BJPS

Brazilian Journal of Pharmaceutical Sciences
vol. 51, n. 3, jul./sep., 2015
http://dx.doi.org/10.1590/S1984-82502015000300020

Article

Pituitary Adenylate Cyclase Activating Peptide (1-38) and its analog (Acetyl-[Ala^{15}, Ala^{20}] PACAP 38-polyamide) reverse methacholine airway hyperresponsiveness in rats

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The aim of this study was to investigate both functionally and structurally bronchodilator effects of Pituitary adenylate cyclase activating peptide (PACAP38) and acetyl-[Ala^{15}, Ala^{20}] PACAP38-polyamide, a potent PACAP38 analog, in rats challenged by methacholine (MeCh). Male Wistar rats were divided randomly into five groups. Groups 1 and 2 inhaled respectively aerosols of saline or increasing doses of MeCh (0.5, 1, 2.12, 4.25, 8.5, 17, 34 and 68mg/L). The other groups received terbutaline (Terb) (250 µg/rat) (10^{-6} M), PACAP38 (50 µg/rat) (0.1 mM) or PACAP38 analog (50 µg/rat) associated to MeCh from the dose of 4.25 mg/L. Total lung resistances (R_L) were recorded before and 2 min after MeCh administration by pneumomultitest equipment. MeCh administration induced a significant and a dose-dependent increase ($p<0.05$) of R_L compared to control rats. Terb, PACAP38 and PACAP38 analog reversed significantly the MeCh-induced bronchial constriction, smooth muscle (SM) layer thickness and bronchial lumen mucus abundance. PACAP38 analog prevents effectively bronchial smooth muscle layer thickness, mucus hypersecretion and lumen decrease. Therefore, it may constitute a potent therapeutic bronchodilator.

Uniterms: Pituitary adenylate cyclase activating peptide/effects/inflammatory lung diseases. Pituitary adenylate cyclase/analogs/effects in inflammatory lung diseases. Methacholine. Bronchodilators/effects. Inflammatory lung diseases/treatment.

O objetivo deste estudo foi investigar funcionalmente e estruturalmente efeito broncodilatador do peptídeo ativador da adenilato ciclase pituitária (PACAP1-38) e da acetil-[Ala^{15}, Ala^{20}]PACAP 38-poliamida, potente análogo do PACAP-38, nos ratos desafiados pelo metacolina (MeCh). Ratos Wistar machos foram aleatoriamente divididos em cinco grupos. Grupos 1 e 2, inalando aerossóis de solução salina ou doses crescentes de MeCh (0,5, 1, 2,12, 4,25, 8,5, 17, 34 e 68 mg/L). Os outros grupos recebendo terbutalina (Terb) (250 µg/rato) (10^{-6}M), PACAP-38 (50 µg/rato) (0.1 mM) ou análogo do PACAP-38 (50 µg/rato) associados a MeCh na dose de 4,25 mg/L. A resistência pulmonar total (R_L) foi registrada antes e 2 min após a administração de Mech pelo equipamento pneumomultiteste. A administração MeCh induziu aumento significativo e dose dependente ($p<0,05$) de R_L em comparação com ratos do grupo controle. Terb e PACAP1-38 e análogo do PACAP-38 reverteram, significativamente, a constrição brônquica induzida por Mech, a espessura do músculo liso (SM) e abundância de muco do lume brônquico. O análogo PACAP-38 do mesmo modo que a Terb impediu a responsividade brônquica a MeCh e pode se constituir em um importante regulador no desenvolvimento da doença inflamatório pulmonar. Contudo, o uso do peptídeo nativo para aplicações terapêuticas é limitado por sua baixa estabilidade metabólica. Consequentemente, o análogo metabolicamente estável representa ferramenta promissora no tratamento de doenças pulmonares inflamatórias.

Unitermos: Adenilato cilase da pituitária/efeitos/doença pulmonar inflamatória. Adenilato cilase da pituitária/análogos/efeitos em doença pulmonar inflamatória. Metacolina. Resistência pulmonar. Broncodilatores/efeitos. Doenças pulmonares inflamatórias/tratamento.

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INTRODUCTION

Pituitary adenylate cyclase activating peptides (PACAPs) are vasoactive intestinal peptide (VIP)-like neuropeptides that have been purified from ovine hypothalami (Miyata *et al*., 1989). Two endogenous forms of PACAP have been identified. PACAP38 and PACAP27 (the N-terminal residue of PACAP38) are powerful stimulants of adenylate cyclase in anterior pituitary cells in culture, a feature from which derives the name (Miyata *et al*., 1989; Arimura, 1998). Different forms of PACAP and its analogs, known for their bronchodilator properties, have been tested for the treatment of obstructive airway diseases such as asthma which is characterized by bronchial hypereractivity and seems to have increasing rates of morbidity and mortality despite progress in therapeutic methods (Okazawa *et al*., 1998; Van Schoor, Joos, Pauwels, 2000).

PACAP38 makes up to 80-90 of the total PACAP in the body and has longer lasting bronchodilator activity on constricted airways than PACAP27. In the guinea-pigs, PACAP38 inhibits smooth muscle tone induced by acetylcholine and histamine and causes more sustained inhibition of bronchoconstriction than VIP without cardiovascular side effects (Lindén *et al*., 1995; 1999). PACAP38 is present in lung and it constitutes a potent endogenous bronchodilator through inhibition of smooth muscle contraction induced by cholinergic and excitatory non-adrenergic and non cholinergic nerves (Shigyo *et al*., 1998; Yoshida *et al*., 2000).

Different studies were conducted to assess the role of PACAPs against pulmonary inflammation (Delgado *et al*., 1999; Vaudry *et al*., 2009; Elekes *et al*., 2011; Reglodi et *al.,* 2012*)* and bronchial hyperresponsiveness and bronchoconstriction (Saguchi *et al.,* 1997; Kinhult *et al*., 2000; Kinhult, Uddman, Cardell, 2001) to various stimuli either *in vivo* or *in vitro*. However, PACAP38 has not been tested against methacholine-induced bronchoconstriction on rat lung *in vivo*. Methacholine (MeCh) is a synthetic muscarinic agonist more stable than acetylcholine and better-tolerated than histamine and carbachol (Van Schoor, Joos, Pauwels, 2000; Sterk *et al*., 2001; Nair, Hanrahan, Hargreave, 2009). On the other hand, as for most natural peptides, the clinical use of PACAP38 is limited by its low metabolic stability (Bourgault *et al*., 2008). The half-life of PACAP38 in the human blood ranges between 5 and 10 min because it is rapidly metabolized by dipeptidyl peptidase IV (DPP IV), a ubiquitous peptidase that liberates dipeptides from the *N*-terminus of regulatory peptides (Mentlein, 1999; Li *et al*., 2007). As a result, the agonistic activity of the peptide is suppressed by the DPP IV-induced cleavage since the amino terminal domain of PACAP is essential for the activation of PAC-1 receptor and N-terminally truncated forms of PACAP behave as antagonists for this receptor which is principally responsible for the biological activity of PACAP. The Acetyl-[Ala^{15}, Ala^{20}] PACAP38-polyamide, a potent PACAP38 analog, has improved plasmatic stability. Indeed, this bi-alanine-substituted analog was more stable than PACAP38 in isolated human plasma. Moreover, it exhibited a complete resistance to DPP IV degradation, a high affinity towards the PAC-1 receptor and a potent activity associated with this receptor (Bourgault *et al*., 2008; Dejda et *al*., 2011). Consequently, the development of modified PACAP analogs with a lower susceptibility to protease degradation represented a promising strategy to further investigate the physiological roles of PACAP. Such stabilized analogs have a potent activity for the PAC-1 receptor and would also facilitate the assessment of the usefulness of PACAP as a drug candidate for the treatment of different pulmonary diseases. To our knowledge, there are no *in vivo* studies on bronchodilator effects and muscarinic airway responsiveness of this novel PACAP38 analog.

The aim of the current study was to examine the bronchodilator effects of inhaled PACAP38 and PACAP38 analog against MeCh challenge compared to terbutaline (Terb), a selective β2-adrenoceptor agonist clinically used for the long-term treatment of obstructive airway diseases and for the treatment of acute bronchospasm effects (Johnson, 2001; Nair, Hanrahan, Hargreave, 2009).

MATERIAL AND METHODS

Animals and chemicals

Male Wistar rats (50 days of age) were purchased from the Tunisian Company of Pharmaceutical Industries (SIPHAT, Rades, Tunis, Tunisia). The animals were housed under controlled conditions of temperature (25 °C) and light (12:12 light:dark). All animals were provided with food and water *ad libitum*.

The 38-amino-acid form of PACAP and acetyl-[Ala^{15}, Ala^{20}] PACAP38-polyamide were synthesized using a solid phase strategy combined with the chemistry methodology (Bourgault *et al*., 2008). Methacholine and Ketamine were purchased from Sigma (St. Louis, MO, USA) and Terbutaline 5mg/2mL solution nebulizer was obtained from AstraZneneca laboratory. All the other reagents were of the highest purity and were purchased from the Sigma Chemical Co. Animals were cared for under the Tunisian Code of Practice for the Care and Use of Animals for Scientific Purposes. Experimental protocols were approved by the Faculty Ethics Committee.

Experimental design and lung resistance (R_L) measurement

Animals were anesthetized with ketamine (100 mg/kg). The necks were opened, the trachea exposed by a mid-line incision and a tracheal cannula was inserted. A second balloon-tipped catheter was inserted into the lower 1/3 of the oesophagus and connected to a pressure transducer to measure the intra-oesophageal pressure. A small pneumotachograph (PTG, 8431B, Hnans Rudoplh, Kansas, USA) was connected to the tracheal cannula at the time of measurement of the flow rate. Period was set at 10 seconds to avoid change in ventilation due to the PTG dead volume. The PTG was connected to two-differential pressure transducers. Both transducers were assembled together with connecting valves to ease the calibration and clearing of the esophageal catheter (Pneumomultitest ERMS, Toulouse, France). Total lung resistance (R_L) was determined using a first order mechanical model of the lung. Aerosols were generated through a DeVibliss nebulizer (Ref.123016 Marquette Medical products, Englewood Co., USA) connected to a compressor (flow rate 100 mL/s) with a flow rate of 0.1 mL/min in a rigid plastic chamber placed over the rat body (Zhao *et al*., 2002).

Rats were randomized into five experimental groups (n=8) as follows: (1) animals received aerosols of MeCh in increasing doses (0.5, 1, 2.12, 4.25, 8.5, 17, 34 and 68 mg/L) within the chamber for 1min with 3min intervals between each dose (Zhao *et al*., 2002), (2) animals were challenged by increasing doses of MeCh and received terbutaline (Terb) (250 µg/rat) in an aerosol form, (3) animals were challenged by increasing doses of MeCh and received aerosols of PACAP dissolved in NaCl 0.9% (50 µg/rat) (Lindén et *al.,* 1999), (4) animals were challenged by increasing doses of MeCh and exposed to aerosols of PACAP-38 analog dissolved in NaCl 0.9% (50 µg/rat), (5) Control rats received aerosols of isotonic saline at the same duration and speed of aersolization. For all-treated groups, exposure to Terb, PACAP or PACAP analog began from the dose of 4.25 mg/mL and the exposure period was 1 minute after each MeCh challenge. Total lung resistances (R_L) were measured before and 2 min after each treatment. At the end of the experiment and after the last R_L measurement, rats were immediately scarified by exsanguinations via cardiac puncture followed by a rapid decapitation.

Histopathology and morphometry

Immediately after sacrifice, lungs were harvested, washed with ice cold saline and fixed overnight at room temperature in paraformaldehyde 4% in 0.1 M phosphate buffer, pH 7.4. The samples were dehydrated with ethanol and toluene series and embedded in paraffin. Serial sections (5µm) were mounted on gelatin-coated glass slides, cut and stained with hematoxylin and eosin for histopathological analysis and using the trichrome Masson's technique for the morphometric study. The quantitative measurements were made with a computerized image analysis system using Image-Pro Plus version 4.5 software (Media Cybernetics Inc, Silver Spring, MD, USA). The protocol was systematized into three phases: capture, processing and quantification. The images were captured at a size of 551/400 pixels at x40, x100 and x200 magnifications. All the bronchi found to be smaller than or equal to the size of the histological field were captured. With the aim of studying only the bronchi cut perpendicularly to their longitudinal axis, all the bronchi whose greatest diameter was at least twice as large as the least diameter were discarded. Airway size defined by the reticular basement membrane perimeter (pbm), the total area of the smooth muscle layer (ASM_{area}) and the internal bronchial lumen diameter were measured by planimetry according to the method of James *et al.* (2012). The main outcome of interest was the ASM thickness which is the ASM_{area} normalized for airway size divided by the pbm (ASM_{area}/ pbm) and expressed in µm. Thereafter, the airways were divided into three categories according to their pbm, as described by Sapienza *et al.* (1991): pbm≤1mm (small), pbm>1mm and ≤ 2mm (medium) and pbm>2mm (large). The measurements were made using 10 randomly chosen medium-sized bronchi per animal (n=8).

Statistics

Data were analyzed using PRISM software (GraphPad Software, San Diego, CA, USA). The results were expressed as means±standard deviations of the mean (SD). Differences between means were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison as the post hoc test. All statistical tests were two-tailed and a *p* value of 0.05 or less was considered significant.

RESULTS

Lung resistance

Lung resistances (R_L) in rats before and after exposure to NaCl aerosols were identical (0.41 ± 0.04 *vs* 0.49 ± 0.05 KPa/L/s), while MeCh inhalation induced a dose-dependent increase of R_L indicating that this agent

is a potent bronchoconstrictor. A significant increase was observed by the dose of 4.25mg/L compared to control (0.853 ± 0.15 *vs* 0.41 ± 0.04 KPa/L/s, $p<0.05$). With the highest dose of MeCh, bronchial resistance reached 1.90 ± 0.20 KPa/L/s. Interestingly, co-exposure to PACAP38 analog (50µg/rat), PACAP38 (50µg/rat) as well as Terb (250 µg/rat) aerosols abolished totally the airway hyperresponsiveness for all doses of MeCh (Figure 1).

Lung histopathology

A histological study revealed that in control rats, lung sections showed large bronchial lumen with little mucus and reduced smooth muscle layer (Figure 2A). After MeCh-challenge, pulmonary sections showed obstructed bronchial lumen with abundant mucus and thick smooth muscle layer (Fig.2.B). Protective effects of PACAP38 and Terb were tested in MeCh-challenged rats. As shown in Figures 2.C, 2.D and 2.E, co-treatment with PACAP38, PACAP38 analog or Terb inhibited airway obstruction in MeCh-inhaled rats and prevented bronchoconstriction and mucus hypersecretion.

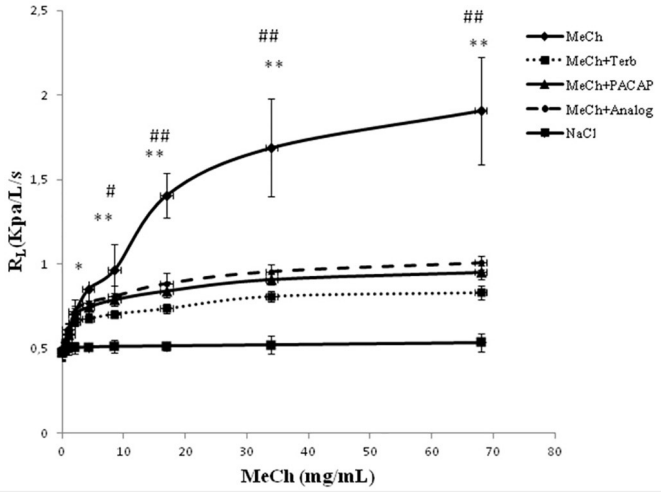

FIGURE 1 - Effect of terbutaline, PACAP38 or PACAP38 analog on lung resistances (R_L) of rats challenged by metacholine (Mech). Each value is the mean ± SD of 8 determinations. * $p \leq 0.05$, ** $p \leq 0.01$ compared to control; # $p \leq 0.05$, ## $p \leq 0.01$ compared to Terb, PACAP38 or PACAP38 analog challenged groups at the corresponding dose (Tukey's multiple comparison post hoc test).

Morphometric analyses

All the morphometric parameters were carried on medium-sized airways. The Table I showed a homogeneity within the range of bronchi sizes measured by their basement membrane parameters (pbm).

Bronchoconstriction was evaluated by measuring bronchial lumen diameter. In MeCh-challenged rats, bronchial lumen diameter decreased significantly compared to control group (64.67 ± 28.86 *vs* 515.70 ± 81.03 µm, $p<0.05$). MeCh effect was abolished by inhaled PACAP38, PACAP38 analog or Terb (Figure 3A). MeCh treatment resulted also in increased smooth muscle layer thickness by about 153 % (48.25 ± 11.26 *vs* 19.06 ± 4.20 µm) and co-administration of PACAP38, PACAP38 analog or Terb prevented this effect (Figure 3B).

DISCUSSION

The present study indicated that inhaled MeCh increased in a dose-dependent manner total lung resistance by the contraction of airway smooth muscle. Histopathological and morphometric observations confirmed this result showing an increase of the smooth muscle layer thickness and accumulation of mucus leading to the obstruction of the bronchial lumen. Previous studies indicated that MeCh effect on bronchial smooth muscle cells, endothelial cells and mucus-producing cells was mediated by muscarinic receptors with an increase in intracellular calcium (Van Schoor, Joos, Pauwels, 2000; Anderson, 2010; Svensson, Bjermer, Tufvesson, 2014). Interestingly, we showed for the first time that PACAP analog as well as PACAP38 and Terb potentially reversed airway resistance induced by MeCh. This result is supported by data demonstrating the presence of PACAP-containing nerve fibers in association with bronchial smooth muscle in primates and rodents (Uddman et *al.,* 1991; Busto *et al.,* 2000). Moreover, a moderate number of vascular smooth muscle and around seromucus glands, suggest that this peptide may play a role in the endogenous regulation of mucus secretion (Kinhult *et al*., 2000). Bronchial relaxant effect of PACAP is mediated through a specific receptor coupled to the adenyl cyclase pathway (Laburthe, Couvineau, 2002; Wilson, Cumming, 2008). Terbutaline a β_2-agonist was used as a reference to assess the bronchodilator action of PACAP. It stabilized the membrane of mast cells and relaxed bronchial smooth muscles thus relieving dyspnea of the patient quickly and improved the respiration (Xiong *et al.,* 2008).

In the present study, we have showed that PACAP38 reversed potentially MeCh-induced bronchoconstriction and mucus hypersecretion in rats. However, the poor metabolic stability of the native peptide limits its therapeutic use because it undergoes rapid enzymatic degradation after systemic introduction (Zhu *et al*., 2003). The Acetyl-[Ala^{15}, Ala^{20}] PACAP38-polyamide, a potent stable PACAP38 analog, which exhibits complete resistance to DPP IV degradation, increases

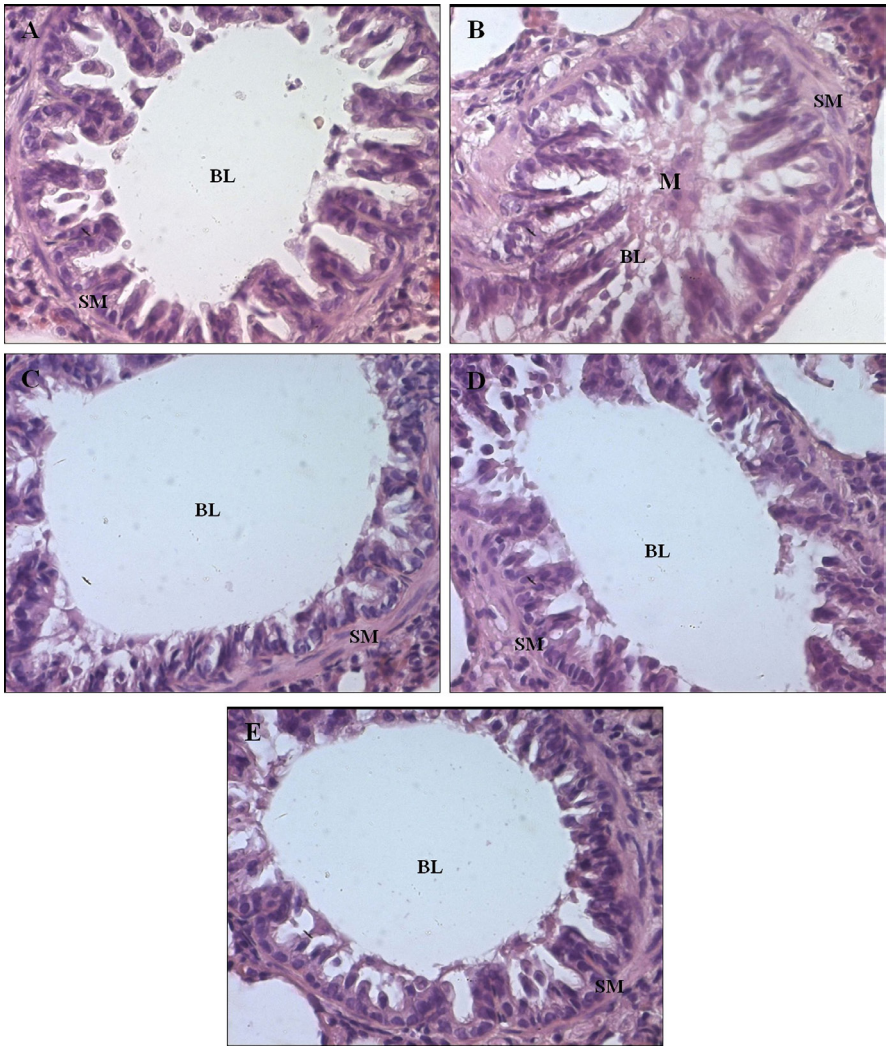

FIGURE 2 - Photomicrographs of hematoxylin-eosin-stained lung sections from rats showing the structure of a representative bronchus. (A) after inhalation of saline. (B) after Mech challenge: note the bronchoconstriction, the increase of the smooth muscle (SM) layer and the abundance of mucus (M) in the bronchial lumen (BL). (C), (D) and (E) after metacholine + terbutaline, metacholine + PACAP and metacholine + PACAP38-analog treatment, respectively: note the bronchodilator effect of Terb, PACAP38 and PACAP38 analog. Magnification: ×400.

TABLE I - Airway sizes of medium-sized bronchi examined for morphometry in the different experimental groups

Experimental groups	Pbm (mm)
NaCl	1.70±0.11
MeCh	1.75±0.13
MeCh+Terb	1.74±0.11
MeCh+PACAP38	1.74±0.08
MeCh+PACAP38 analog	1.76±0.11

Airway sizes were determined by measuring basement membrane diameters (pbm). Values (means ±standard deviations, n=10 bronchi per animal (n=8)) are not significantly different (Tukey's multiple comparison post hoc test).

metabolic activity and behaves as an agonist of the PAC1 receptor. Our results indicate, for the first time, that the PACAP-38 analog produces a significant inhibitory effect on muscarinic airway responsiveness *in vivo* after airway administration. Indeed, the Acetyl-[Ala^{15}, Ala^{20}] PACAP38-polyamide could have improved pharmacokinetic effects compared to the native peptide because of its high metabolic stability.

In conclusion, although further investigations are needed in rodents and humans to clarify the bronchodilator mechanism of PACAP38 analog and to assess its potential side effects, the use of such a novel DPP IV-resistant analog represented a promising tool for the *in vivo* exploration of the physiological roles of PACAP and for

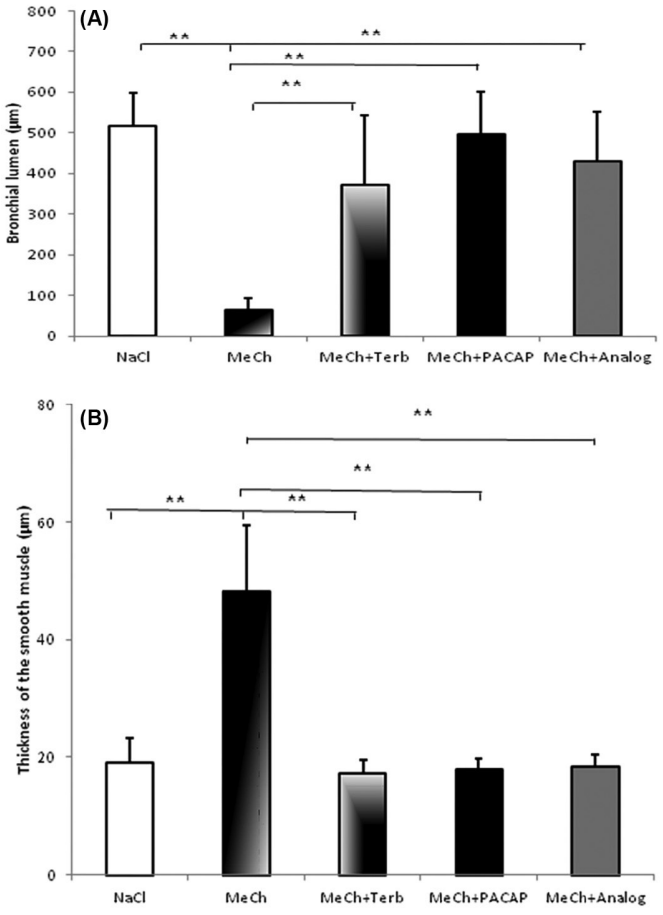

FIGURE 3 - Effect of terbutaline or PACAP on bronchial morphometry in lungs of rats challenged by metacholine (Mech). (A) Bronchial lumen diameter (B) bronchial smooth muscle (SM) layer thickness. Each value is the mean ± SD of 8 determinations. * $p \leq 0.05$, ** $p \leq 0.01$ between experimental groups.

future pharmacological interventions in the treatment of obstructive lung diseases.

ACKNOWLEDGMENTS

This work was supported by the Tunisian Ministry of Higher Education and Scientific Research and Technology and Carthage University. The authors gratefully acknowledge the technical assistance of Béchir Azib. The authors also thank Dr Abdelwahed Azib for his valuable review of the manuscript.

REFRENCES

ANDERSON, S.D. Indirect challenge tests: Airway hyperresponsiveness in asthma: its measurement and clinical significance. *Chest*, v.138, n.2, p.25-30, 2010.

ARIMURA, A. Perspectives on pituitary adenylate cyclase activating polypeptide (PACAP) in the neuroendocrine, endocrine, and nervous systems. *Jpn. J. Physiol*., v.48, n.5, p.301-331, 1998.

BOURGAULT, S.; VAUDRY, D.; BOTIA, B.; COUVINEAU, A.; LABURTHE, M.; VAUDRY, H. Novel stable PACAP analogs with potent activity towards the PAC1 receptor. *Peptides*, v.29, n.6, p.919-932, 2008.

BUSTO, R.; PRIETO, J.C.; BODEGA, G.; ZAPATERO, J.; CARRERO, I. Immunohistochemical localization and distribution of VIP/PACAP receptors in human lung. *Peptides*, v.21, n.2, p.265-269,2000.

DEJDA, A.; SEABORN, T.; BOURGAULT, S.; TOUZANI, O.; FOURNIER, A.; VAUDRY,H.; . VAUDRY, D. PACAP and a novel stable analog protect rat brain from ischemia:Insight into the mechanisms of action. *Peptides*, v.29.n.2, p.1207-1216, 2011.

DELGADO, M.; GANEA, D. Inhibition of endotoxin-induced macrophage chemokine production by VIP and PACAP *in vitro* and *in vivo*. *Arch. Physiol. Biochem*., v.109, n.4, p.377-382, 2001.

DELGADO, M.; POZO, D.; MARTINEZ, C.; LECETA, J.; CALVO, J.R.; GANEA, D. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit endotoxin-induced TNF-alpha production by macrophages: *in vitro* and *in vivo* studies. *J. Immunol*., v.162, n.4, p.2358-2367, 1999.

ELEKES, K.; SANDOR, K.; MORICZ, A.; KERESKAI, .; KEMENY, A.; SZOKE, E.; PERKECZ, A.; REGLODI, D.; HASHIMOTO, H.; PINTER, E.; SZOLCSANYI, J.; HELYES, Z. Pituitary adenylate cyclase-activating polypeptide plays an anti-inflammatory role in endotoxin-induced airway inflammation: In vivo study with gene-deleted mice. *Peptides*, v.32, n.7, p. 1439-1446, 2011.

JAMES, A.L.; ELLIOT, J.G.; JONES, R.L.; CARROLL, M.L.; MAUAD, M.; BAI, T.R.; ABRAMSON, M.J.; MCKAY, K.O., GREEN, F.H. Airway Smooth Muscle Hypertrophy and Hyperplasia in Asthma. *Am. J. Respir. Crit. Care Med*., v.185, n.10, p.1058–1064, 2012.

JOHNSON, M. Beta2-adrenoceptors: mechanisms of action of beta2-agonists. *Paediatr. Respir. Rev*., v.2, n.1, p.57-62, 2001.

KINHULT, J.; ANDERSSON, J.A.; UDDMAN, R.; STJÄRNE, P.; CARDELL, L.O. Pituitary adenylate cyclase-activating peptide 38 a potent endogenously produced dilator of human airways. *Eur. Respir. J*., v.15, n.2, p.243-247, 2000.

KINHULT, J.; UDDMAN, R.; CARDELL, L.O. The induction of carbon monoxide-mediated airway relaxation by PACAP 38 in isolated guinea pig airways. *Lung*, v.179, n.1, p.1-8, 2001.

LABURTHE, M.; COUVINEAU, A. Molecular pharmacology and structure of VPAC Receptors for VIP and PACAP. *Regul. Pept.*, v.108, n.2-3, p.16-173, 2002.

LI, M.; MADERDRUT, J.L.; LERTORA, J.J.; BATUMAN, V. Intravenous infusion of pituitary adenylate cyclase-activating polypeptide (PACAP) in a patient with multiple myeloma and myeloma kidney: a case study. *Peptides*, v.28, n.9, p.1891-1895, 2007.

LINDÉN, A.; CARDELL, L.O.; YOSHIHARA, S.; NADEL, J.A. Bronchodilation by pituitary adenylate cyclase-activating peptide and related peptides. *Eur. Respir. J*., v.14, n.2, p.443-451, 1999.

LINDÉN, A.; CARDELL, L.O.; YOSHIHARA, S.; STJÄRNE, P.; NADEL, J.A. PACAP 1-38 as an inhaled bronchodilator in guinea pigs *in vivo*. *Peptides*, v.19, n.1, p.93-98, 1998.

LINDÉN, A.; YOSHIHARA, S.; CHAN, B.; NADEL, J.A. Inhibition of bronchoconstriction by pituitary adenylate cyclase activating polypeptide (PACAP 1-27) in guinea-pigs *in vivo*. *Br. J. Pharmacol*., v.115, n.6, p.913-916, 1995.

MENTLEIN, R. Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptides. *Regul. Pept.*, v.85, n.1, p.9-24, 1999.

MIYATA, A.; ARIMURA, A.; DAHL, R.R.; MINAMINO, N.; UEHARA, A.; JIANG, L. Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. *Biochem. Biophys. Res. Commun.*, v.164, n.1, p.567-574, 1989.

MIYATA,A.; JIANG, L.; DAHL, R.D.; KITADA, C.; KUBO, K.; FUJINO.; M. Isolation of a neuropeptide corresponding to the N-terminal 27 residues of the pituitary adenylate cyclase activating polypeptide with 38 residues (PACAP 38). *Biochem. Biophys. Res. Commun*., v.170, n.2, p.643-648, 1990.

NAIR, P.; HANRAHAN, J.; HARGREAVE, F.E. Clinical equivalence testing of inhaled bronchodilators. *Pol. Arch. Med. Wewn*., v.119, n.11, p.731-735, 2009.

OKAZAWA, A.; CUI, Z.H.; LÖTVALL, J.; YOSHIHARA, S.; SKOOGH, B.E.; KASHIMOTO, K. Effect of a novel PACAP-27 analogue on muscarinic airway responsiveness in guinea-pigs *in vivo*. *Eur. Respir. J*., v.12, n.5, p.1062-1066, 1998.

REGLODI, D.; KISS, P. ; SZABADFI, K. ; ATLASZ, T.; GABRIEL, R.; HORVATH, G.; SZAKALY, P.; SANDOR, B.; LUBICS, A.; LASZLO, E.; FARKAS, J.; MATKOVITS, A.; BRUBEL, R.; HASHIMOTO, H.; FERENCZ, A.; VINCZE, A.; HELYES, Z.; WELKE, L.; LAKATOS, A.; TAMAS A. PACAP is an endogenous protective factor-insights from PACAP-deficient mice. *J. Mol. Neurosci.*, v.43, n. 3, p. 482-492, 2012.

SAGUCHI, Y.; ANDO, T.; WATANABE, T.; YAMAKI, K.; SUZUKI, R.; TAKAGI, K. Inhibitory effects of pituitary adenylate cyclase activating polypeptide on histamine-induced respiratory resistance in anesthetized guinea pigs. *Regul. Pept*., v.70, n.1, p.9-13,1997.

SAPIENZA, S.; DU, T.; EIDELMAN, D.H.; WANG, NS.; MARTIN, J.G. Structural changes in the airways of sensitized Brown Norway rats after antigen challenge. *Am. J. Respir. Crit. Care Med*., v.144, n.2, p.423-427, 1991.

SHIGYO, M.; AIZAWA, H.; INOUE, H.; MATSUMOTO, K, TAKATA, S, HARA, N. Pituitary adenylate cyclase activating peptide regulates neurally mediated airway response. *Eur. Respir. J*., v.12, n.1, p.64-70, 1998.

STEPHENS, N.L. Airway smooth muscle. *Lung*, v.179, n.6, p.333-373, 2001.

STERK, P.J.; FABBRI, L.M.; QUANJER, P.H.; COCKCROFT, D.W.; ANDERSON, S.D.; MALO, J.L. Tests normalisés chez l'adulte: stimulus pharmacologique, physique et sensibilisant. *Rev. Mal. Respir*., v.18, n.3, p.67-113, 2001.

SVENSSON, H.; BJERMER, L.; TUFVESSON, E. Exhaled breath temperature in asthmatics and controls after eucapnic voluntary hyperventilation and a methacholine challenge test. *Respiration*, v.87, n.2, p. 149-157, 2014.

UDDMAN, R.; LUTS, A.; ARIMURA, A., SUNDLER , F. Pituitary adenylate cyclase-activating peptide (PACAP), a new vasoactive intestinal peptide (VIP)-like peptide in the respiratory tract. *Cell Tissue Res*., v.265, n.1, p.197-201, 1991.

VAN SCHOOR, J.; JOOS, G.F.; PAUWELS, R.A. Indirect bronchial hyperresponsiveness in asthma: mechanisms, pharmacology and implications for clinical research. *Eur. Respir. J.*, v.16, n.3, p.514-533, 2000.

VAUDRY, D.; FALLUE, D.; MOREL, A.; BOURGAULT, S.; BASILLE, M.; BUREL, D. Pituitary adenylate cyclase-activating polypeptide and its receptors: 20 years after the discovery. *Pharmacol. Rev*., v.61, n3, p.283-357, 2009.

WILSON, R.J.; CUMMING, K.J. Pituitary adenylate cyclase-activating polypeptide is vital for neonatal survival and the neuronal control of breathing. *Respir. Physiol. Neurobiol*., v.164, n.1-2, p.168-178, 2008.

XIONG, G.; XU, L.; WEI, L.; LI, X. Atomization inhalation of terbutaline and budesonide efficiently improved immunity and lung function of AECOPD patients. *Cell. Mol. Immunol*., v.5, n.4, p.287-291, 2008.

YOSHIDA, M.; AIZAWA, H.; TAKAHASHI, N.; SHIGYO, M.; HARA, N. Pituitary adenylate cyclase activating peptide mediates inhibitory nonadrenergic noncholinergic relaxation. *Eur. J. Pharmacol*., v.39, n.1, p.77-83, 2000.

YOSHIHARA, S.; YAMADA, Y.; ABE, T.; KASHIMOTO, K.; LINDÉN, A.; ARISAKA, O. Long-lasting smooth-muscle relaxation by a novel PACAP analogue in human bronchi. *Regul. Pept*., v.123, n1-3, p.161-165, 2004.

ZHAO, W.; ROUATBI, S.; TABKA, Z.; GUÉNARD, H. Inhaled sodium fluoride decreases airway responsiveness to acetylcholine analogs *in vivo*. *Respir. Physiol. Neurobiol*., v.131, n.3, p.245-253, 2002.

ZHU, L.; TAMVAKOPOULOS, C.; XIE, D.; DRAGOVIC, J.; SHEN, X.; FENYK-MELODY, J.E. The role of dipeptidyl peptidase IV in the cleavage of glucagon family peptides: *in vivo* metabolism of pituitary adenylate cyclase activating polypeptide-(1-38). *J. Biol. Chem*., v.278, n.25, p.22418-22423, 2003.

Received for publication on 07th November 2013
Accepted for publication on 09th December 2014