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# Inhibition of pituitary hormone exocytosis by a synthetic peptide related to the rab effector domain

J.S. Davidson[a], A. Eales[a], R.W. Roeske[b] and R.P. Millar[a]

[a]*Regulatory Peptides Research Unit, Department of Chemical Pathology, University of Cape Town Medical School, Cape Town 7925, South Africa* and [b]*Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN 46202–5122, USA*

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GTP-binding proteins of the rab family are believed to function at several steps in intracellular vesicular transport. We examined the effects of a rab-related peptide in permeabilized pituitary cells, in which exocytosis can be triggered by distinct $Ca^{2+}$-dependent or $Ca^{2+}$-independent pathways. We report that a synthetic peptide of 18 amino acids related to the rab effector domain, rab3AL(30–47) inhibited luteinizing hormone (LH) and growth hormone (GH) exocytosis triggered by either pathway. $Ca^{2+}$-stimulated LH and GH release were inhibited by more than 80% and 50%, respectively, by 100 $\mu$M peptide. The peptide (100 $\mu$M) also inhibited LH and GH exocytosis stimulated by phorbol myristate acetate plus cAMP by more than 45% and 80%, respectively. The effect was sequence-specific since a second peptide, lacking the first 3 amino acids but otherwise identical failed to inhibit exocytosis. These results suggest that a protein of the rab family is involved in regulated pituitary hormone exocytosis, and they identify 3 amino acids of the putative rab effector domain which may be functionally important in exocytosis.

Exocytosis; Rab; GTP-binding protein; Secretion; Pituitary; Luteinizing hormone; Growth hormone

## 1. INTRODUCTION

The effects of hydrolysis-resistant GTP analogs in a variety of secretory cell types have provided evidence that GTP-binding proteins are involved in regulated exocytosis at a site distal to second messenger generation [1–9]. The identity of these protein(s) is unclear, and GTP-binding proteins belonging to both the heterotrimeric and the small monomeric classes are reported to be associated with secretory vesicles [10].

The rab family of small GTP-binding proteins are known to be involved at several stages in vesicular transport in mammalian cells [11,12]. In constitutive exocytosis in yeast, a monomeric GTP-binding protein, SEC4p, functions in the targeting or fusion of secretory vesicles with the plasma membrane [13]. Rab3a appears to be a likely candidate for a similar role in regulated exocytosis in mammalian cells, because of its association with synaptic vesicles and its dissociation during exocytosis [14,15]. Although this is an attractive hypothesis, functional studies are needed to confirm the involvement of a rab-related protein in either constitutive or regulated exocytosis in mammalian cells.

*Correspondence address:* J.S. Davidson, Regulatory Peptides Research Unit, Department of Chemical Pathology, University of Cape Town Medical School, Cape Town 7925, South Africa. Fax: (27) (21) 478 955.

We report here that in permeabilized pituitary cells, exocytosis of luteinizing hormone (LH) and growth hormone (GH) stimulated by both $Ca^{2+}$-dependent and $Ca^{2+}$-independent mechanisms was inhibited by a synthetic peptide related in sequence to the rab effector domain.

## 2. MATERIALS AND METHODS

Primary sheep pituitary cells were dispersed with collagenase and cultured in 12-well plates for 48h as previously described [16]. Attached cells were washed twice with HEPES-buffered saline (buffer I, ref. 16) and once in $Ca^{2+}$-free buffer I. Cells were incubated for 10 min with ice-cold buffer IC containing 0.5 mM EGTA, 1 mM $MgCl_2$ and 1 U/ml streptolysin O (SLO, Wellcome Diagnostics cat. no. MR 16) to allow binding of SLO to cells. Buffer IC consisted of 140 mM Na propionate, 25 mM PIPES (pH 6.6). Excess SLO-containing buffer was removed and cells were permeabilized and stimulated simultaneously by addition of 0.4 ml/well buffer IC at 37°C, containing 5 mM sodium ATP, 10 mM creatine phosphate, 7 mM $MgCl_2$ and Ca-EGTA mixture (total EGTA 30 mM) formulated to give free $Ca^{2+}$ 0.1 $\mu$M (unstimulated) or 10 $\mu$M ($Ca^{2+}$-stimulated). An alternative stimulation utilized 0.1 $\mu$M phorbol myristate acetate (PMA) plus 30 $\mu$M cAMP, with 0.1 $\mu$M free $Ca^{2+}$. After 15 min the medium was collected, centrifuged and stored at −20°C prior to assay for LH and GH.

Ovine LH and GH were determined by radioimmunoassay using reagents provided by the National Institute for Diabetic, Digestive and Kidney Diseases, using the recommended procedures. Hormone release is expressed as% of total cellular hormone content determined after lysis of cells from 4 unstimulated (control) wells with 1% Triton X-100. Data points show the mean ± difference of duplicate wells in a representative experiment. Each experiment was performed independently 2 to 4 times with similar results.

The peptide nomenclature used follows that of Plutner et al. [17]. The numbering refers to the homologous amino acid positions in

Ha-ras. Peptides Rab3AL(30–47) (PAFVSALGIDFKVKTIYR) and Rab3AL(33–47) (VSALGIDFKVKTIYR) were synthesized by the solid phase method using Boc for α-amino protection and benzyl-based protecting groups for side chain functional groups. Peptides were assembled on a Boc Arg (MTS) Pam resin using an Applied Biosystems Model 431A automated synthesizer, with couplings mediated by DCC/HOBt. After treatment of the resin with HF at 0°C for 45 min the peptides were extracted into 50% HOAc, diluted with water and lyophilized to a white powder. Crude peptides were purified to homogeneity by preparative reversed-phase chromatography on $C_{18}$ silica gel, using isocratic elution with acetonitrile/water/ 0.1% TFA.

## 3. RESULTS AND DISCUSSION

Rab proteins contain a highly conserved region (homologous to amino acids 33–48 in Ha-ras) which, by analogy with the ras proteins, is thought to be the effector domain which mediates the functions of these proteins in vesicular transport [17]. It was recently reported that synthetic peptides related to the putative effector domain of the rab proteins inhibited endoplasmic reticulum to Golgi and intra-Golgi vesicular transport in cell-free assay systems [17]. The most potent inhibitory peptide in that study was rab3AL(33–48), which is derived from the rab3 sequence with two amino acid substitutions. We therefore chose a similar peptide, rab3AL(33–47) for the present study, together with a longer peptide, rab3AL(30–47).

We previously showed that in permeabilized pituitary cells LH exocytosis can be stimulated by two distinct triggering mechanisms, (a) a $Ca^{2+}$-dependent pathway maximally stimulated by 10 μM free $Ca^{2+}$ and (b) a $Ca^{2+}$-independent pathway maximally stimulated by a combination of the protein kinase C activator phorbol myristate acetate (PMA) plus cAMP [16,18]. The effect of rab-related peptides on both of these modes of stimulation was investigated in the present study.

Peptide Rab3AL(30–47) inhibited both $Ca^{2+}$ and PMA/cAMP stimulation of LH and GH exocytosis in a dose-dependent manner (Figs. 1 and 2). The peptide had no effect on basal release of either hormone. At a concentration of 100 μM, $Ca^{2+}$-stimulated LH release was inhibited by more than 80% in all 4 experiments.

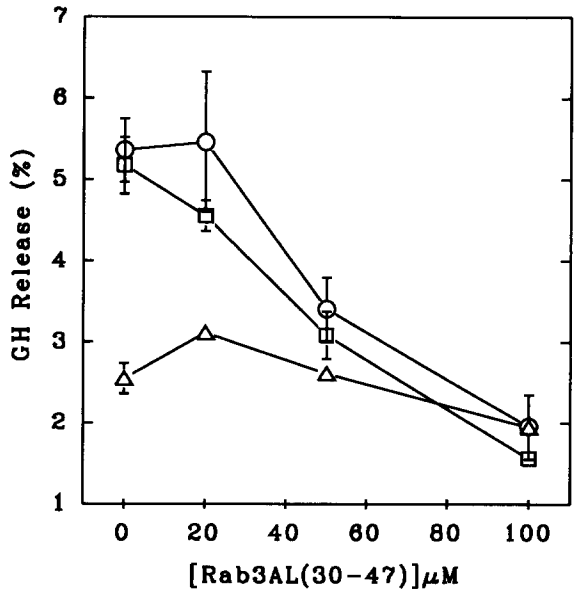


Fig. 2. Effect of peptide rab3AL(30–47) on GH exocytosis stimulated by $Ca^{2+}$ (circles), phorbol ester plus cAMP (squares) or unstimulated (triangles).

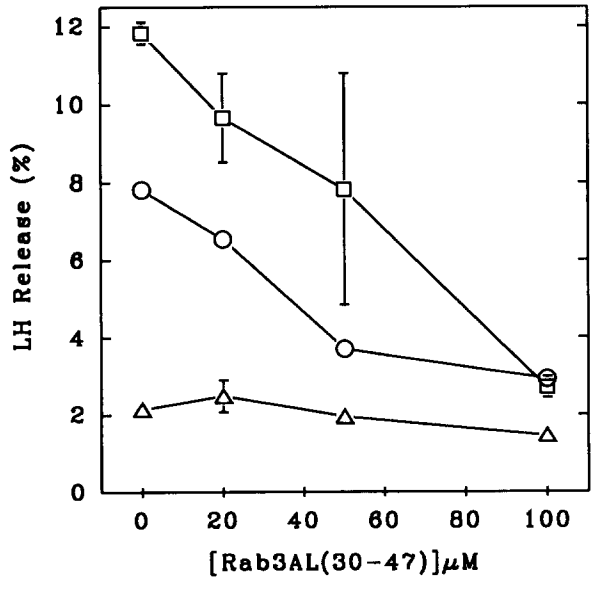


Fig. 1. Effect of peptide rab3AL(30–47) on LH exocytosis stimulated by $Ca^{2+}$ (circles), phorbol ester plus cAMP (squares) or unstimulated (triangles).

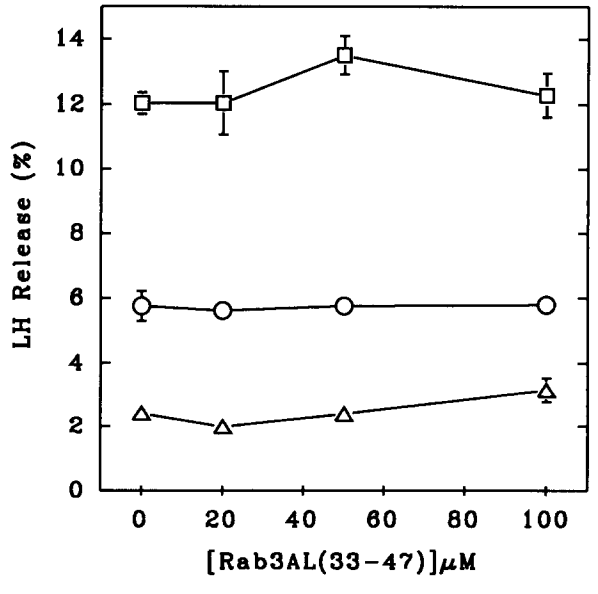


Fig. 3. Effect of peptide rab3AL(33–47) on LH exocytosis stimulated by $Ca^{2+}$ (circles), phorbol ester plus cAMP (squares) or unstimulated (triangles).

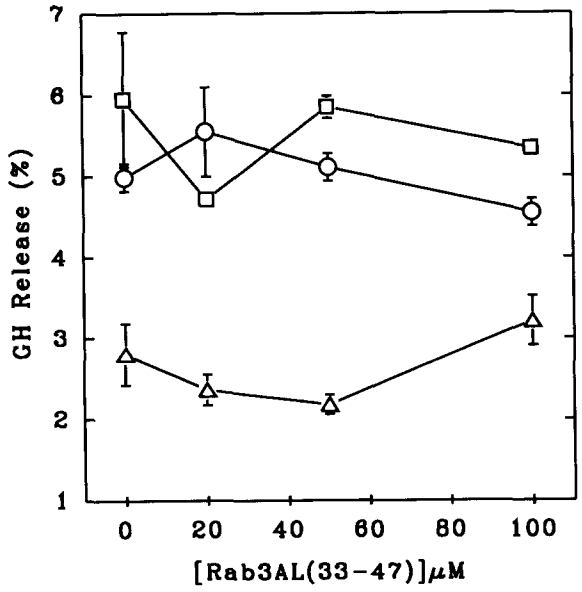


Fig. 4. Effect of peptide Rab3AL(30–47) on GH exocytosis stimulated by $Ca^{2+}$ (circles), phorbol ester plus cAMP (squares) or unstimulated (triangles).

Inhibition of PMA/cAMP-stimulated LH release was more variable, ranging from 45% to 85% inhibition by 100 μM peptide. The same concentration of peptide inhibited $Ca^{2+}$-stimulated GH exocytosis by 50 to 100%, and PMA/cAMP-stimulated GH exocytosis by 80 to 100% (2 experiments). These concentrations of peptide are in the same range as reported for inhibition of ER to Golgi and intra-Golgi transport by rab3AL(33–48) [17].

In contrast, the shorter peptide Rab3AL(33–47) at concentrations up to 100 μM was without any effect on LH or GH release stimulated by either triggering pathway (Figs. 3 and 4).

These results indicate that the inhibitory effect has an absolute requirement for the N-terminal 3 amino acids of the longer peptide. Inhibition of pituitary hormone exocytosis therefore requires a sequence specificity different from that for inhibition of ER to Golgi and intra-Golgi transport, since the latter processes were inhibited by rab3AL(33–48) [17].

In two recent studies the peptide rab3AL(33–48) was surprisingly found to enhance, rather than inhibit regulated exocytosis in permeabilized pancreatic acini [19], and chromaffin cells [20]. Peptides extending further in the N-terminal direction were not examined in those studies, and it would therefore be of interest to determine whether rab3AL(30–47) also inhibits exocytosis in these cell types.

In summary, a peptide related to the rab effector domain inhibited exocytosis of LH and GH triggered by $Ca^{2+}$-dependent and $Ca^{2+}$-independent pathways, suggesting the involvement of a rab-like protein in regulated exocytosis. A larger number of peptides are currently being examined in order to determine the precise sequence specificity of the inhibitory effect.

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