

Galanin evokes a cytosolic calcium bursting mode and hormone release in GH3/B6 pituitary cells

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The effects of galanin on secretion and cytosolic free Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) have been studied in GH3/B6 pituitary cells. Prolactin (PRL) and growth hormone (GH) release was measured in column perfusion experiments; $[\text{Ca}^{2+}]_i$ was monitored in single cells by dual emission microspectrofluorimetry using indo-1 as intracellular Ca^{2+} probe. Galanin (0.1–1 nM) caused PRL and GH release coincident with a modest rise in $[\text{Ca}^{2+}]_i$. The increase in $[\text{Ca}^{2+}]_i$ comprises the establishment of characteristic long-lasting bursts of $[\text{Ca}^{2+}]_i$ transients. Galanin acts on Ca^{2+} entry through voltage-gated Ca^{2+} channels since there was no response to the peptide when Cd^{2+} – a Ca^{2+} channel blocker – was added to the bath solution. The stimulation of bursting activity by galanin may provide a fine Ca^{2+} -signalling mechanism which maximally stimulates hormone release while avoiding refractory periods.

Galanin; Calcium; Prolactin; Growth hormone; GH3 cell

1. INTRODUCTION

Galanin, a newly discovered neuropeptide [1], present in high concentration in the hypothalamus [2], has recently emerged as a putative stimulant of PRL and GH release from anterior pituitary [3,4]. Although the sequence of signal transduction solicited by galanin is still unknown at the pituitary cell level, studies performed in other cell types have reported that galanin may act through a variety of pathways, such as, inositol phospholipid breakdown [5], adenylate cyclase activity [6], ionic (K^+) channels [7] and cytosolic free Ca^{2+} concentration, $[\text{Ca}^{2+}]_i$ [8].

Regulation of $[\text{Ca}^{2+}]_i$ is well known as an important avenue by which transmitters promote hormone release from pituitary cells. Fluorescent methods recently developed allow the monitoring of $[\text{Ca}^{2+}]_i$ in individual cells loaded with either fura-2 or indo-1, as intracellular Ca^{2+} probes [9,10]. Using such technologies, we have previously provided evidence that the firing of action potentials spontaneously occurring in PRL- and GH-secreting clonal pituitary cells (GH3 cells), leads to well-defined transient elevations of $[\text{Ca}^{2+}]_i$ of a magnitude sufficient to cause hormone release [11,12]. Furthermore, an important part of pituitary cell signalling is based on the modulation of these $[\text{Ca}^{2+}]_i$ oscillations. Indeed, it was found that hormones and neurotransmitters affect $[\text{Ca}^{2+}]_i$ oscillations in agreement with their major influence on secretion [11,13,14].

In the present report, galanin action was studied in GH3/B6 pituitary cells (a GH3 sub-clone) in which (i) secretion was measured using column perfusion experiments from cell populations, and (ii) $[\text{Ca}^{2+}]_i$ was monitored using dual emission microspectrofluorimetry in individual cells loaded with indo-1.

2. MATERIALS AND METHODS

GH3/B6 pituitary cells were cultured in a Ham's F10 nutrient medium supplemented with 12.5% inactivated horse serum and 2.5% fetal calf serum and maintained at 37°C in a humidified atmosphere gassed with 95% air–5% CO_2 . Hormone secretion experiments and microfluorimetric recordings were conducted 5–10 days after replating on to Cytodex 1 microcarriers and glass coverslips, respectively. To determine the effects of galanin on secretion, cells were perfused with modified M199 medium containing 0.4% BSA and buffered to pH 7.2 with $\text{Na}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ under standard column perfusion experiments [15]. PRL and GH were assayed by radioimmunoassay using materials kindly provided by NIDDK through the courtesy of Dr Parlow. For the dye loading with the Ca^{2+} probe indo-1, the nutrient medium was replaced with modified Hank's solution containing 142.6 mM NaCl, 5.6 mM KCl, 2 mM CaCl_2 , 0.8 mM MgCl_2 , 5 mM glucose, 10 mM Hepes, and buffered to pH 7.3 with NaOH. The cells were loaded with indo-1 by exposure to 5 μM indo-1 penta-acetoxymethylester (indo-1/AM, Calbiochem, USA) and 0.02% Pluronic F-127 (Molecular Probes, USA) in Hank's solution for 30 min at 20°C. $[\text{Ca}^{2+}]_i$ was estimated in individual cells from indo-1 fluorescence by the ratio method using single wavelength excitation (355 nm) and dual emission (405 nm and 480 nm) exactly as previously described [12]. Porcine galanin was purchased from UCB-Bioproducts (Belgium) and salts from Sigma (USA). Test compounds were applied during fluorescent experiments by pressure ejection from micropipettes (tip diameter, 2–5 μm) positioned close to the recorded cell (within 10–30 μm).

Results were expressed as means \pm SD when appropriate. Student's *t*-test was used for statistical comparison among means and differences with $P < 0.01$ were considered significant.

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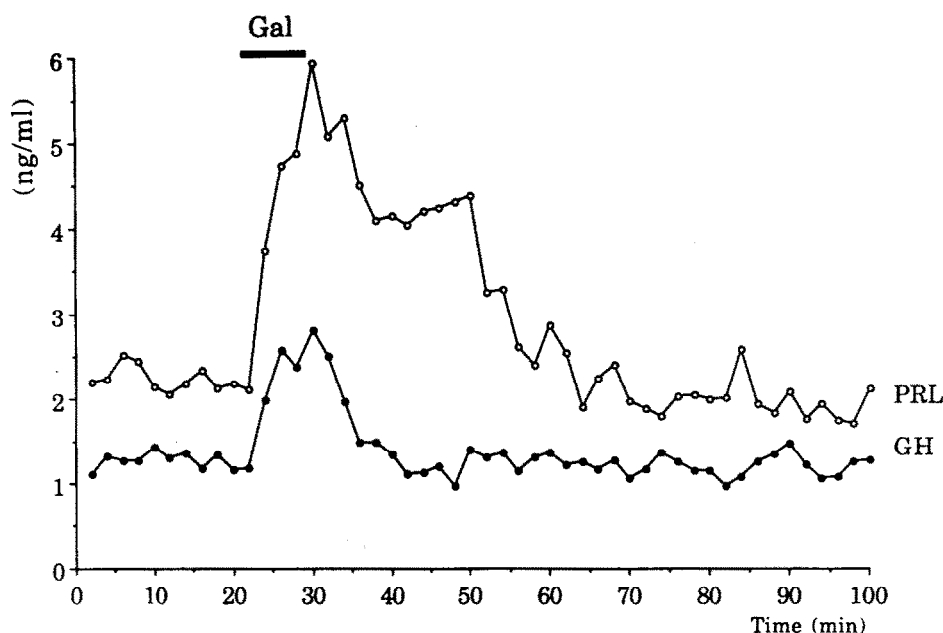


Fig. 1. Effects of galanin on secretion from GH3/B6 cells. PRL (unfilled circles) and GH (filled circles) release was measured in column perfusion experiments. Galanin (0.2 nM) induced a clear-cut rise in PRL and GH release. Each secretion profile was representative of five experiments made in duplicate.

3. RESULTS

We first examined under column perfusion experiments whether galanin would affect secretion from GH3/B6 cells as recently described in normal rat somatotrophs [4]. Galanin (0.1–1 nM) induced a reversible enhancement of PRL and GH release from GH3/B6 cells. An example of such an effect is shown in Fig. 1.

Single cell $[Ca^{2+}]_i$ recordings addressed the question of whether galanin action on secretion might be related to a $[Ca^{2+}]_i$ signal. In total, 58 separate cells were investigated. It was demonstrated that this peptide consistently evoked a characteristic change in firing of $[Ca^{2+}]_i$ transients at concentrations effective in eliciting secretion. In cells showing spontaneously a near-constant firing of single $[Ca^{2+}]_i$ transients, galanin (1 nM) induced an enhancement of frequency (Fig. 2A) (0.16 ± 0.12 Hz vs 0.29 ± 0.15 Hz for galanin-treated, $P < 0.001$, $n = 27$) associated with a change in firing pattern, i.e. $[Ca^{2+}]_i$ oscillations occurred frequently as bursts of spikes (2–15 transients) separated with silent intervals. It should be noted that $[Ca^{2+}]_i$ did not return to pre-stimulated basal level during bursts. Randomly grouped $[Ca^{2+}]_i$ transients (2–5 transient) were detected in some cells prior to galanin stimulation. In such cells, galanin caused prolonged bursts (27 ± 6 s vs 61 ± 10 s, $P < 0.001$, $n = 17$) which each resulted from a package of 3–5 groups of $[Ca^{2+}]_i$ transients (Fig. 2B). Computed integration of $[Ca^{2+}]_i$ above pre-stimulated basal level, calculated during 2 min prior and after peptide application, revealed that galanin caused – via bursting activity

– a modest, but significant increase in $[Ca^{2+}]_i$ from 147 ± 85 nM·s to 229 ± 113 nM·s ($P < 0.001$, $n = 24$). Furthermore, galanin triggered a burst-firing of $[Ca^{2+}]_i$ transients in silent cells (Fig. 2C, $n = 14$).

The link between galanin-evoked $[Ca^{2+}]_i$ response and secretion was investigated by use of Ca^{2+} channel blockers. Cadmium was used as a powerful blocker of the two distinct types of Ca^{2+} channels, namely T and L, expressed in GH3 cells [16]. When Cd^{2+} (200 μ M) was applied, the spontaneous $[Ca^{2+}]_i$ oscillations and

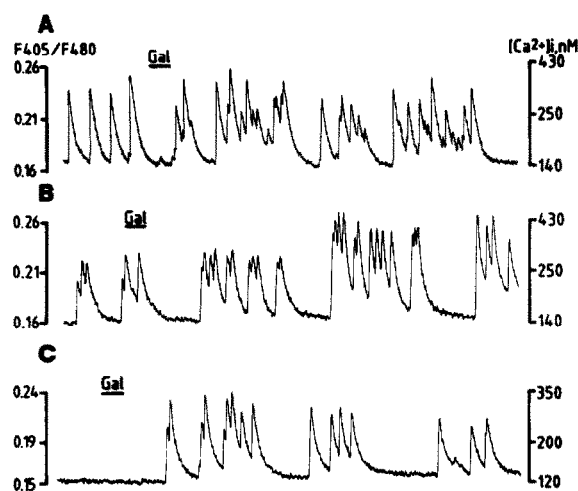


Fig. 2. Effects of galanin on $[Ca^{2+}]_i$ in GH3/B6 cells. $[Ca^{2+}]_i$ was recorded from individual cells loaded with indo-1. Galanin (1 nM) was applied by low-pressure ejection from external micropipettes. (A and B) Two different cells undergoing characteristic oscillations in $[Ca^{2+}]_i$ linked to spontaneous action potentials [11]. (C) A silent cell.

hormone release were suppressed and decreased by about 70% respectively. Furthermore, both galanin-stimulated $[Ca^{2+}]_i$ transients and galanin-induced hormone release were completely blocked. The inhibitory action of Cd^{2+} on $[Ca^{2+}]_i$ bursting was reversible (Fig. 3A). The recovery of hormone release was incomplete under our experimental conditions (Fig. 3B), as expected following a long-lasting Cd^{2+} application. In contrast, application of nickel, Ni^{2+} (200 μM), a weaker blocker of Ca^{2+} channels in GH3/B6 cells, appeared to be much less effective than Cd^{2+} in reducing galanin action (data not shown). Thus, a voltage-gated Ca^{2+} -entry may be considered as a prerequisite for the galanin action on both $[Ca^{2+}]_i$ and subsequent secretion.

4. DISCUSSION

Our findings provide evidence that galanin evokes hormone release in GH3/B6 pituitary cells. Furthermore, this peptide enhances or even triggers the firing of $[Ca^{2+}]_i$ transients that appears consistently as a bursting activity in the same cell line.

We have previously shown that transient rises in $[Ca^{2+}]_i$ caused by firing of action potentials are sufficient to evoke an exocytotic process from GH3/B6 cells [11]. It seems reasonable to propose that galanin action on secretion may occur by a direct facilitation of

bursting patterns of electrical activity and ensuing enhancement of $[Ca^{2+}]_i$ firing because of the similar sensitivities to Ca^{2+} channel blockers of both of these processes. Note, that the apparent discrepancy between the response time for the onset of $[Ca^{2+}]_i$ bursting and the increase in hormone release would argue against such a relationship. However, the $[Ca^{2+}]_i$ data and the hormone release data were collected in experiments performed on individual cells and cell populations, respectively. The slower response time observed for hormone release may reflect a delay due to solution mixing in the perfusion chamber and asynchronous hormone release from a cell population which may mask the kinetics of hormone release of individual cells [13,15].

Stimulated bursting activity as evoked in response to galanin has not yet been described in endocrine pituitary cells. The modulation of firing activity at the pituitary cell level has been frequently discussed in terms of frequency or amplitude of action potentials and/or $[Ca^{2+}]_i$ transients [11,17]. The present findings suggest that a temporal organization of $[Ca^{2+}]_i$ oscillations – as a bursting mode – may also play an important role in hormone release. Indeed, galanin evokes a large enhancement of hormone release associated with a modest increase in $[Ca^{2+}]_i$. Thus, $[Ca^{2+}]_i$ bursting may provide a fine mechanism to stimulate the secretory process without large $[Ca^{2+}]_i$ elevations.

Burst-firing activity and its regulation have been well described in other cell types such as glucose-stimulated insulin-secreting β cells and 'bursting' neurones in which bursting electrical activity is generated by a complex interplay of ionic channel activities [18,19]. The link between bursting mode and the secretory process in 'bursting' neurone endings has been recently opened to investigation under simultaneous measurements of voltage-gated Ca^{2+} -entry (patch-clamp recordings) and exocytotic fusion of secretory granules (membrane capacitance recordings). These combined experiments strengthened the suggestion that intermittent bursts of repetitive Ca^{2+} -entry is an effective way to periodically trigger exocytosis without any attenuation [20]. A bursting mode may thus provide a frequency-encoded Ca^{2+} -signalling to maximally stimulate release while avoiding the adaptation that often occurs at a sustained high frequency. Such refractory states are seen in studies on populations of pituitary cells where large $[Ca^{2+}]_i$ transients cause hormone release but may also lead to adaptation when evoked at high frequency [21]. As the establishment of $[Ca^{2+}]_i$ bursting is the characteristic of galanin action on Ca^{2+} homeostasis, we propose that the $[Ca^{2+}]_i$ bursting mode is an efficient way to pace the various steps of the exocytotic process. Further exploration of the link between bursting activity and its influence on hormone release may extend our understanding of the range of mechanisms whereby hormones are released from pituitary cells. Such a study will be attempted by a

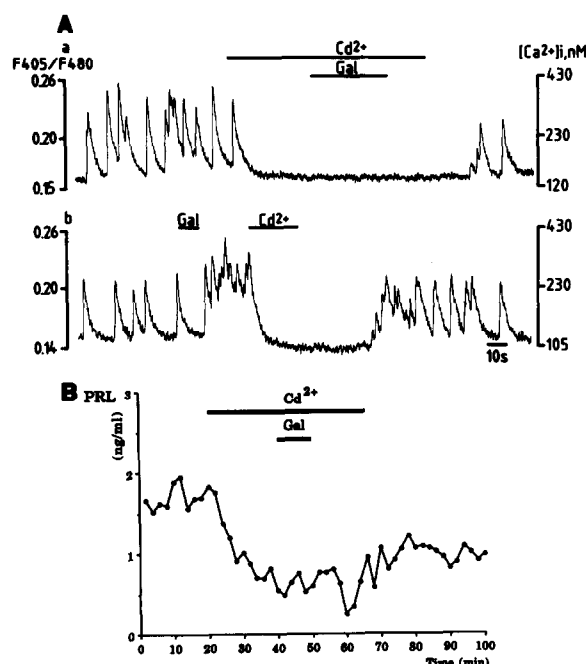


Fig. 3. Effects of Cd^{2+} ions on both $[Ca^{2+}]_i$ and secretion. (A) $[Ca^{2+}]_i$ measurements came from two separate cells. (Aa) Cd^{2+} (200 μM) completely abolished spontaneous $[Ca^{2+}]_i$ oscillations as well as the $[Ca^{2+}]_i$ response to 1 nM galanin; (Ab) Cd^{2+} (200 μM) reversibly blocked $[Ca^{2+}]_i$ oscillations during galanin action. (B) Cd^{2+} (200 μM) reduced basal PRL release and prevented PRL response to 0.2 nM galanin. A similar GH release profile was observed (not shown).

direct measurement of exocytosis using membrane capacitance measurements combined simultaneously with single cell $[Ca^{2+}]_i$ recordings.

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