ELSEVIER

Biochimica et Biophysica Acta 1449 (1999) 186–193

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Involvement of phosphodiesterase-cGMP-PKG pathway in intracellular Ca^{2+} oscillations in pituitary GH_3 cells

M. Cataldi [a], A. Secondo [a], A. D'Alessio [a], F. Sarnacchiaro [a], A.M. Colao [a], S. Amoroso [a], G.F. Di Renzo [b], L. Annunziato [a,*]

[a] *Section of Pharmacology, Department of Neuroscience, School of Medicine, Federico II University of Naples, Via S. Pansini 5, 80131 Naples, Italy*

[b] *School of Pharmacy, University of Catanzaro, Catanzaro, Italy*

Received 10 November 1998; received in revised form 19 January 1999; accepted 22 January 1999

Abstract

The present study investigates the potential role of the Ca^{2+}-calmodulin-dependent type I phosphodiesterase (PDE)-cGMP-protein kinase G (PKG) pathway in spontaneous $[Ca^{2+}]_i$ oscillations in GH_3 cells using fura-2 single cell videoimaging. Vinpocetine (2.5–50 μM), a selective inhibitor of type I PDE, induced a concentration-dependent inhibition of spontaneous $[Ca^{2+}]_i$ oscillations in these pituitary cells, and at the same time produced an increase of the intracellular cGMP content. The cell permeable cGMP analog N^2,2′-*O*-dibutyryl-cGMP (dB-cGMP) (1 mM) caused a progressive reduction of the frequency and the amplitude of spontaneous $[Ca^{2+}]_i$ oscillations when added to the medium. KT5823 (400 nM), a selective inhibitor of cGMP-dependent protein kinase (PKG), produced an increase of baseline $[Ca^{2+}]_i$ and the disappearance of spontaneous $[Ca^{2+}]_i$ oscillations. When KT5823 was added before vinpocetine, the PKG inhibitor counteracted the $[Ca^{2+}]_i$ lowering effect of the cGMP catabolism inhibitor. Finally, the removal of extracellular Ca^{2+} or the blockade of L-type voltage-sensitive calcium channels (VSCC) by nimodipine produced a decrease of cytosolic cGMP levels. Collectively, the results of the present study suggest that spontaneous $[Ca^{2+}]_i$ oscillations in GH_3 cells may be regulated by the activity of type I PDE-cGMP-PKG pathway.

Keywords: Pituitary; Intracellular Ca^{2+} concentration; Phosphodiesterase; cGMP; Oscillation; Protein kinase G

1. Introduction

Intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) in pituitary cells display wide fluctuations which occur spontaneously and in a periodic oscillatory pattern [1,2]. These $[Ca^{2+}]_i$ oscillations are involved in some relevant biological functions, such as the spontaneous release of anterior pituitary hormones [3,4], as well as the transcription of the mRNAs encoding for PRL and glycoproteic hormones [5,6].

The mechanisms underlying $[Ca^{2+}]_i$ oscillations in pituitary cells are only partially understood [1,2]. It has been proposed that two different oscillators located at the level of plasmamembrane (PM oscillator) and of endoplasmic reticulum Ca^{2+} stores (ER oscillator) are involved in the genesis of $[Ca^{2+}]_i$ oscillations [7]. In GH_3 pituitary cells $[Ca^{2+}]_i$ oscillations seem to depend on a complex interplay between intracellular Ca^{2+} stores which alternatively release and recapture Ca^{2+} ions from the cytoplasm, and

* Corresponding author. Fax: +39 (81) 746-3323; E-mail: farmacol@unina.it

0167-4889/99/$ – see front matter
PII: S0167-4889(99)00013-0

voltage-sensitive Ca^{2+} channels (VSCC) which open and close spontaneously allowing the periodical entrance of Ca^{2+} ions into the cytoplasm [8,9]. Since cAMP and cGMP through the activation of cAMP dependent- and cGMP-dependent protein kinases (PKA and PKG) can modify the activity of channels and pumps located both on the 'PM' and on the 'ER' oscillators [10–14], it has been suggested that $[Ca^{2+}]_i$ oscillations may be linked to changes in the intracellular contents of these nucleotides [15]. Steady-state levels of cAMP and cGMP seem to be mostly regulated by phosphodiesterases (PDE) [16]. Seven different isoforms of these enzymes have been cloned until now. One of these seven isoenzymes, type I PDE, is activated by Ca^{2+}-calmodulin [17] and therefore may translate changes in $[Ca^{2+}]_i$, in variations of intracellular cGMP and/or cAMP contents as well as play a role in the genesis of spontaneous $[Ca^{2+}]_i$ oscillations. In order to explore the potential involvement of type I PDE-cGMP-PKG pathway in this process, spontaneous $[Ca^{2+}]_i$ oscillations have been continuously monitored in single fura-2-loaded GH_3 cells using a real time videoimaging apparatus after type I PDE inhibition with the vinka alkaloid vinpocetine [18], the infusion of the cell-permeable cGMP analog dB-cGMP and of the selective PKG inhibitor KT5823. Furthermore, the changes in cGMP intracellular content induced by this drug have also been evaluated.

2. Materials and methods

2.1. Cell culture

GH_3 cells were obtained from Flow Laboratories (Irvine, UK) and grown on plastic dishes in Ham's F10 medium (Gibco-BRL, San Giuliano Milanese, Italy) with 15% horse serum (Flow, Irvine, UK), 2.5% fetal calf serum (Hyclone, Logan, UT, USA), 100 IU penicillin/ml and 100 μg streptomycin/ml. Cells were cultured in a humidified 5% CO_2 atmosphere. Culture medium was changed every 2 days. For microfluorimetric studies, cells were seeded on glass coverslips (Fisher, Springfield, NJ, USA) coated with poly-L-lysine (30 μg/ml) (Sigma, St. Louis, MO, USA). All the experiments were performed 2–4 days after seeding. The cells were at a culture passage between 34 and 60.

2.2. Intracellular calcium measurements

$[Ca^{2+}]_i$ was measured using a microfluorimetric technique, as previously reported [20]. Briefly: the cells grown on glass coverslips were loaded with 5 μM fura-2 AM for 1 h at room temperature in Krebs–Ringer saline solution (5.5 mM KCl, 160 mM NaCl, 1.2 mM $MgCl_2$, 1.5 mM $CaCl_2$, 10 mM glucose, and 10 mM HEPES-NaOH, pH 7.4). At the end of fura-2 AM loading, the coverslip was mounted in a microscope chamber (Medical System, Greenvale, NY) on an inverted Nikon Diaphot fluorescence microscope. Throughout the experiment, the cells were kept in Krebs–Ringer saline solution. All the drugs tested were introduced into the microscope chamber by fast injection. A 100-W Xenon Lamp (Osram, Germany) with a computer-operated filter wheel bearing two different interference filters (340 and 380 nm) illuminated the microscopic field with UV light alternatively at the wavelength of 340 and 380 nm, with an interval of 500 ms between lighting at 340 and 380 nm. The interval between each pair of lighting was chosen according to the experimental protocol. Emitted light was passed through a 400-nm dichroic mirror, filtered at 510 nm, and collected by a CCD camera (Photonic Science, Robertsbridge, East Sussex, UK) connected to a light amplifier (Applied Imaging, Gateshead, UK). Images were digitized and analyzed with a Magiscan image processor (Applied Imaging, Gateshead, UK). The Tardis software (Applied Imaging, Gateshead, UK) calculated using a calibration curve the $[Ca^{2+}]_i$ corresponding to each pair of images, from the ratio between the intensity of the light emitted when the cells were lit at 340 and 380 nm.

2.3. Determination of intracellular cAMP and cGMP content

The intracellular content of cGMP was determined using a radioimmunoassay kit (Amersham, Milan, Italy). GH_3 cells grown on 100-mm plastic dishes, were incubated with or without the appropriate drug in Krebs–Ringer saline solution for 2 min. At

the end of the incubation, the medium was removed and cells were scraped in ice-cold 60% ethanol. The cells were then transferred into a polypropylene tube and allowed to settle for 15 min in ice. At the end of this period, the tubes were spun for 15 min at 14 000 rpm in a microfuge. The resulting pellet was used for protein determination while the supernatant was transferred into a fresh tube and concentrated under a vacuum using a speed-vac concentrator (Savant, Springfield). The dried extracts were resuspended in a buffer consisting of 0.05 M sodium acetate, pH 5.8 with 0.01% sodium azide and then acetylated using a freshly prepared triethylamine/acetic anydride mixture (2:1). One hundred microliters of the acetylation product was assayed in duplicate using a double antibody RIA protocol. The standard curve ranged from 2 to 128 pmol/ml. Results were normalized for protein content. Protein determination was carried out using the method of Bradford [21].

cAMP concentrations were determined using a commercial [^{3}H]cAMP assay system (Amersham, Milan, Italy). The cells, grown in 100-mm plastic dishes, were incubated with vinpocetine or vehicle in Krebs–Ringer solution for 2 min. The reaction was stopped placing the dishes in ice; then the medium was removed and substituted with ice-cold acid ethanol (1 ml 1 N HCl/100 ml ethanol). Intracellular cAMP was extracted overnight under shaking at 4°C. Then ethanol was neutralized with Tris-HCl, transferred into a fresh tube, and concentrated under vac-

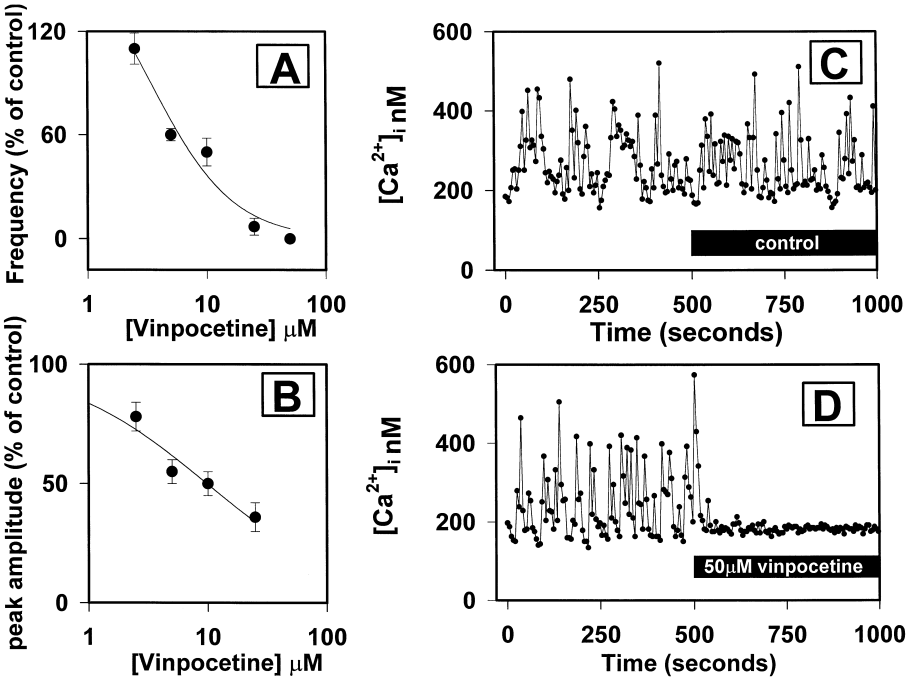

Fig. 1. Concentration-dependency of vinpocetine-induced inhibition of $[Ca^{2+}]_i$ oscillations in GH_3 cells. (A) Concentration–effect curve of vinpocetine-induced inhibition of $[Ca^{2+}]_i$ transient frequency. $[Ca^{2+}]_i$ oscillation inhibition was quantified as the ratio between pulse frequency (peaks/min) in the presence of increasing vinpocetine concentrations and in its absence. The plot was fitted to the equation $y = \max/[1+(x/k)^n]$, where max was the maximal ratio value, k was 3.24 and Hill coefficient (n_H) was 1.24. (B) Concentration–effect dependency of the vinpocetine-induced suppression of $[Ca^{2+}]_i$ oscillation amplitude. $[Ca^{2+}]_i$ transient suppression was quantified as the ratio between pulse amplitude ($[Ca^{2+}]_i$ nM) in the presence of increasing concentrations of vinpocetine and in its absence. The plot was fitted to the equation $y = \max/[1+(x/k)^n]$, where max was the maximal ratio value, k was 9.66 and Hill coefficient (n_H) was 0.75. The point representing the effect of 50 μM vinpocetine was omitted in this panel, since no peak was detected at this concentration. (C) Effect of vinpocetine vehicle (0.2% of a 1:1 solution of acetone and DMSO) on $[Ca^{2+}]_i$ oscillations. The vehicle was added to the chamber 500 s after the beginning of the experiment and left another 500 s before the wash. (D) Effect of 50 μM vinpocetine on $[Ca^{2+}]_i$ oscillations in GH_3 cells. Vinpocetine was added after 500 s of baseline $[Ca^{2+}]_i$ monitoring and left in the chamber for 500 s. Each point of A and B represents the mean ± S.E. of at least 30 determinations performed in two different experimental sessions. To calculate both the frequency and the amplitude of $[Ca^{2+}]_i$ oscillations, the entire traces were considered and the statistical analysis was performed on the raw data. The traces shown in C and D are single cell recordings and are representative of the pattern of 50 cells recorded in at least three different experimental sessions.

uum using a speed-vac concentrator. The dried extracts were resuspended in Tris EDTA buffer. cAMP was determined from the extent of [^{3}H]cAMP binding to a cAMP-binding protein in the presence of the sample. Briefly, samples and standard tubes were incubated with cAMP-binding protein in the presence of [^{3}H]cAMP for 2 h. To separate free and bound cAMP, the samples were incubated with a charcoal suspension and centrifuged at 3000 rpm. A 200-μl aliquot of the supernatant was counted in a scintillation counter after the addition of a liquid scintillation solution. The calibration curve of the assay ranged from 1.0 to 16.0 pmol/tube. All the data were normalized for protein content determined with the Bradford method [21].

2.4. Mathematical analysis of spontaneous $[Ca^{2+}]_i$ oscillations

$[Ca^{2+}]_i$ oscillations, defined as an increase of $[Ca^{2+}]_i$ above the baseline followed by a rapid return to baseline values, were analyzed using the DETECT software, which is widely used for the detection of hormonal pulses [22]. This program, through the analysis of the first derivatives of the data and the fitting of straight line segments through local regions of the data, allows for the identification of those $[Ca^{2+}]_i$ variations which assume the significance of $[Ca^{2+}]_i$ oscillations, and excludes those $[Ca^{2+}]_i$ variations due to technical noise. The frequency was calculated as the number of peaks occurring per minute, while the amplitude was determined as the difference between the maximal $[Ca^{2+}]_i$ value of the peak and the minimal $[Ca^{2+}]_i$ value just before the occurrence of the peak.

2.5. Statistical analysis of the data

All the numerical data are reported as mean ± S.E.M. The statistical significance of the data was calculated using the Student's *t*-test for paired data. The threshold for significance was set at $P < 0.05$.

2.6. Drugs and chemicals

All the chemicals were of analytical grade and were purchased from Sigma (St. Louis, MO, USA). Fura-2 AM, dB-cGMP, vinpocetine, KT5823 and KT5720 were obtained from Calbiochem (La Jolla, CA, USA).

3. Results

3.1. Effect of different concentrations of the type I phosphodiesterase inhibitor vinpocetine on $[Ca^{2+}]_i$ oscillations and on intracellular content of cGMP and cAMP

Vinpocetine, when added to the medium for 8.5 min in concentrations ranging from 2.5 to 50 μM, inhibited in a concentration-dependent manner, both the frequency and the peak amplitude of $[Ca^{2+}]_i$ oscillations in GH_3 cells (Fig. 1A,B). The IC_{50} of vinpocetine-induced inhibition of both the frequency and of the peak amplitude of $[Ca^{2+}]_i$ oscillations was approximately 10 μM. The maximal inhibiting effect was achieved at 50 μM. The time course of vinpocetine (50 μM) and its vehicle effect on $[Ca^{2+}]_i$ is depicted in Fig. 1C,D.

The same concentration of vinpocetine (50 μM), which exerted the maximal inhibitory effect on $[Ca^{2+}]_i$ oscillations in GH_3 cells, caused a doubling of basal cGMP content in GH_3 cells; whereas it did not modify cAMP levels (Fig. 2).

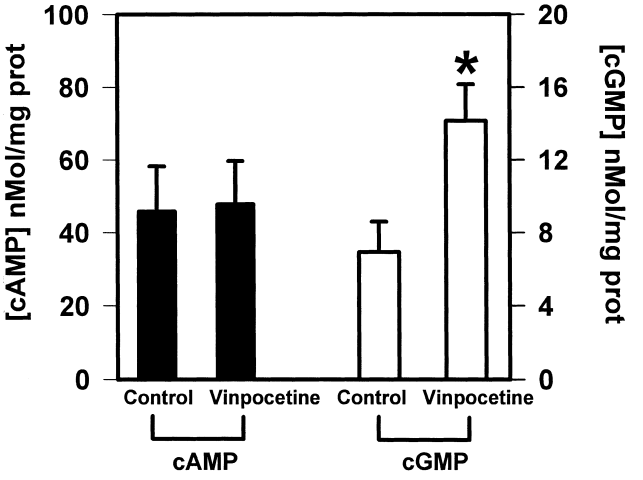

Fig. 2. Effect of 50 μM vinpocetine on intracellular content of cAMP and cGMP in GH_3 cells. cAMP and cGMP were extracted and quantified as described in Section 2. The results represent the means with S.E. (vertical bars) of three separate determinations. Asterisks indicate a significant difference from control values ($P < 0.05$).

3.2. Effect of the PKG activator, dB-cGMP and of the PKG inhibitor KT5823 on $[Ca^{2+}]_i$ oscillations in GH_3 cells

As shown in Fig. 3A, when dB-cGMP was added to the medium at a concentration of 1 mM, which is widely used to activate PKG [23,24], a progressive reduction in both the frequency and the amplitude of spontaneous $[Ca^{2+}]_i$ oscillations was observed. The complete suppression of spontaneous $[Ca^{2+}]_i$ oscillations occurred approximately after 7 min of incubation with this PKG activator.

By contrast, KT5823, a selective inhibitor of PKG, at a concentration of 400 nM, a value two times higher than the IC_{50} [19], induced a progressive increase in baseline $[Ca^{2+}]_i$ and a reduction of the amplitude of $[Ca^{2+}]_i$ oscillations.

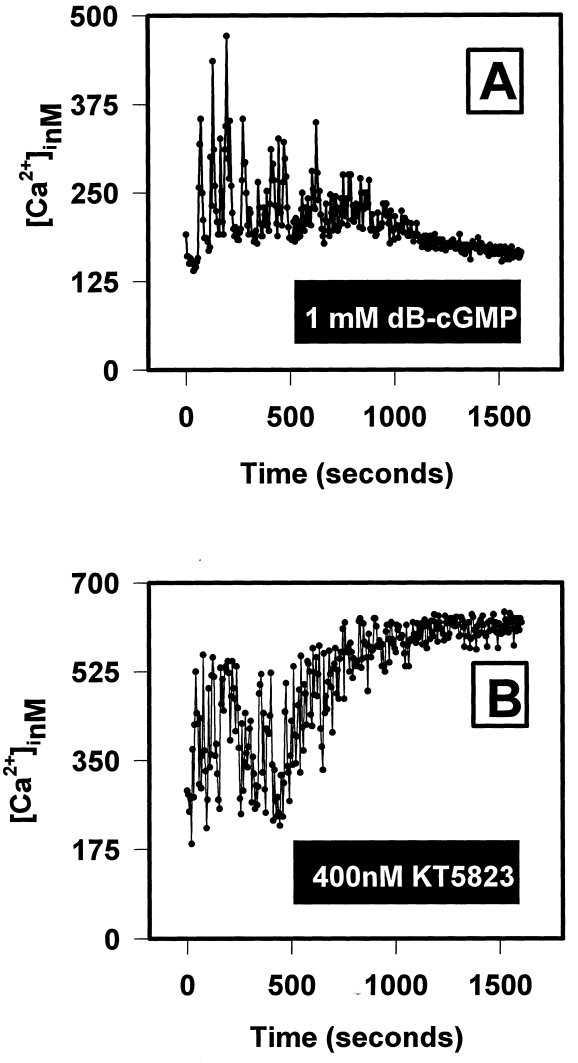

Fig. 3. Effect of the cell permeable cGMP-analog dB-cGMP and of the PKG inhibitor KT5823 on the occurrence of spontaneous $[Ca^{2+}]_i$ oscillations in GH_3 cells. (A) Effect of 1 mM dB-cGMP on spontaneous $[Ca^{2+}]_i$ oscillations in GH_3 cells. dB-cGMP (1 mM) was added to the chamber after 500 s of monitoring of $[Ca^{2+}]_i$ oscillation occurrence in basal conditions, and left in the chamber for 1100 s. (B) Effect of 400 nM KT5823 on spontaneous $[Ca^{2+}]_i$ oscillations in GH_3 cells. KT5823 (400 nM) was added after 500 s of monitoring of $[Ca^{2+}]_i$ transient occurrence in basal conditions and left in the chamber for another 1000 s. All traces shown in the figure are single cell recordings and are representative of the pattern of 30 cells recorded in three different experimental sessions.

3.3. Effect of the selective PKG inhibitor KT5823 and of the selective PKA inhibitor KT5720 on vinpocetine-induced suppression of spontaneous $[Ca^{2+}]_i$ oscillations

When KT5823 (400 nM) was preincubated 6 min before vinpocetine, it prevented the $[Ca^{2+}]_i$ lowering effect of the type I PDE inhibitor. In fact, in the presence of KT5823 $[Ca^{2+}]_i$ increased and $[Ca^{2+}]_i$ oscillations disappeared (Fig. 4A).

By contrast, the specific PKA inhibitor KT5720 at a concentration of 200 nM, four times higher than its IC_{50} [19], was unable to prevent the vinpocetine-induced suppression of $[Ca^{2+}]_i$ oscillations and did not modify by itself the amplitude and the frequency of $[Ca^{2+}]_i$ oscillations (Fig. 4B)

3.4. Effect of extracellular Ca^{2+} removal or nimodipine blockade of L-type VSCC on intracellular cGMP and cAMP levels

Extracellular Ca^{2+} removal or the incubation with 10 μM nimodipine induced a statistically significant decrease (70–80%) of intracellular cGMP levels (Fig. 5A). By contrast, both Ca^{2+} removal and L-type VSCC blockade did not produce any change in GH_3 cell cAMP contents (Fig. 5B).

4. Discussion

The results obtained in the present study showed that the vinca alkaloid derivative vinpocetine, an inhibitor of the Ca^{2+}/calmodulin-dependent type I PDE [18], abolished the occurrence of $[Ca^{2+}]_i$ oscil-

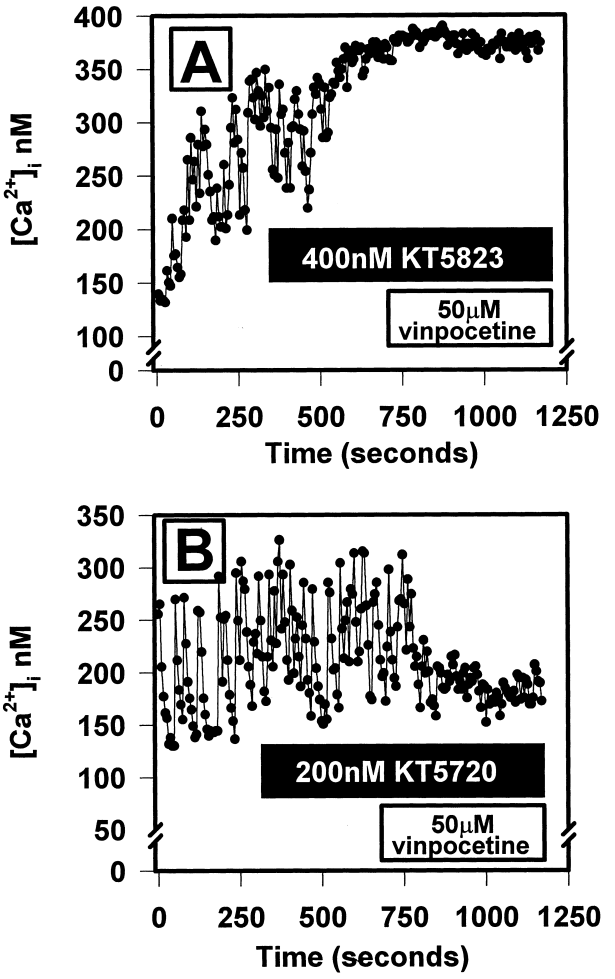

Fig. 4. Effect of the selective PKG inhibitor KT5823 and of the selective PKA inhibitor KT5720 on vinpocetine-induced suppression of spontaneous $[Ca^{2+}]_i$ oscillations in GH_3 cells. (A) Effect of KT5823 preincubation on vinpocetine-induced $[Ca^{2+}]_i$ transient suppression. KT5823 (400 nM) was added to the medium 360 s before vinpocetine. (B) Effect of KT5720. KT5720 was added to the medium 350 s before vinpocetine. The traces are representative of 30 cells recorded in at least three separate experimental sessions.

lations lowering $[Ca^{2+}]_i$ to baseline values in GH_3 cells. Since the inhibition of type I PDE produced a selective elevation of intracellular cGMP content, the effect of vinpocetine might be attributed to the increase of this cyclic nucleotide. This hypothesis was supported by the evidence that the cell-permeable cGMP-analog dB-cGMP was also effective in lowering $[Ca^{2+}]_i$ to baseline values and in suppressing $[Ca^{2+}]_i$ oscillations. Therefore, the data here reported suggest that type I PDE could be involved in the genesis of $[Ca^{2+}]_i$ oscillations through a cGMP-dependent pathway. Since both cGMP and dB-cGMP are potent activators of the PKG [23], the effect of vinpocetine on $[Ca^{2+}]_i$ oscillation occurrence could be attributed to PKG activation. In fact, when PKG was inhibited by its specific blocker KT5823, $[Ca^{2+}]_i$ oscillations disappeared even though it induced a constant elevation of baseline $[Ca^{2+}]_i$. This ability is compatible with the hypothesis suggesting the existence of an inhibitory and fluctuating function exerted by PKG on VSCC. Thus when PKG

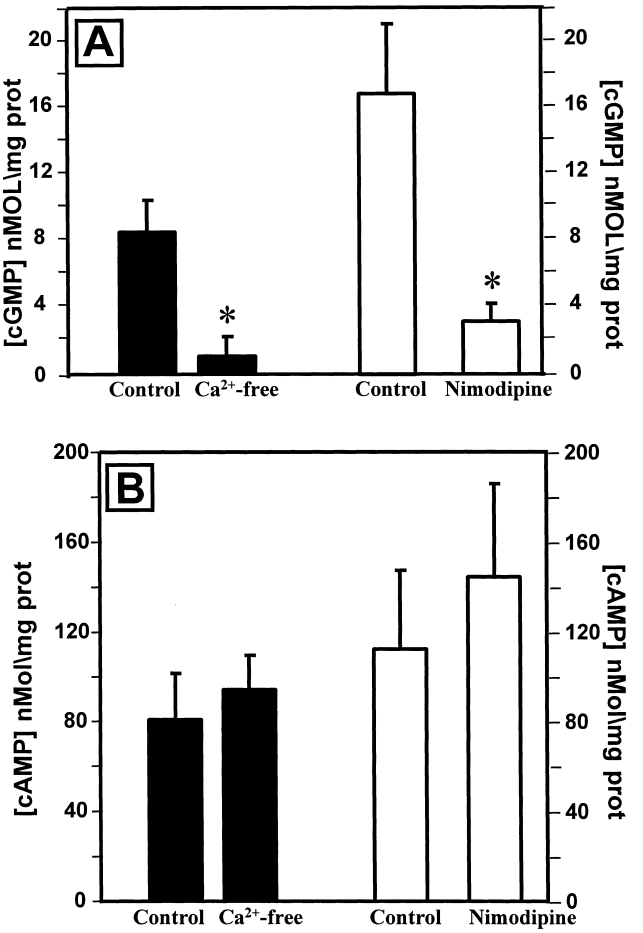

Fig. 5. Effect of extracellular Ca^{2+} removal or nimodipine blockade of L-type VSCC on intracellular cGMP and cAMP levels. (A) Effect of extracellular Ca^{2+} removal or nimodipine blockade on cGMP levels. Intracellular cGMP concentrations were detected after 1 h of Ca^{2+}-free incubation and after 5 min of nimodipine addition. (B) Effect of extracellular Ca^{2+} removal or nimodipine-induced VSCC blockade on cAMP levels. The times of incubation were the same as reported for cGMP determinations. The results represent the means with S.E. (vertical bars) of six determinations. Asterisks indicate a significant difference from respective control values ($P < 0.05$).

activity is constantly blocked by KT5823 the fluctuating and inhibitory role played by the kinase cannot be exerted, and GH_3 cells do not display oscillations, but set up baseline $[Ca^{2+}]_i$ at a higher level. In light of this model by which PKG controls $[Ca^{2+}]_i$ oscillations through the modulation of VSCC activity, the persistence of high baseline $[Ca^{2+}]_i$ when both the PKG inhibitor and vinpocetine were present can be explained by the predominant effect of the PKG inhibitor KT5823 that overcame the increased levels of cGMP induced by vinpocetine. The specificity of vinpocetine in activating the cGMP-PKG pathway in GH_3 cells was further supported by the failure of the PKA inhibitor KT5720 to modify spontaneous $[Ca^{2+}]_i$ oscillations and to prevent the $[Ca^{2+}]_i$ lowering effect of vinpocetine. Interestingly, the physiological relevance of type I PDE-cGMP-PKG pathway in the occurrence of $[Ca^{2+}]_i$ oscillation in GH_3 cells was further demonstrated by the evidence that the blockade of extracellular Ca^{2+} entrance decreased intracellular steady-state levels of cGMP. This role seems to be selective for the type I PDE-cGMP-PKG pathway since cAMP levels were not affected by the blockade of Ca^{2+} entrance into the cells.

In order to explain the present results, several mechanisms can be taken into consideration. PKG could indeed interfere with the components of both the plasmamembrane oscillator, like L-type VSCC and Ca^{2+}-ATPases, as well as the endoplasmic reticulum oscillator, like sarcoplasmic Ca^{2+}-ATPases and ionic channels. In particular, it has been demonstrated that in mammalian cardiac myocytes, L-type Ca^{2+} currents are markedly inhibited by PKG activation obtained either through exogenous administration of cGMP or through cGMP elevation induced by the unspecific blocker of PDE isomethylbutylxanthine (IBMX) [25,26]. In addition, evidence has been provided showing that PKG can reduce $[Ca^{2+}]_i$ by activating Ca^{2+} extrusion mechanisms, including the plasmamembrane-associated calmodulin-stimulated Ca^{2+}-ATPase [27–29] and the Na^+/Ca^{2+} exchanger [30]. Furthermore, the possibility exists that PKG activation can promote Ca^{2+} removal from the cytosol into the intracellular stores by stimulating sarcoplasmic Ca^{2+}-ATPase [31,32], and/or reduce Ca^{2+} ions mobilization from the IP_3-sensitive Ca^{2+} stores by inhibiting IP_3 production [33].

The results obtained from the present study suggest that the occurrence of $[Ca^{2+}]_i$ oscillations in GH_3 cells may be regulated by the activity of type I PDE-cGMP-PKG pathway which seems to be controlled by $[Ca^{2+}]_i$.

In fact, when the Ca^{2+} entrance is blocked, this pathway is inhibited, whereas, when the bivalent cation entrance is not prevented, this biochemical pathway might exert its inhibitory action on L-type VSCC.

Acknowledgements

This work was supported by the CNR Grants 95.04501.ST74, 95.02857.CT04 and 96.02074.P504 to L.A. by the CNR Grant 96.02060.CT04 to S.A. and by MURST 60 and 40% grants to L.A. We acknowledge Prof. Angela Acquaviva and Dr. Annamaria Catanzano (Department of Biology and of Cellular and Molecular Pathology, at the Federico II University of Naples) for their help in performing the cAMP determinations. Special thanks to Dr. Sandra Zavaleta and Mrs. Marcella Donato for their editorial help in the elaboration of the manuscript.

References

[1] S.S. Stojilkovic, K.J. Catt, Calcium oscillations in anterior pituitary cells, Endocr. Rev. 13 (1992) 256–280.
[2] S.S. Stojilkovic (Ed.), Calcium oscillations in pituitary cells (special issue), Trends Endocrinol. Metab. 7 (1996) 357–360.
[3] Y. Kidokoro, Spontaneous calcium action potentials in a clonal pituitary cell line and their relationship to prolactin secretion, Nature 258 (1975) 741–742.
[4] R.W. Holl, M.O. Thorner, G.L. Mandell, J.A. Sullivan, Y.N. Sinha, D.A. Leong, Spontaneous oscillations of intracellular calcium and growth hormone secretion, J. Biol. Chem. 263 (1988) 9682–9685.
[5] D.J. Haisenleder, G.A. Ortolano, A.C. Dalkin, M. Yasin, J.C. Marshall, Differential actions of thyrotropin (TSH)-releasing hormone pulses in the expression of prolactin and TSH subunit messenger ribonucleic acid in rat pituitary cells in vitro, Endocrinology 130 (1992) 2917–2923.
[6] D.J. Haisenleder, A. Yasin, M. Yasin, J.C. Marshall, Regulation of prolactin, thyrotropin subunit and gonadotropin subunit gene expression by pulsatile or continuous calcium signals, Endocrinology 133 (1993) 2055–2061.
[7] S.S. Stojilkovic (Ed.), Calcium oscillations in pituitary cells (special issue), Trends Endocrinol. Metab. 7 (1996) 357–360.

[8] W. Schlegel, B.P. Winiger, P. Mollard, P. Vacher, F. Wuarin, G.R. Zahnd, C.B. Wollheim, B. Dufuy, Oscillation of cytosolic Ca^{2+} in pituitary cells due to action potentials, Nature 329 (1987) 719–721.
[9] K.A. Wagner, P.W. Yacono, D.E. Golan, A.H. Tashjian, Mechanism of spontaneous intracellular calcium fluctuations in single GH_4C_1 rat pituitary cells, Biochem. J. 292 (1993) 175–182.
[10] A.C. Dolphin, Facilitation of Ca^{2+} current in excitable cells, Trends Neurosci. 19 (1996) 35–43.
[11] T.F. Mcdonald, S. Pelzer, W. Trautwein, D.J. Pelzer, Regulation of calcium channels in cardiac skeletal and smooth muscle cells, Physiol. Rev. 74 (1994) 366–507.
[12] P. Komalavilas, T. Lincoln, Phosphorylation of the inositol 1,4,5-triphosphate receptor. Cyclic GMP-dependent protein kinase mediates cAMP and cGMP dependent phosphorylation in the intact rat aorta, J. Biol. Chem. 271 (1996) 21933–21938.
[13] F. Hofmann, W. Dostmann, A. Keilbach, W. Landgraf, P. Ruth, Structure and physiological role of cGMP-dependent protein-kinase, Biochem. Biophys. Acta 1135 (1992) 51–60.
[14] X. Wang, P.J. Robinson, Cyclic GMP-dependent protein kinase and cellular signaling in the nervous system, J. Neurochem. 68 (1997) 443–456.
[15] D.M.F. Cooper, N. Mons, J.W. Karpen, Adenylyl cyclases and the interaction between calcium and cAMP signalling, Nature 374 (1995) 421–424.
[16] J.A. Beavo, Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms, Pharmacol. Rev. 75 (1995) 725–748.
[17] H. Charbonneau, S. Kumar, J.P. Novack, D.K. Blume, K.A. Walsh, Evidence for domain organization within the 61 kDa calmodulin-dependent cyclic nucleotide phosphodiesterase from bovine brain, Biochemistry 30 (1991) 7931–7940.
[18] M. Hagiwara, T. Endo, H. Hidaka, Effects of vinpocetine on cyclic nucleotide metabolism in vascular smooth muscle, Biochem. Pharmacol. 33 (1984) 453–457.
[19] H. Kase, K. Iwahashi, S. Nakanishi, Y. Matsuda, K. Yamada, M. Takahashi, C. Murakata, A. Sato, M. Kaneko, K-252 compounds novel and potent inhibitors of protein kinase C and cyclic nucleotide-dependent protein kinases, Biochem. Biophys. Res. Commun. 142 (1987) 436–440.
[20] M. Cataldi, M. Taglialatela, S. Guerriero, S. Amoroso, G. Lombardi, G.F. Di Renzo, L. Annunziato, Protein tyrosine kinases activate while protein tyrosine phosphatases inhibit L-type calcium channel activity in pituitary GH_3 cells, J. Biol. Chem. 271 (1996) 9441–9446.
[21] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.
[22] K.E. Oerter, V. Guardabasso, D. Rodbard, Detection and characterization of peaks and estimation of instantaneous secretory rate for episodic pulsatile hormone secretion, Comput. Biomed. Res. 19 (1986) 171–191.
[23] J.D. Corbin, D. Ogreid, J.P. Miller, R.H. Suva, B. Jastorff, S.O. Doskeland, Studies of cGMP analog specificity and function of the two intrasubunit binding sites of cGMP-dependent protein kinase, J. Biol. Chem. 261 (1986) 1208–1214.
[24] D.C. Sane, A. Bielawska, C.S. Greenberg, Y.A. Hannun, Cyclic GMP analogs inhibit gamma thrombin-induced arachidonic acid-release in human platelets, Biochem. Biophys. Res. Commun. 165 (1989) 708–714.
[25] P.F. Méry, S.M. Lohman, U. Walter, R. Fischmeister, Ca^{2+} current is regulated by cGMP-dependent protein kinase in mammalian cardiac myocytes, Proc. Natl. Acad. Sci. USA 88 (1991) 1197–1201.
[26] K. Sumii, N. Sperelakis, c-GMP dependent kinase regulation of the L-type Ca^{2+} current in ventricular myocytes, Circ. Res. 77 (1995) 803–812.
[27] K.I. Furukawa, H. Nakamura, Cyclic GMP regulation of the plasmamembrane (Ca^{2+}/Mg^{2+}) ATPase in vascular smooth muscle cells, J. Biochem. 101 (1987) 287–290.
[28] Y. Yoshida, H.T. Sun, J.Q. Cai, S. Imai, Cyclic GMP-dependent protein kinase stimulates the plasma membrane Ca^{2+} pump ATPase of vascular smooth muscle via phosphorylation of a 240-kDa protein, J. Biol. Chem. 266 (1991) 19819–19825.
[29] S.S. Rashatwar, T.l. Cornwell, T.M. Lincoln, Effects of 8-bromo-cGMP on Ca^{2+} levels in vascular smooth muscle cells: possible regulation of Ca^{2+}-ATPase by cGMP-dependent protein kinase, Proc. Natl. Acad. Sci. USA 84 (1987) 5685–5689.
[30] K.I. Furukawa, N. Ohshima, Y. Tawada-Iwata, M. Shigekawa, Cyclic GMP stimulates Na^{+}/Ca^{2+} exchange in vascular smooth muscle cells in primary culture, J. Biol. Chem. 266 (1991) 12337–12341.
[31] T. Cornwell, K. Pryzwansky, T.A. Wyatt, T.M. Lincoln, Regulation of sarcoplasmic reticulum protein phosphorylation by localized cyclic GMP-dependent protein kinase in vascular smooth muscle cells, Mol. Pharmacol. 40 (1991) 923–931.
[32] B. Sarcevic, V. Brooks, T.J. Martin, B.E. Kemp, P.J. Robinson, Atrial natriuretic peptide-dependent phosphorylation of smooth muscle cell particulate fraction proteins is mediated by cGMP-dependent protein kinase, J. Biol. Chem. 264 (1989) 20648–20654.
[33] P. Ruth, I. Boekhoff, B. May, R. Pfeifer, M. Korth, H. Breer, F. Hofmann, Transfected cGMP-dependent protein kinase suppresses calcium transients by inhibition of inositol 1,4,5 triphosphate production, Proc. Natl. Acad. Sci. USA 90 (1993) 26263–26267.