PACAP Protects Against Salsolinol-Induced Toxicity in Dopaminergic SH-SY5Y Cells: Implication for Parkinson's Disease

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Abstract Pituitary adenylate cyclase-activating polypeptide (PACAP) is an endogenous 38 amino acid containing neuropeptide with various cytoprotective functions including neuroprotection. Administration of PACAP has been shown to reduce damage induced by ischemia, trauma, or exogenous toxic substances. Moreover, mice deficient in PACAP are more vulnerable to damaging insults. In this study, we sought to determine whether PACAP may also be protective against salsolinol-induced toxicity in SH-SY5Y cells and, if so, elucidate its mechanism(s) of action. Salsolinol (SALS) is an endogenous dopamine metabolite with selective toxicity to nigral dopaminergic neurons, which are directly implicated in Parkinson's disease (PD). SH-SY5Y cells, derived from human neuroblastoma cells, express high levels of dopaminergic activity and are used extensively as a model to study these neurons. Exposure of SH-SY5Y cells to 400 μM SALS for 24 h resulted in approximately 50 % cell death that was mediated by apoptosis as determined by cell flow cytometry and increases in caspase-3 levels. Cellular toxicity was also associated with reductions in brain-derived neurotrophic factor and phosphorylated cyclic AMP response element-binding protein. Pretreatment with PACAP dose-dependently attenuated SALS-induced toxicity and the associated apoptosis and the chemical changes. PACAP receptor antagonist PACAP6-38, in turn, dose-dependently blocked the effects of PACAP. Neither PACAP nor PACAP antagonist had any effect of its own on cellular viability. These results suggest the protective effects of PACAP in a cellular model of PD. Hence, PACAP or its agonists could be of therapeutic benefit in PD.

Keywords PACAP · Salsolinol · SH-SY5Y cell line · Neuroprotection · Apoptosis · BDNF · p-CREB

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by movement disorders, resulting from damage or destruction of dopaminergic neurons in the substantia nigra (Jankovic 2008; Samii et al. 2004). Later, cognitive and behavioral problems may also arise. Although available medications provide some symptomatic relief in almost all patients, none has been shown to significantly slow or stop the disease progression (Lang 2009; Fernandez 2012). Consequently, there is a dire need for more effective therapeutic interventions. The cause of PD is unknown, but some atypical cases seem to have a genetic origin. Although numerous genes responsible for familial PD have been identified, the etiology of sporadic PD, which accounts for the majority of PD cases, is still unknown (Healy et al. 2004; Morris 2005). Recent advances in PD pathology suggest that the neuronal degeneration in this disease likely involves several cellular and molecular events, including oxidative stress, microglia-mediated inflammation as well as proapoptotic mechanisms (von Bohlen et al. 2004). As such, there is a lot of effort being put into finding new therapies targeting these pathways.

Pituitary adenylate cyclase-activating polypeptide (PACAP), originally isolated from the sheep hypothalamic extract, is a widespread neuropeptide with diverse actions (Masuo et al.1993). PACAP acts through the specific PAC1 receptor and the VPAC1/2 receptors, which also bind vasoactive intestinal

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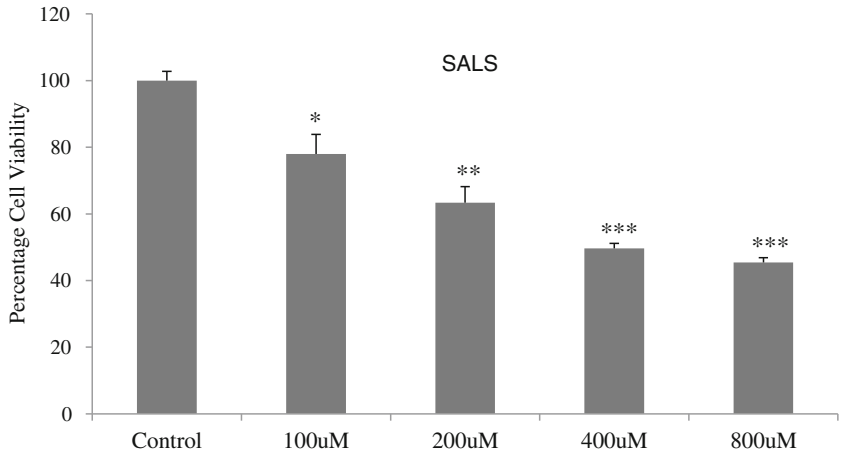


Fig. 1 Dose–response effect of salsolinol (SALS) on SH-SY5Y cells. MTT assay was conducted 24 h after SALS treatment. Values are mean ± SEM, $n=5$. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to control

peptide (Vaudry et al. 2009). PACAP and its closest structural related peptide VIP have been shown to possess potent neuroprotective properties against ischemia, trauma, or exogenous toxic substances such as 6-hydroxy-dopamine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and rotenone both in vivo and in neuronal cultures (Offen et al. 2000; Reglödi et al. 2000; Reglödi et al. 2004; Somogyvari-Vigh and Reglödi 2004; Wang et al. 2005, 2008; Botia et al. 2011; Rat et al. 2011; Reglödi et al. 2011; Nakamachi et al. 2012; Tamas et al. 2012a; Tuncel et al. 2012; Tsuchikawa et al. 2012). Moreover, mice deficient in PACAP are more vulnerable to damaging insults (Reglödi et al. 2012; Szabadfi et al. 2012; Tamas et al. 2012a, b). Based on these findings, it has been suggested that PACAP may offer a novel therapeutic approach in the treatment of neurodegenerative diseases including Parkinson's disease (Dejda et al. 2005; Vaudry et al. 2009; Reglödi et al. 2011). PACAP and its major receptor PAC1, postulated to be the primary target of PACAP, are highly expressed in the substantia nigra (Masuo et al. 1992; Reglödi et al. 2011). It has been documented that at least some of the protective effects of PACAP are due to its anti-apoptotic effects, reflected in caspase-3 reduction, as well as up-regulation of brain-derived neurotrophic factor (BDNF) and enhancement of its signal transduction mediated via phosphorylation of cyclic AMP response element-binding protein (CREB) (Vaudry et al. 2000; Frechilla et al. 2001; Yaka et al. 2003; Racz et al. 2006; Botia et al. 2011; Rat et al. 2011; Lazarovici et al. 2012). PACAP may also influence dopamine synthesis via activation of tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine synthesis (Reglödi et al. 2011).

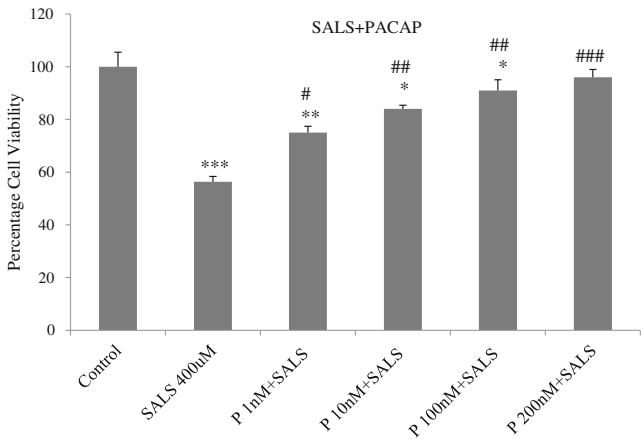


Fig. 2 Dose–response effect of PACAP (P) pretreatment against salsolinol (SALS)-induced toxicity in SH-SY5Y cells. PACAP was applied 2 h prior to salsolinol treatment and the cell viability was determined 24 h after SALS. Values are mean ± SEM, $n=5$. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to control; #$p<0.05$, ##$p<0.01$, ###$p<0.001$ compared to SALS 400 μM

In this study, we first sought to determine whether PACAP might have protective effects against salsolinol-induced toxicity in SH-SY5Y cells. Salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, SALS) is an endogenous dopamine metabolite with selective toxicity to nigral dopaminergic neurons. Indeed, many Parkinson patients show high levels of SALS in their urine and cerebrospinal fluid, which has led to the suggestion that SALS might be involved in the etiology or loss of dopamine neurons in at least some of these patients (Storch et al. 2002; Maruyama et al. 2004). SH-SY5Y cells, derived from human neuroblastoma cells, express high levels of dopaminergic activity and are used extensively as a cellular model to study the mechanism(s) of toxicity and protection in nigral dopaminergic neurons (Storch et al. 2002; Maruyama et al. 2004; Naoi et al. 2004; Copeland et al. 2007; Das and Tizabi 2009; Ramlochansingh et al. 2011). Once the protective effects of PACAP were established, we were interested in determining the mechanism of such protection and whether the effects of PACAP could be attenuated or blocked by a PACAP antagonist.

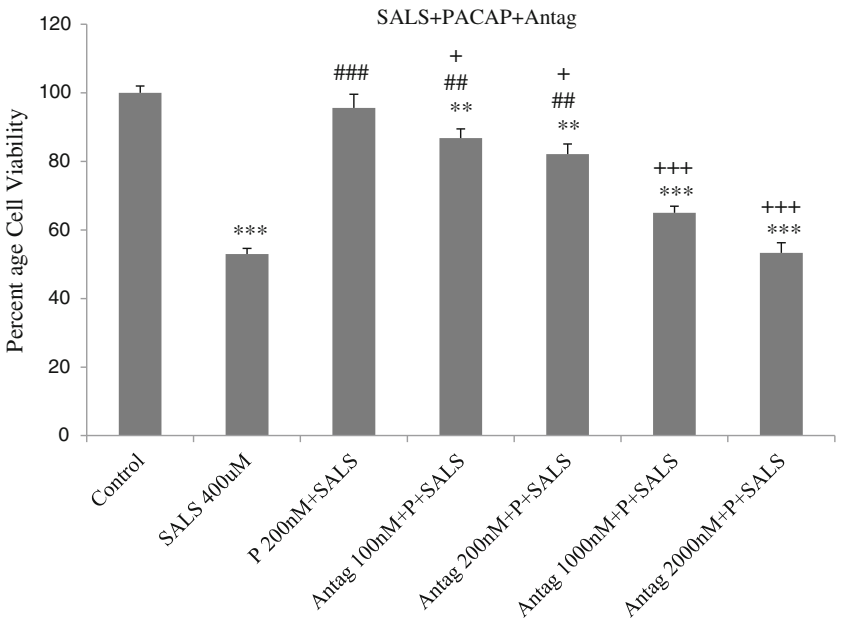


Fig. 3 Dose–response effect of PACAP antagonist (Antag) on the protective effects of PACAP (P) against salsolinol (SALS)-induced toxicity in SH-SY5Y cells. PACAP antagonist was applied 2 h prior to PACAP, which was applied 2 h prior to SALS. Cell viability was determined 24 h after SALS. Values are mean ± SEM, $n=5$. $^{**}p<0.01$, $^{***}p<0.001$ compared to control; $\#\#p<0.01$, $\#\#\#p<0.001$ compared to SALS 400 μM; $+p<0.05$, $+++p<0.001$ compared to P+SALS

Methods

Drugs

Salsolinol, PACAP, and its antagonist (PACAP6-38) as well as other analytical reagents were purchased from Sigma Chemical Company (Sigma Aldrich, St. Louis, MO, USA).

Cell Culture

SH-SY5Y cells were purchased from the American Type Culture Collection in Manassas, VA, USA and were cultivated in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 supplemented with 10 % fetal bovine serum, penicillin/streptomycin (1,000 IU/ml), and gentamicin (50 μg/ml) at 37 °C in an incubator. The cells were harvested approximately 5 days later when confluent and plated in a 96-well plate (1.6×10^4 cells/well). Cells were allowed to adhere to the plate for 24 h.

Salsolinol Treatment

To determine the dose–response effects of salsolinol, following the adherence of the cells to the plate, the media were aspirated and fresh media containing different concentrations of salsolinol were added. After 24 h, cell viability was measured via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

PACAP Treatment

To determine the protective effects of PACAP, 2 h prior to salsolinol (400 μM, a concentration that yielded approximately 50 % toxicity), various concentrations of PACAP were added. Again, after 24 h, cell viability was determined via the MTT assay.

PACAP Antagonist Treatment

To determine the effects of PACAP antagonist on the protective effects of PACAP, 2 h prior to PACAP, cells were treated with PACAP6-38, followed by salsolinol (400 μM) and determination of cell viability after 24 h via the MTT assay.

MTT Assay for Cell Viability

Determination of cell viability was done by the MTT colorimetric assay. The yellow MTT tetrazolium salt was dissolved in phosphate-buffered saline (PBS) with 10 mM HEPES, and 30 μl of this reagent was added to each well for 3 h and incubated at 37 °C. The live cells caused a reduction of the yellow salt to insoluble purple formazan crystals. The wells were then aspirated and 50 μl of dimethyl sulfoxide was added to the wells to solubilize the crystals. The plates were shaken for 1 h and read spectrophotometrically at 570 nm in a plate reader. The data were analyzed and represented as percent cell viability.

Cell Flow Cytometry

Cell flow cytometry was used to detect apoptosis vs necrosis by measuring and sorting cells by fluorescent labeling of markers on cell surface. The cells were grown and treated as described above. In this case, however, following harvesting, the cells were washed twice with cold PBS and then gently suspended in a solution that consisted of 100 μl Annexin V-Fluos labeling solution, 5 μl of fluorescein

isothiocyanate labeled by Annexin V-FITC, and 5 μl of propidium iodide (PI). Afterwards, the cells were incubated in the dark at room temperature for 15 min before adding 500 μl of Annexin V-Fluos labeling solution to each well. Finally, the cells were subjected to flow cytometry using a cellometer machine (Nexcelom, Lawrence, MA, USA) followed by analysis of apoptotic and necrotic fraction using FCS express software.

BDNF, p-CREB, and Caspase-3 Measurement

Western blot was used to determine the levels of BDNF, p-CREB, and caspase-3 in SH-SY5Y cells. Briefly, cells were homogenized in lysis buffer (10 mM Tris buffer, 5 mM EDTA, 150 mM NaCl, 0.5 % Triton X-100 (*v*/*v*)) with protease inhibitors (Sigma-Aldrich, St. Louis, MO, USA). The protein concentration in each sample was determined using a BCA protein assay kit (Pierce Biotechnology Inc., IL, USA), and an equal protein amount (as confirmed by β-actin) was loaded in each immunoblot. The proteins were separated using 12 % SDS-PAGE gel and transferred onto a nitrocellulose membrane. The membranes were blocked with a blocking reagent (5 % nonfat milk in TBS buffer) for 0.5 h and incubated at 4 °C overnight with the primary antibody against BDNF, p-CREB, and caspase-3 (1:1,000). The membranes were washed with TBST (TBS buffer with 1 % Tween-20) and blocked with the blocking reagent. Membranes were then incubated for 1 h at room temperature in goat antirabbit–HRP conjugated secondary antibody (1:3,000 in TBS, Bio-Rad Laboratories, CA, USA). The membranes were then washed in the TBST washing solution and then visualized using enhanced chemiluminescent kits (Bio-Rad Laboratories). The intensity of the protein bands on the gel was quantified using ChemiDoc XRS system (Bio-Rad Laboratories).

Statistical Analysis

Statistical difference between treatment groups was determined by one-way ANOVA followed by post hoc Tukey comparison test to determine which groups differed. Significant difference was considered a priori at $p<0.05$. Data were analyzed using GraphPad Prism 3 (San Diego, CA, USA) and expressed as mean ± SEM.

Results

Salsolinol Effect

As observed previously (Ramlochansingh et al. 2011), SALS caused a dose-dependent toxicity with near EC50 at 400 μM concentration (Fig. 1). Hence, this concentration of SALS was used in subsequent studies.

Fig. 4 Effects of PACAP (P, 200 nM) and PACAP antagonist (Antag, 2 μM) on salsolinol (SALS)-induced apoptosis/necrosis as determined by cell flow cytometry (**a** cell flow diagram; **b** percent change in apoptosis/necrosis). Living cells ($FITC^-/PI^-$) are represented in the *lower left quadrant*; early apoptotic cells ($FITC^+/PI^-$) are represented in the *lower right quadrant*; late apoptotic cells ($FITC^+/PI^+$) are represented in the *upper right quadrant*; and necrotic cells ($FITC^-/PI^+$) are represented in the *upper left quadrant*. Antagonist was applied 2 h before PACAP, which was applied 2 h prior to SALS. Cell flow analysis was performed 24 h after treatment. Values are mean ± SEM, $n=5$. ***$p<0.001$ compared to control

PACAP Effect

PACAP pretreatment resulted in a dose-dependent protection against SALS-induced toxicity where the highest concentration (200 nM) completely blocked SALS effect (Fig. 2). PACAP by itself had no effect on cell viability (data not shown).

PACAP Antagonist Effect

PACAP antagonist (PACAP6-38) dose-dependently blocked the effects of PACAP (200 nM). At 2 μM, PACAP antagonist totally blocked the effect of PACAP (Fig. 3). PACAP antagonist by itself had no effect on cell viability (data not shown).

Cell Flow Results

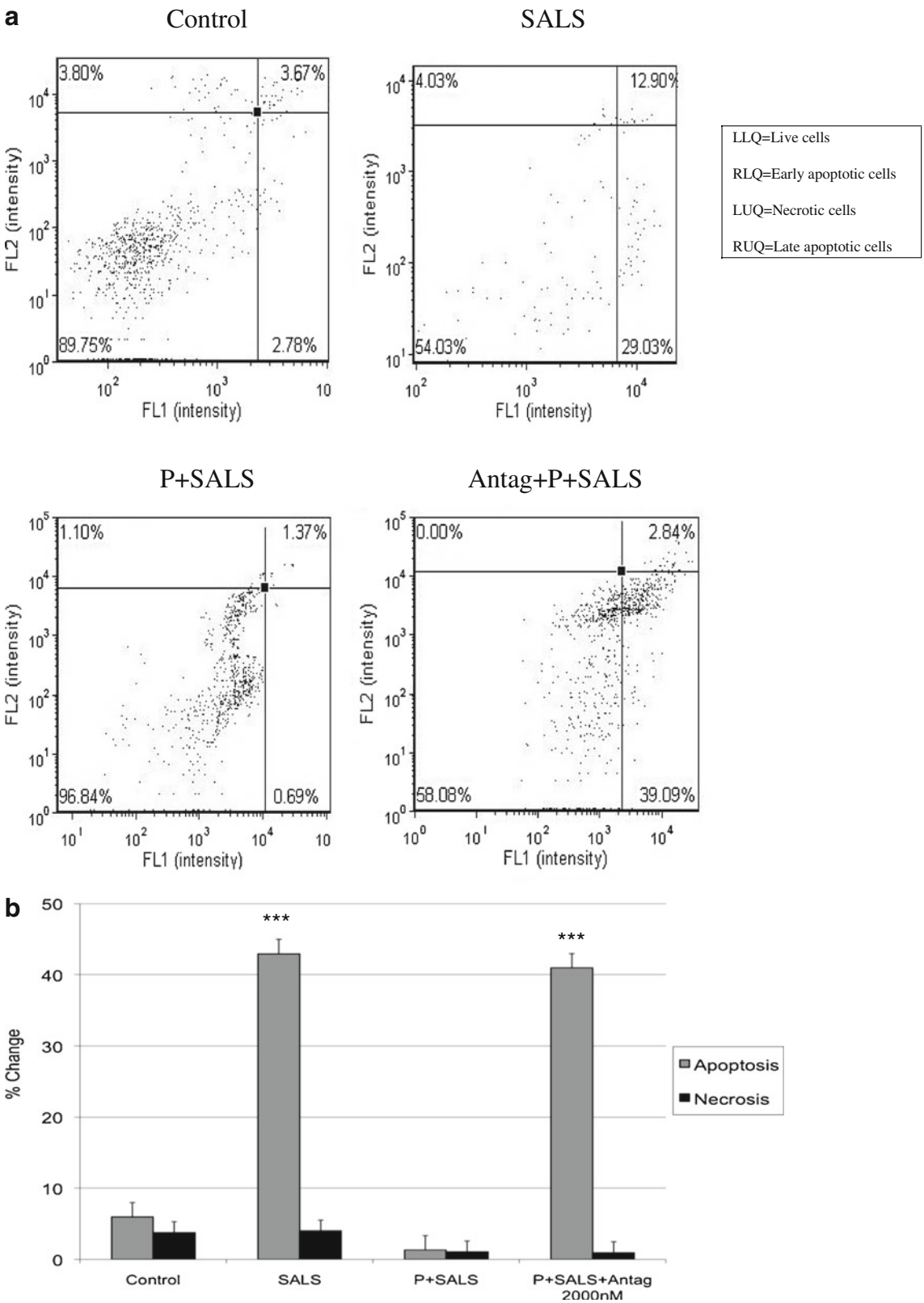
Cell flow cytometry indicates that the major effects of SALS (400 μM) were due to the apoptotic mechanism. These effects were blocked by PACAP (200 nM), and the PACAP antagonist (2 μM) in turn blocked the effects of PACAP (Fig. 4).

BDNF and p-CREB Results

Panels b and c of Fig. 5 depict that SALS resulted in approximately 25 % reduction in BDNF and approximately 15 % reduction in p-CREB levels. PACAP pretreatment (200 nM) completely blocked the effect of SALS. The PACAP antagonist (2 μM) in turn blocked the effects of PACAP. PACAP or its antagonist did not have any effect of their own on the levels of BDNF or p-CREB (data not shown).

Caspase-3 Results

Figure 5d depicts that SALS resulted in approximately 40 % increase in caspase-3 levels. PACAP pretreatment (200 nM) completely reversed the effect of SALS. The PACAP



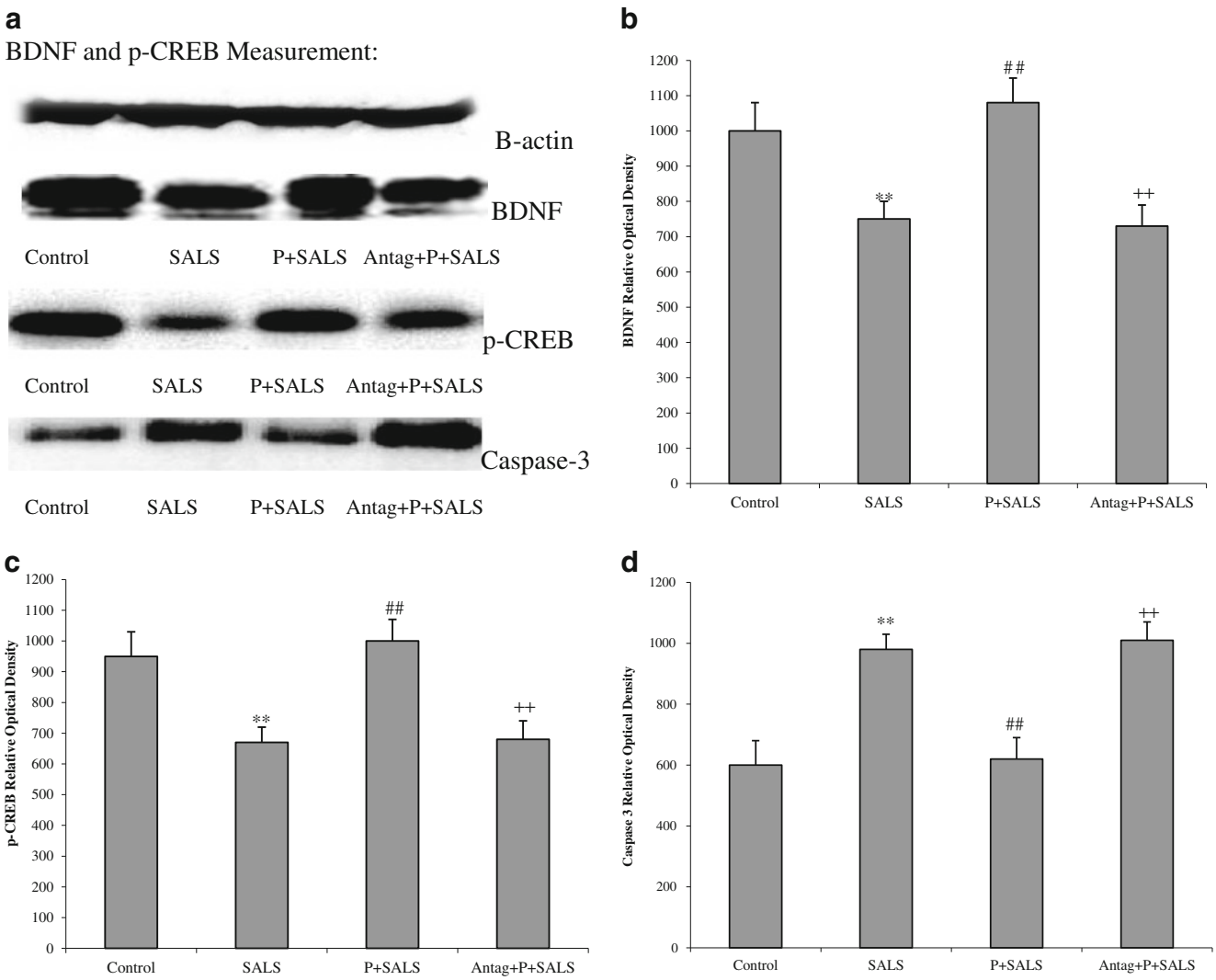


Fig. 5 Effects of PACAP (P, 200 nM) and PACAP antagonist (Antag, 2 μ) on salsolinol (SALS)-induced changes in BDNF, p-CREB, and caspase-3 as determined by western blot (**a**). Antag was applied 2 h before P, which was applied 2 h prior to SALS. BDNF (**b**), p-CREB (**c**), and caspase-3 (**d**) relative optical density. Values are mean ± SEM, $n=5$. $^{**}p<0.01$ compared to control; $^{\#\#}p<0.01$ compared to SALS 400 μM; $^{++}p<0.01$ compared to P+SALS

antagonist (2 μM) in turn blocked the effect of PACAP. PACAP or its antagonist did not have any effect of their own on caspase-3 levels (data not shown).

Discussion

The results of the current study provide further proof of the neuroprotective effects of PACAP and suggest that PACAP or its receptor agonist could be of therapeutic potential in retarding the progressive, neurodegenerative nature of PD. This contention is based on the finding that PACAP dose-dependently blocked the cytotoxicity induced by salsolinol and that PACAP effects in turn were blocked by its antagonist, PACAP6-38. Although PACAP6-38 may also antagonize VPAC2 receptor, it is likely that the protection seen in our studies is primarily mediated by PAC1 receptor as numerous studies have implicated this receptor in neuroprotective and cell survival promoting effects of PACAP (Somogyvari-Vigh and Reglödi 2004; Vaudry et al. 2009). The results also confirm the reported apoptotic effects of SALS via caspase-3 up-regulation (Bollimuntha et al. 2006; Copeland et al. 2007; Das and Tizabi 2009; Jantas et al. 2008; Ramlochansingh et al. 2011) and extend those findings to include involvement of the neurotrophic pathway in SALS-induced neurotoxicity. Thus, SALS caused a reduction in BDNF and its signal transduction protein, p-CREB, both of which were attenuated by PACAP. Since stimulation of BDNF/CREB signaling can lead to down-regulation of caspase-3 levels and inhibition of neuronal apoptosis (Han et al. 2000; Kim and Zhao 2005; Li and Liu 2010), it may be suggested that the anti-apoptotic effects of PACAP may be a

major mechanism of its neuroprotection against SALS-induced toxicity.

In addition to the aforementioned mechanisms, PACAP utility in PD may also be enhanced by its activation of TH. Interestingly, both BDNF and TH are regulated by the transduction signal CREB, the phosphorylation of which can lead to enhanced synthesis of both proteins (Lewis-Tuffin et al. 2004; Purves et al. 2008). Hence, the dual effects of PACAP on the prevention or delay of dopaminergic neuronal degeneration while simultaneously enhancing the DA synthesis capacity may offer a unique advantage in PD treatment. However, as with any peptide therapy, the questions of bioavailability and penetration of PACAP into the brain remain important concerns for the actual intervention in PD (Dogrukol-Ak et al. 2009). Nonetheless, possible development of ligands that may directly activate PAC1 receptor and/or enhance the signal transduction mechanisms involved in PACAP effectiveness may prove feasible and, hence, of significant therapeutic potential.

Our findings complement previous studies where PACAP has been shown to protect against various neuronal toxins in animal models of PD. Thus, PACAP can rescue dopaminergic neurons in rats subjected to unilateral injection of 6-hydroxydopamine, a commonly used model of PD (Reglödi et al. 2004, 2006). Additionally, PACAP is able to protect against methamphetamine as well as MPTP-induced toxicity in a mouse model of PD (Guillot et al. 2008; Wang et al. 2008). Altogether, the findings provide the possible utility of this peptide in the treatment of PD.

It has been recently demonstrated that a number of established as well as novel drugs with utility in neurodegenerative conditions may share a common mechanism of enhancing CREB–BDNF signaling (Li and Liu 2010; Puerta et al. 2010; see also the review by Bitner 2012). In this regard, a combination of such drugs, where the triggering mechanism for signal transduction may differ, could prove of superior intervention than a monotherapy (Priestley et al. 2012). Thus, combining PACAP which activates PAC1 receptor to inhibit caspase-3 (Dejda et al. 2005) with sildenafil, a phosphodiesterase 5 inhibitor which inhibits calpain, an intracellular Ca^{2+}-dependent Cys protease (Puerta et al. 2010; Sorimachi et al. 2012), both of which eventually lead to an increase in signal transduction, may be a highly effective therapy in PD. PACAP utility in neurodegenerative disorders in general and PD in particular may also be aided by its suppression of inflammatory mediators that have been implicated in such diseases (Suk et al. 2004). In summary, PACAP or its receptor agonists, by enhancing the neurotrophic pathway and inhibition of apoptosis, may be of therapeutic benefit in PD.

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References

Bitner S (2012) Cyclic AMP response element-binding protein (CREB) phosphorylation: a mechanistic marker in the development of memory enhancing Alzheimer's disease therapeutics. Biochem Pharmacol 83:705–714

Bollimuntha S, Ebadi M, Singh BB (2006) TRPC1 protects human SH-SY5Y cells against salsolinol-induced cytotoxicity by inhibiting apoptosis. Brain Res 1099:141–149

Botia B, Jolivel V, Burel D et al (2011) Neuroprotective effects of PACAP against ethanol-induced toxicity in the developing rat cerebellum. Neurotox Res 19:423–434

Copeland RL, Das JR, Kanaan YM (2007) Antiapoptotic effects of nicotine in its protection against salsolinol-induced cytotoxicity. Neurotox Res 12:61–69

Das JR, Tizabi Y (2009) Additive protective effects of donepezil and nicotine against salsolinol-induced cytotoxicity in SH-SY5Y cells. Neurotox Res 16:194–204

Dejda A, Sokolowska P, Nowak JZ (2005) Neuroprotective potential of three neuropeptides PACAP, VIP and PHI. Pharmacol Rep 57:307–320

Dogrukol-Ak D, Kumar VB, Ryerse JS, Farr SA, Verma S, Nonaka N (2009) Isolation of peptide transport system-6 from brain endothelial cells: therapeutic effects with antisense inhibition in Alzheimer and stroke models. J Cereb Blood Flow Metab 29:411–422

Fernandez HH (2012) Updates in the medical management of Parkinson disease. Cleve Clin J Med 79:28–35

Frechilla D, Garcia-Osta A, Palacios S, Cenarruzabebeitia E, Del Rio J (2001) BDNF mediates the neuroprotective effect of PACAP-38 on rat cortical neurons. Neuroreport 12:919–923

Guillot TS, Richardson JR, Wang MZ et al (2008) PACAP38 increases vesicular monoamine transporter 2 (VMAT2) expression and attenuates methamphetamine toxicity. Neuropeptides 42:423–434

Han BH, D'Costa A, Back SA et al (2000) BDNF blocks caspase-3 activation in neonatal hypoxia–ischemia. Neurobiol Dis 7:38–53

Healy DG, Abou-Sleiman PM, Wood NW (2004) PINK, PANK, or PARK? A clinicians' guide to familial parkinsonism. Lancet Neurol 3:652–662

Jankovic J (2008) Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry 79:368–376

Jantas D, Pytel M, Mozrzymas JW et al (2008) The attenuating effect of memantine on staurosporine-, salsolinol- and doxorubicin-induced apoptosis in human neuroblastoma SH-SY5Y cells. Eurochem Int 52:864–877

Kim DH, Zhao X (2005) BDNF protects neurons following injury by modulation of caspase activity. Neurocrit Care 3:71–76

Lang AE (2009) When and how should treatment be started in Parkinson disease. Neurology 72:S39–S43

Lazarovici P, Cohen G, Arien-Zakay H et al (2012) Multimodal neuroprotection induced by PACAP38 in oxygen–glucose deprivation and middle cerebral artery occlusion stroke models. J Mol Neurosci 48:526–540

Lewis-Tuffin LJ, Quinn PG, Chikaraishi DM (2004) Tyrosine hydroxylase transcription depends primarily on cAMP response element activity, regardless of the type of inducing stimulus. Mol Cell Neurosci 25:536–547

Li N, Liu GT (2010) The novel squamosamide derivative FLZ enhances BDNF/TrkB/CREB signaling and inhibits neuronal apoptosis in APP/PS1 mice. Acta Pharmacol Sin 31:265–272

Maruyama W, Yi H, Takahashi T et al (2004) Neuroprotective function of R-(–)-1-(benzofuran-2-yl)-2-propylaminopentane, [R-(–)-BPAP], against apoptosis induced by N-methyl(R)salsolinol, an endogenous dopaminergic neurotoxin, in human dopaminergic neuroblastoma SH-SY5Y cells. Life Sci 75:107–117

Masuo Y, Ohtaki T, Masuda Y, Tsuda M, Fujino M (1992) Binding sites for pituitary adenylate cyclase activating polypeptide (PACAP):

comparison with vasoactive intestinal polypeptide (VIP) binding site localization in rat brain sections. Brain Res 575:113–123

Masuo Y, Suzuki N, Matsumoto H et al (1993) Regional distribution of pituitary adenylate cyclase activating polypeptide (PACAP) in the rat central nervous system as determined by sandwich-enzyme immunoassay. Brain Res 602:657–663

Morris HR (2005) Genetics of Parkinson's disease. Ann Med 37:86–96

Nakamachi T, Tsuchida M, Kagami N et al (2012) IL-6 and PACAP receptor expression and localization after global brain ischemia in mice. J Mol Neurosci 48:518–525

Naoi M, Maruyama W, Nagy GM (2004) Dopamine-derived salsolinol derivatives as endogenous monoamine oxidase inhibitors: occurrence, metabolism and function in human brains. Neurotoxicology 25:193–204

Offen D, Sherki Y, Melamed E, Fridkin M, Brenneman DE, Gozes I (2000) Vasoactive intestinal peptide (VIP) prevents neurotoxicity in neuronal cultures: relevance to neuroprotection in Parkinson's disease. Brain Res 854:257–262

Priestley JV, Michael-Titus AT, Tetziaff W (2012) Limiting spinal cord injury by pharmacological intervention. Handb Clin Neurol 109:463–484

Puerta E, Hervias I, Barros-Minones L et al (2010) Sildenafil protects against 3-nitropropionic acid neurotoxicity through the modulation of calpain, CREB, and BDNF. Neurobiol Dis 38:237–245

Purves D, Augustine GJ, Fitzpatrick D, Hall WC, LaMantia A, White LE (2008) Neuroscience, 4th edn. Sinauer Associates, Sunderland, pp 170–176

Racz B, Tamas A, Kiss P et al (2006) Involvement of ERK and CREB signaling pathways in the protective effect of PACAP in monosodium glutamate-induced retinal lesion. Ann N Y Acad Sci 1070:507–511

Ramlochansingh C, Taylor RE, Tizabi Y (2011) Toxic effects of low alcohol and nicotine combinations in SH-SY5Y cells are apoptotically mediated. Neurotox Res 20:263–269

Rat D, Schmitt U, Tippmann F et al (2011) Neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) slows down Alzheimer's disease-like pathology in amyloid precursor protein-transgenic mice. FASEB J 25:3208–3218

Reglödi D, Somogyvari-Vigh A, Vigh S, Kozicz T, Arimura A (2000) Delayed systemic administration of PACAP38 is neuroprotective in transient middle cerebral artery occlusion in the rat. Stroke 31:1411–1417

Reglödi D, Tamas A, Lubics A, Szalontay L, Lengvari I (2004) Morphological and functional effects of PACAP in 6-hydroxydopamine-induced lesion of the substantia nigra in rats. Regul Pept 123:85–94

Reglödi D, Tamas A, Lengvari I, Toth G, Szalontay L, Lubics A (2006) Comparative study on the effects of PACAP in young, aging, and castrated males in a rat model of Parkinson's disease. Ann NY Acad Sci 1070:518–524

Reglödi D, Kiss P, Lubics A, Tamas A (2011) Review on the protective effect of PACAP in models of neurodegenerative diseases in vitro and in vivo. Curr Pharm Des 17:962–972

Reglödi D, Kiss P, Szabadfi K et al (2012) PACAP is an endogenous protective factor-insights from PACAP-deficient mice. J Mol Neurosci 48:482–492

Samii A, Nutt JG, Ransom BR (2004) Parkinson's disease. Lancet 363:1783–1793

Somogyvari-Vigh A, Reglödi D (2004) Pituitary adenylate cyclase activating polypeptide: a potential neuroprotective peptide. Curr Pharm Des 10:2861–2889

Sorimachi H, Mamitsuka H, Ono Y (2012) Understanding the substrate specificity of conventional calpains. Biol Chem 393:853–871

Storch A, Ott S, Hwang YI et al (2002) Selective dopaminergic neurotoxicity of isoquinoline derivatives related to Parkinson's disease: studies using heterologous expression systems of the dopamine transporter. Biochem Pharmacol 63:909–920

Suk K, Park JH, Lee WH (2004) Neuropeptide PACAP inhibits hypoxic activation of brain microglia: a protective mechanism against microglial neurotoxicity in ischemia. Brain Res 1026:151–156

Szabadfi K, Atlasz T, Kiss P et al (2012) Mice deficient in pituitary adenylate cyclase activating polypeptide (PACAP) are more susceptible to retinal ischemic injury in vivo. Neurotox Res 21:41–48

Tamas A, Reglödi D, Farkas O et al (2012a) Effect of PACAP in central and peripheral nerve injuries. Int J Mol Sci 13:8430–8448

Tamas A, Szabadfi K, Nemeth A et al (2012b) Comparative examination of inner ear in wild type and pituitary adenylate cyclase activating polypeptide (PACAP)-deficient mice. Neurotox Res 21:435–444

Tsuchikawa D, Nakamachi T, Tsuchida M et al (2012) Neuroprotective effect of endogenous pituitary adenylate cyclase-activating polypeptide on spinal cord injury. J Mol Neurosci 48:508–517

Tuncel N, Korkmaz OT, Tekin N, Sener E, Akyuz F, Inal M (2012) Antioxidant and anti-apoptotic activity of vasoactive intestinal peptide (VIP) against 6-hydroxy dopamine toxicity in the rat corpus striatum. J Mol Neurosci 46:51–57

Vaudry D, Gonzalez BJ, Basille M et al (2000) The neuroprotective effect of pituitary adenylate cyclase-activating polypeptide on cerebellar granule cells in mediated through inhibition of the CED3-related cysteine protease caspase-3/CPP32. Proc Natl Acad Sci USA 97:13390–13395

Vaudry D, Falluel-Morel A, Bougault S et al (2009) Pituitary adenylate cyclase-activating polypeptide and its receptors: 20 years after the discovery. Pharmacol Rev 61:283–357

von Bohlen, Halbach O, Schober A, Krieglstein K (2004) Genes, proteins, and neurotoxins involved in Parkinson's disease. Prog Neurobiol 73:151–177

Wang G, Qi C, Fan GH, Zhou HY, Chen SD (2005) PACAP protects neuronal differentiated PC12 cells against the neurotoxicity induced by a mitochondrial complex I inhibitor, rotenone. FEBS Lett 579:4005–4011

Wang G, Pan J, Tan YY et al (2008) Neuroprotective effects of PACAP27 in mice model of Parkinson's disease involved in the modulation of K (ATP) subunits and D2 receptors in the striatum. Neuropeptides 42:267–276

Yaka R, He DY, Phamluong K, Ron D (2003) Pituitary adenylate cyclase-activating polypeptide (PACAP(1-38)) enhances *N*-methyl-D-aspartate receptor function and brain-derived neurotrophic factor expression via RACK1. J Biol Chem 278:9630–9963