either by inhibiting growth or direct killing. Inhibition of the budded-to-hyphal-form transition in combination with common cytotoxic drugs like fluconazole might lower the risk of pathogen dissemination and could have a preventive effect in high-risk patients. Furthermore, rising problems with resistance to antifungal drugs currently in use might be solved by using drugs with a different mode of action, such as GMAP, presented here.

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