PACAP protects neuronal PC12 cells from the cytotoxicity of human prion protein fragment 106–126

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Abstract Misfolding of the prion protein yields amyloidogenic isoforms, and it shows exacerbating neuronal damage in neurodegenerative disorders including prion diseases. Pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) potently stimulate neuritogenesis and survival of neuronal cells in the central nervous system. Here, we tested these neuropeptides on neurotoxicity in PC12 cells induced by the prion protein fragment 106–126 [PrP (106–126)]. Concomitant application of neuropeptide with PrP(106–126) (5×10^{-5} M) inhibited the delayed death of neuron-like PC12 cells. In particular, PACAP27 inhibited the neurotoxicity of PrP(106–126) at low concentrations ($>10^{-15}$ M), characterized by the deactivation of PrP(106–126)-stimulated caspase-3. The neuroprotective effect of PACAP27 was antagonized by the selective PKA inhibitor, H89, or the MAP kinase inhibitor, U0126. These results suggest that PACAP27 attenuates PrP(106–126)-induced delayed neurotoxicity in PC12 cells by activating both PKA and MAP kinases mediated by PAC1 receptor.

Key words: Pituitary adenylate cyclase-activating polypeptide; Vasoactive intestinal peptide; PC12 cell; Prion protein-106–126; Caspase; Circular dichroism

1. Introduction

Prion diseases are unusual fetal neurodegenerative disorders, including Creutzfeldt–Jakob disease and Gerstmann–Straussler–Scheinker syndrome in humans, scrapie in sheep and goats, and spongiform encephalopathy in cattle [1]. These diseases are characterized by the accumulation of large aggregates of the prion protein (PrP) in both human and animal brains. PrP tends to adopt a β-sheet conformation in buffered solutions, and aggregates into amyloid fibrils that are partly resistant to protease digestion [2]. The prion protein fragment PrP(106–126), corresponding to a putative transmembrane region of cellular PrP, is believed to be one of the key regions where conformational changes are initiated, leading to the conversion of native cellular prion protein (PrP^c) to scrapie isoform of prion protein (PrP^{sc}) [3]. This peptide shows a remarkable conformational polymorphism, acquiring different secondary structures in various environments, and it has been shown to induce cell death in neurons likely via the programmed cell death pathway. The importance of the 106–126 sequence of PrP makes it a useful model for the in vitro study of prion-induced cell death. Although evidence is accumulating for the hypothesis that inflammatory components such as cytokines and complement proteins play a significant role in exacerbating neuronal damage in neurodegenerative disorders including prion diseases [4], it is still unclear how PrP^{sc} accumulation gives rise to the profound neurodegeneration characteristic of scrapie.

Pituitary adenylate cyclase-activating polypeptide (PACAP) [5] and vasoactive intestinal peptide (VIP) [6] are two neuropeptides that perform a broad spectrum of biological functions affecting both natural and acquired immunity, primarily as regulatory agents. There are two forms of mammal PACAP, PACAP38 and a shorter peptide with the same N-terminal 27 residue, PACAP27, and they have been shown to have the same biological activity and receptor-binding activity [7]. PACAP and VIP have been shown to act as survival factors against ischemia and glutamate-induced neurotoxicities [8], and in vitro studies have demonstrated that they can stimulate neurite outgrowth [9].

These observations prompted us to investigate the effect of neuropeptides including PACAP and VIP on the PrP-induced neurotoxicity using neuron-like PC12 cells, in which the expression of PACAP-specific PAC1 receptor was confirmed [10]. In addition, we evaluated how PACAP/VIP influence the cytotoxicity level, characterized by caspase cascades and protein kinase signaling pathways.

2. Materials and methods

2.1. Chemicals

The neuropeptides PACAP and VIP were synthesized by the solid-

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Abbreviations: Ac-DEVD-CHO, acetyl-Asp-Glu-Val-Asp-1-al; CD, circular dichroism; db-cAMP, dibutyryl-cAMP; DMEM, Dulbecco's modified Eagle's medium; H89, *N*-(2-[*p*-bromocinnamylamino]ethyl)-5-isoquinolinesulfonamide; MAP, mitogen-activated protein; Myr-ψPKC, myristoyl-Gly-Arg-Arg-Asn-Ala-Ile-His-Asp-Ile; LDH, lactate dehydrogenase; PACAP, pituitary adenylate cyclase activating polypeptide; PKA, protein kinase A; PKC, protein kinase C; PrP, prion protein; PrP^c, native cellular prion protein; PrP^{sc}, scrapie isoform of prion protein; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate; U0126, bis[amino[(2-aminophenyl)thio]methylene]butanedinitrile; VIP, vasoactive intestinal peptide; WST-8, 4-[3-(2-methoxy-4-nitrophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate sodium salt; Z-VAD-FMK, *N*-benzyloxycarbonyl-Val-Ala-Asp(O-Me) fluoromethyl ketone

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phase strategy employing optimal side-chain protection as reported previously [11]. Human PrP(106–126) was purchased from American Peptide Company (Sunnyvale, CA, USA). 4-[3-(2-Methoxy-4-nitrophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate sodium salt (WST-8) was purchased from Dojindo (Kumamoto, Japan). Dibutyryl-cAMP (db-cAMP), 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA), bis[amino [(2-aminophenyl)thio]methylene]butanedinitrile (U0126) and *N*-(2-[*p*-bromocinnamylamino]ethyl)-5-isoquinolinesulfonamide (H-89) were purchased from Sigma (St. Louis, MO, USA). Myristoyl-Gly-Arg-Arg-Asn-Ala-Ile-His-Asp-Ile (Myr-ψPKC), acetyl-Asp-Glu-Val-Asp-1-al (Ac-DEVD-CHO) and *N*-benzyloxycarbonyl-Val-Ala-Asp(O-Me) fluoromethyl ketone (Z-VAD-FMK) were purchased from Promega (Madison, WI, USA).

2.2. Cell cultures

Rat pheochromocytoma (PC12) cells were obtained from the RIKEN Cell Bank (Ibaraki, Japan). The PC12 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma) supplemented with 5% (v/v) horse serum (Gibco BRL, Grand Island, NY, USA) and 5% (v/v) newborn calf serum (Gibco BRL) as described previously [9]. The cultures were maintained in 5% CO_2/95% humidified air at 37°C.

2.3. Treatment with PrP(106–126)

PrP(106–126) was prepared as a 10 mg/ml stock in sterile water and incubated at 37°C for 72 h, and then the solution of PrP(106–126) was diluted to the required concentration with serum-free DMEM containing 2 μM insulin.

2.4. Circular dichroism analysis of synthetic prion peptide

For circular dichroism (CD) analysis, the prion peptide was dissolved in 20 mM Tris–HCl buffer (pH 7.4). The CD spectra were baseline-corrected and smoothed by the algorithm provided by the manufacturer, and they were recorded at room temperature in a Jasco model J-720 spectropolarimeter (Jasco, Tokyo, Japan) with a cell path length of 10 mm. Five scans each of duplicate samples were measured and averaged. Ellipticity was calculated as mean residue ellipticity [θ] (degrees cm^2 $dmol^{-1}$).

2.5. Lactate dehydrogenase and WST-8 assay

The PC12 cells were seeded at 1×10^4 cells/well in 96-well plates coated with type I collagen (Becton Dickinson Labware, Bedford, MA, USA) at least 24 h before the experiment and cultured in serum-free DMEM supplemented with 2 μM insulin. PrP(106–126) was added to the cultures with or without stimulants, and the extent of cell death was assessed by measurement of the activity of lactate dehydrogenase (LDH) released from the dead cells. The LDH activity in the culture medium was determined using a commercially available kit (Wako, Osaka, Japan) according to the manufacturer's protocol. In addition to the LDH measurement in the medium, cell mortality was also assayed by WST-8 conversion [12]. A volume of 10 μl of WST-8 (5 mM WST-8, 0.2 mM 1-methoxy-5-methylphenazinium methylsulfate, and 150 mM NaCl) was added to each well and the incubation was carried out for 4 h at 37°C. The absorbance of the sample at 450 nm was measured using a microplate reader (Bio-Tek, Winooski, VT, USA) with a reference wavelength of 720 nm.

2.6. Caspase-3-like activity

The caspase-3-like activity in culture was measured with the use of an Apo-ONE™ Homogeneous Caspase-3/7 Assay Kit (Promega) according to the manufacturer's instructions. Briefly, the cells (5×10^4 cells/well) in type I collagen-coated 96-well plates (Becton Dickinson Labware) were rinsed twice with phosphate-buffered saline (PBS). The cultures were incubated with or without the indicated stimulants in DMEM (50 μl) at 37°C in an atmosphere of 95% air and 5% CO_2. The cells were lysed in 50 μl of Homogeneous Caspase-3/7 Buffer containing the caspase-3 substrate Z-DEVD-rhodamine 110, and the cell lysates were incubated for 12 h at room temperature. After incubation, the cell lysates (50 μl) were diluted with 2 ml of PBS, and the fluorescence (excitation at 480 nm and emission at 535 nm) was measured with an RF-5000 spectrofluorophotometer (Shimadzu, Tokyo, Japan).

2.7. Statistical analysis

For statistical comparisons, one-way analysis of variance with pairwise comparison by the Fisher's least significant difference procedure was used. A P value of less than 0.05 was considered significant for all analyses.

3. Results

3.1. The protective effects of PACAP27 on PrP(106–126)-induced neurocytotoxicity

CD spectral analysis of PrP(106–126), prepared at a concentration of 80 μg/ml, showed a combination of random-coil and β-sheet structures, and the aggregation properties of this PrP(106–126) were confirmed in the polyacrylamide gel electrophoresis analyses [13]. To achieve complete conformational conversion into abnormal β-sheet-rich structures that have propensities to fibrillize, PrP(106–126) was aged in an aqueous solution at a high concentration (>10 mg/ml). It is well-established that the CD spectrum of the random-coiled structure shows an intense negative band at 198 nm and a weak positive band at 220 nm, and that of the β-sheet structure indicates an intense positive band at 198 nm and a negative extremal band at 218 nm [14]. According to this interpretation, our CD spectral analysis indicated that normal PrP(106–126), which exhibited a mixture of abundant random-coil and a few β-sheet structures, was converted to the β-sheet-rich structure after aging in our conditions (Fig. 1A). Therefore, aged PrP(106–126) was used as a toxic agent throughout this investigation.

Chronic exposure of PC12 cells to PrP(106–126) resulted in a concentration-dependent decrease of cell viability assayed by WST-8, which indicated a reduction in the mitochondria of living cells (Fig. 1B). PrP(106–126) also stimulated the gradual release of cellular LDH into the culture medium (Fig. 1C), indicating the loss of cell membrane integrity in both necrotic and apoptotic cells. The increase in LDH release by PrP(106–126) was evident at 48 h after the treatment, although the control medium treatment led to a slight induction of the release of LDH. PACAP27 is believed to act as a potent neurotrophic factor in the nervous system. When PACAP27 (10^{-9} M) was administered to PC12 cells with PrP(106–126), a significant decrease of LDH release was observed. PACAP27 is well-known as a potent activator of adenylate cyclase [5], and db-cAMP, a cell-permeable cAMP analogue, mimicked the neuroprotective effect of PACAP27. PACAP27 suppressed PrP(106–126)-induced cell death at concentrations as low as 10^{-15} M, and this cytoprotective effect was diminished at 10^{-7} M, providing a bell-shaped dose dependence (Fig. 1D). Incubation of PC12 cells with VIP (10^{-7} M) also attenuated the cytotoxicity of PrP(106–126), and these dose–response fashions were consistent with our previous data of the PACAP27-evoked activation of adenylate cyclase [15]. The present study revealed that PACAP27 was 10^4-fold higher than VIP in neuroprotection against PrP(106–126) cytotoxicity. In addition, PACAP was much higher than VIP in activation of adenylate cyclase, catecholamine secretion and neurite outgrowth in PC12 cells [9,10]. These results, taken together with the previous results showing the predominant expression of PAC1 receptor in PC12 cells [10], indicated that the effect of PACAP/VIP is mediated through the activation of 'PACAP-preferring' PAC1 receptors in PC12 cells. Hence, this is the reason why PACAP27 at an extremely low concentration shows significant biological activities.

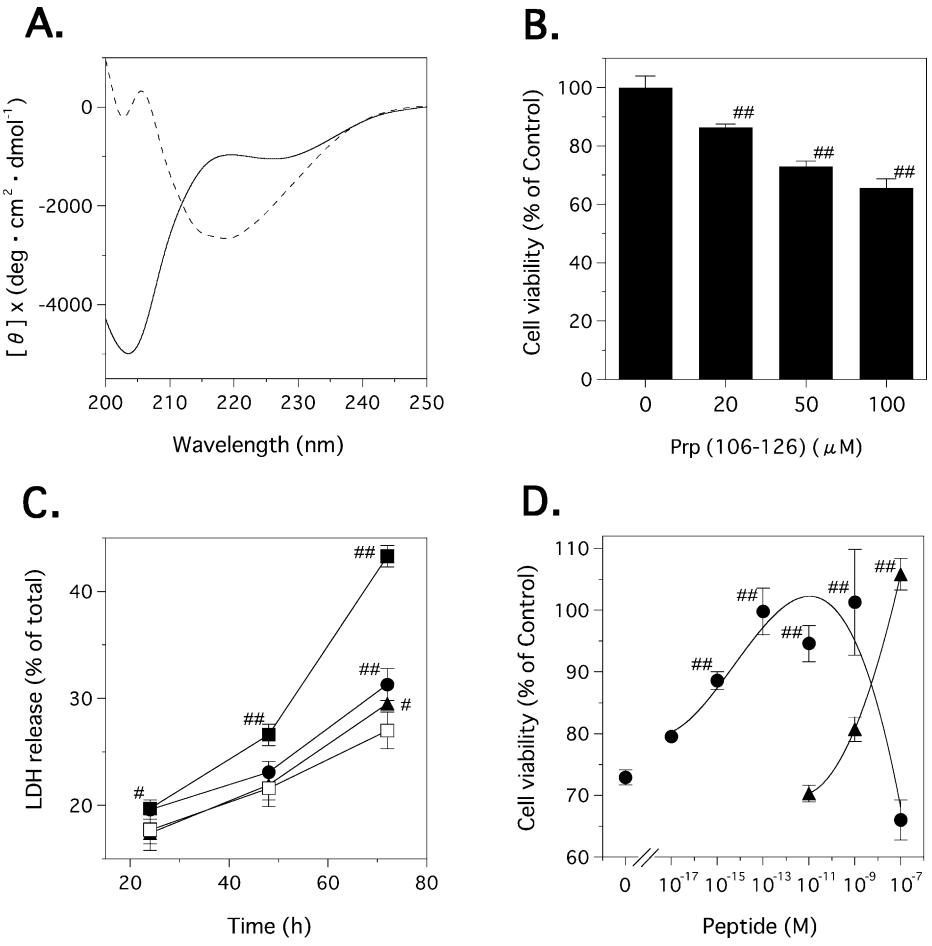

Fig. 1. PrP(106–126)-induced cytotoxicity and the effects of neuropeptides on the cell viability in PC12 cells. A: Representative CD spectra of the prion peptide in 20 mM Tris–HCl (pH 7.4). Solid line, normal PrP(106–126); dashed line, aged PrP(106–126). B: Dose-dependent neurotoxicity of PrP(106–126) measured by WST-8 assay 72 h after chronic exposure of PC12 cells. Each bar represents the means ± S.D. of four experiments. $^{\#\#}P < 0.01$ with respect to control (vehicle alone). C: PC12 cells were treated with PrP(106–126) (5×10^{-5} M) for 72 h along with dibutyryl-cAMP (10^{-4} M) or synthetic PACAP27 (10^{-9} M), and the medium was assayed by the release of LDH every 24 h. □, control (vehicle alone); ■, PrP(106–126)-treated,; ●, PrP(106–126)-treated with PACAP27; ▲, PrP(106–126)-treated with dibutyryl-cAMP. The results are the means ± S.D. of four experiments. $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ with respect to the control group. D: Dose-response curves (10^{-17}–10^{-7} M) of PACAP27 (●) or VIP (▲) on PrP(106–126) (5×10^{-5} M)-treated PC12 cells. After 72-h incubation, the cell viability was assessed by measuring the reduction of WST-8, and each value is the means ± S.D. of four experiments. $^{\#\#}P < 0.01$ with respect to the PrP(106–126)-treated group (without neuropeptides).

3.2. Signaling pathways involved in the neuroprotective effect of PACAP27

PACAP stimulates various protein kinases, including the adenylate cyclase/protein kinase A (PKA), the phospholipase C/protein kinase C (PKC) and the mitogen-activated protein (MAP) kinase pathways [7]. PACAP exerts its neuronal actions through the activation of these kinases in the nervous system. In order to clarify the possible signaling pathway involved in the neuroprotective effect of PACAP27, the effect of stimulators/inhibitors of protein kinases were examined. db-cAMP, a potent PKA stimulator [16], showed a dose-dependent neuroprotection against the toxicity of PrP(106–126) (5×10^{-5} M) (Fig. 2A). db-cAMP at the concentration of 10^{-4} M resulted in a 120% survival of PC12 cells, probably reflecting defense against naturally occurring cell death in this system. A potent PKC activator, TPA (10^{-7} M) [17], reduced the cytotoxicity of PrP(106–126), whereas TPA at higher concentrations ($>10^{-6}$ M) showed no neuroprotection due to the cytotoxicity itself (Fig. 2A). Next, when the selective PKA inhibitor, *N*-(2-[*p*-bromocinnamylamino]ethyl)-5-isoquinolinesulfonamide (H89), was added simultaneously with PrP(106–126) and PACAP27 (10^{-9} M), the protective effect of PACAP27 against PrP(106–126)-induced neurotoxicity was inhibited significantly. U0126, a specific MAP kinase inhibitor [18], caused a marked attenuation of the PACAP27-evoked neuroprotection. On the other hand, the selective PKC inhibitor, Myr-ψPKC (10^{-6} M) [19], showed no response against the effect of PACAP27 in this manipulation. Hence, the neuroprotection of PACAP27 was mediated through PKA and MAP kinase, but not through PKC signaling pathways.

3.3. Effect of PACAP27 on caspase-3-like activity

Since the activation of caspase-3 is involved in neuronal death during nervous system development and under certain pathological conditions [20], we examined the caspase-3-like activity in PC12 cells by measuring the ability of cell lysates to cleave a fluorometric caspase-3 substrate, Z-DEVD-Rhodamine 110 [21]. Cellular extracts from untreated control cul-

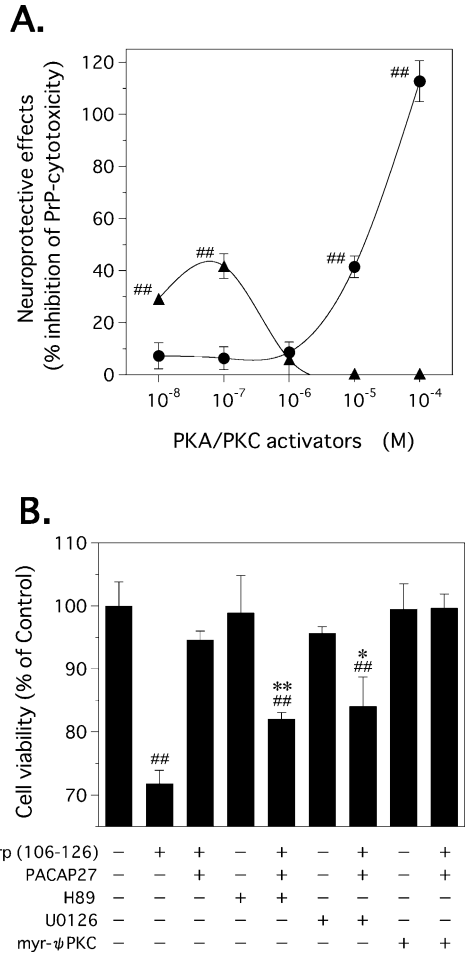

Fig. 2. Involvement of protein kinase cascades in the neuroprotective effect of PACAP27. A: Effect of PKA/PKC activator on PrP(106–126)-induced cell death in PC12 cells. The cells were cultured for 72 h with PrP(106–126) (5×10^{-5} M) in the presence or absence of db-cAMP (●) or TPA (▲) at a final concentration between 10^{-8} and 10^{-4} M. The cell viability was assessed by the reduction of WST-8. The data represent the means ± S.D. of four experiments. $^{\#\#}P<0.01$ with respect to the control group. B: Effects of the selective protein kinase inhibitors on PACAP27-evoked neuroprotection. PC12 cells were treated with PrP(106–126) (5×10^{-5} M) and PACAP27 (10^{-9} M) in the absence or presence of the protein kinase inhibitors (10^{-6} M) for 72 h, and the cell viability was assessed by WST-8 reduction analysis. The data represent the means ± S.D. of four experiments. $^{\#\#}P<0.01$ with respect to the control group. $^{**}P<0.01$ and $^{*}P<0.05$ between PACAP27-treated group with and without protein kinase inhibitor.

tures showed a slight cleavage, while the activity of the Z-DEVD-rhodamine 110 cleavage was elevated after treatment with 5×10^{-5} M PrP(106–126) (data not shown). This significant elevation in caspase-3-like activity was blocked by caspase inhibitors, including Ac-DEVD-CHO, a caspase-3-specific inhibitor [22], and Z-VAD-FMK, an irreversible inhibitor of several members of the caspase family [23] (data not shown). The activation of caspase-3 reached the maximal level at 12 h, and this elevated caspase-3 activity decreased gradually to the same level as control at 48 h (Fig. 3A). At 12 h incubation, PACAP27, at the concentrations between 10^{-13} and 10^{-7} M, significantly deactivated the PrP(106–126)-evoked caspase-3 activity up to 60% inhibition, producing a dose-dependent curve (Fig. 3B). db-cAMP (10^{-4} M) mimicked this effect of PACAP27 (data not shown). Although these data suggested the involvement of deactivation of caspase-3 in the neuroprotective effect of PACAP27, the addition of Ac-DEVD-CHO resulted in partial inhibition (26%) of the PrP(106–126)-induced cell death (Fig. 3C). Z-VAD-FMK (10^{-4} M) prevented cell death up to 77% inhibition after treatment with PrP(106–126) (5×10^{-5} M). These findings indicated that at least caspase-3 was involved in the PrP(106–126)-induced neuronal apoptosis, but other signaling pathways may be linked to the neuroprotection of PACAP27.

4. Discussion

Prion diseases are associated with conformational changes of the prion protein into an abnormal β-sheet-rich structure, which often has a tendency to accumulate as amyloid. The structure of PrP(106–126) is also variable depending on the conditions used [13]. Here, we demonstrated the marked conversion of PrP(106–126) into the β-sheet-rich structure by aging at a high concentration, which showed neurotoxicity in neuron-like PC12 cells. In addition, we demonstrated the neuroprotective effect of PACAP27 on PrP(106–126)-induced neuronal cell death. The neuroprotection of PACAP27 was significantly effective at extremely low concentrations (10^{-15} M). The PACAP-related neuropeptide, VIP, also showed a similar neuroprotective effect only at the higher concentrations ($>10^{-9}$ M). PACAP/VIP are known to activate adenylate cyclase, leading to increased levels of cAMP. With respect to the effect of PACAP/VIP on cAMP production in PC12 cells, these two neuropeptides showed similar dose-response curves in terms of the neuroprotective effect, suggesting that they exerted their neuronal action through cAMP production [15]. In our investigation, db-cAMP (10^{-4} M) mimicked the effect of PACAP27 (10^{-9} M), and the PKA inhibitor, H89, prevented the neuroprotective effect of PACAP27. In addition to the PKA signaling pathway, MAP kinase is a key regulatory enzyme in the final common signaling pathway for PACAP27-induced cellular proliferation, differentiation and neuroprotection in PC12 cells, and this kinase is activated by PKA followed by the activation of adenylate cyclase [24]. We showed that the MAP kinase inhibitor, U0126, attenuated the PACAP-evoked neuroprotection in PC12 cells. Hence, the activation of MAP kinase by PKA is likely to be involved in the signaling pathways for the neuroprotective effect of PACAP27 against PrP(106–126)-induced cytotoxicity.

There are at least three specific receptors for PACAP/VIP, such as PAC1 for PACAP, VPAC1 for VIP, and VPAC2 for helodermin [7]. PACAP27 and VIP display similar affinities for VPAC1 and 2 receptors, whereas the binding activity of PACAP toward PAC1 receptors is 10^3-10^4-fold higher than VIP. With respect to the expression of PACAP/VIP receptors in PC12 cells, an RT-PCR experiment clearly indicated the dominant expression of the PAC1 receptor [10]. This result was consistent with the present data showing that PACAP27 was more potent than VIP in the stimulation of adenylate cyclase and the neuroprotection against the cytotoxicity of PrP(106–126) in PC12 cells. As well as the activation of adenylate cyclase, PACAP27 stimulates the accumulation of intracellular calcium mediated via the PAC1 receptor, resulting in the increase of inositol phosphates in neuronal cells [7]. We demonstrated that low concentrations of TPA, a PKC activator, provoked the inhibition of PrP(106–126)-induced cell death. On the contrary, Myr-ψPKC, a potent PKC inhibitor,

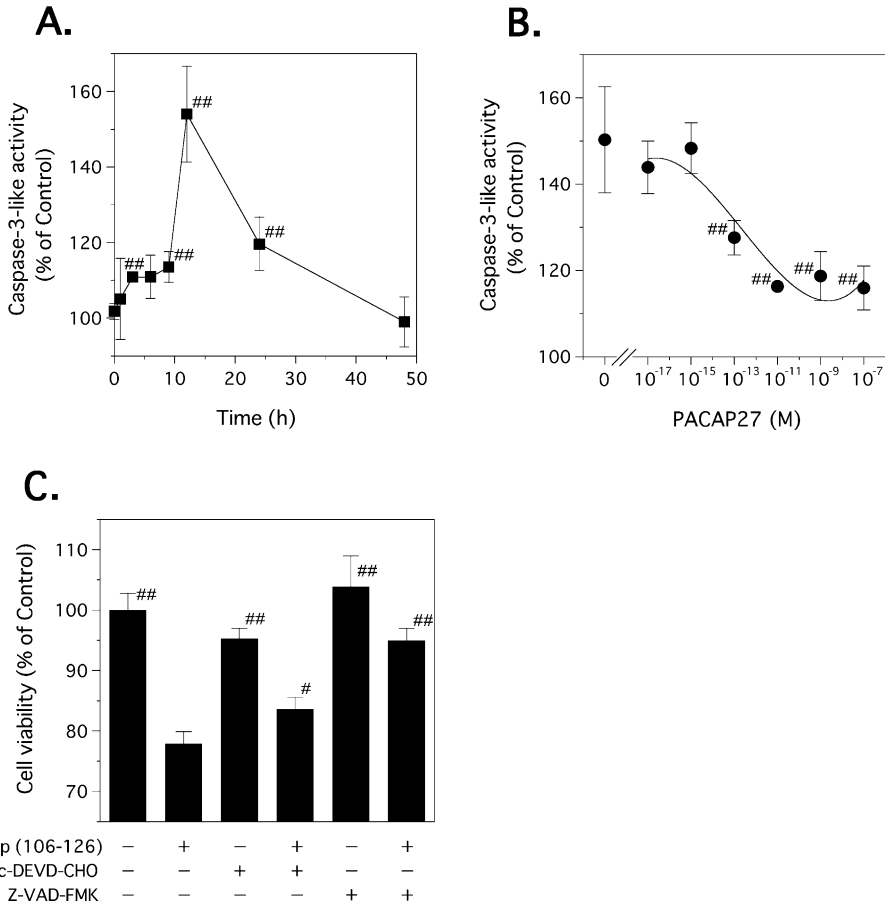

Fig. 3. Effects of PACAP27 on PrP(106–126)-evoked caspase-3 activity. A: PC12 cells were treated with PrP(106–126) (5×10^{-5} M) and lysed at the indicated period. Caspase-3-like activity in the cell lysate was determined fluorometrically using Z-DEVD-Rhodamine 110. The data are expressed as percentage of the control value (means ± S.D. of four experiments). $^{**}P<0.01$ with respect to the control group. B: Effect of graded concentration of PACAP27 on PrP(106–126)-stimulated caspase-3-like activity. PC12 cells were exposed for 12 h to PrP(106–126) (5×10^{-5} M) and PACAP27 at the indicated concentration, followed by measurement of the caspase-3-like activity. Each value is the means ± S.D. of four experiments. $^{**}P<0.01$ with respect to the PrP(106–126)-treated group. C: Effects of caspase inhibitors on cell viability in PrP(106–126)-treated cultures. PC12 cells were treated with PrP(106–126) (5×10^{-5} M) for 72 h with or without the caspase inhibitors Ac-DEVD-CHO (10^{-4} M) or Z-VAD-FMK (10^{-4} M). The cell viability was assessed by WST-8 reduction analysis, and each bar represents the means ± S.D. of four experiments. $^{**}P<0.01$ and $^{*}P<0.05$ with respect to the PrP(106–126)-treated group.

failed to suppress the effect of PACAP27 against the cytotoxicity of the PrP fragment. These data suggested that the effect of PACAP27 on PrP(106–126)-induced cell death in PC12 cells is mediated through the PAC1 receptor, followed by activation of the PKA and MAP kinase signaling pathways, but not through the PKC signaling pathway.

Apoptosis is a physiologically important cellular suicide pathway, and there is some evidence to indicate that the mechanism of neuronal cell death in prion disease is apoptosis, as apoptotic neurons have been observed in the brain of scrapie-infected sheep [25]. Apoptosis is mediated by the family of cysteine, aspartyl-specific proteases known as caspases, and the activation of caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis [20]. In this study, PrP(106–126) activated the basal caspase-3-like activity up to 155% at 12 h after PrP(106–126) challenge, and then this elevated activity decreased gradually to the same level as the control at 48 h. LDH release from PrP(106–126)-treated PC12 cells occurred after 48-h incubation. This result indicated that caspase-3 is activated prior to the loss of membrane integrity. The addition of PACAP27 ($>10^{-13}$ M) resulted in a significant decrease of catalytic activity of caspase-3, suggesting that PACAP27 acted as an anti-apoptotic agent through the deactivation of caspase-3. A caspase-3-specific inhibitor slightly deactivated the caspase-3 in PrP(106–126)-stimulated PC12 cells to increase the cell viability, while the broad-spectrum caspase inhibitor, Z-VAD-FMK, showed both clear prevention of caspase-activation and cell death. Other groups have also reported that the inhibition of caspase-3 was insufficient to prevent PrP(106–126)-induced neuronal cell death [26]. Taken together, these results suggest that there is activation of critical cytotoxic factors upstream of caspase activation, and that PACAP27 inhibits the neurotoxicity of PrP(106–126) through caspase-3-dependent and independent pathways.

In conclusion, the present study demonstrated, for the first time, that PACAP27 acts as a neuroprotective agent against PrP(106–126)-induced cell death. PACAP27 activates both the PKA and MAP kinase signaling pathways through the PAC1 receptor, which may lead to the rescue of apoptotic cell death induced by PrP as well as ischemia [27], glutamate [8], or β-amyloid [15].

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