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ORIGINAL ARTICLE

Alteration of the PAC1 Receptor Expression in the Basal Ganglia of MPTP-Induced Parkinsonian Macaque Monkeys

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Abstract

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a well-known neuropeptide with strong neurotrophic and neuroprotective effects. PACAP exerts its protective actions via three G protein-coupled receptors: the specific Pac1 receptor (Pac1R) and the Vpac1/Vpac2 receptors, the neuroprotective effects being mainly mediated by the Pac1R. The protective role of PACAP in models of Parkinson's disease and other neurodegenerative diseases is now well-established in both in vitro and in vivo studies. PACAP and its receptors occur in the mammalian brain, including regions associated with Parkinson's disease. PACAP receptor upregulation or downregulation has been reported in several injury models or human diseases, but no data are available on alterations of receptor expression in Parkinson's disease. The model closest to the human disease is the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced macaque model. Therefore, our present aim was to evaluate changes in Pac1R expression in basal ganglia related to Parkinson's disease in a macaque model. Monkeys were rendered parkinsonian with MPTP, and striatum, pallidum, and cortex were evaluated for Pac1R immunostaining. We found that Pac1R immunosignal was markedly reduced in the caudate nucleus, putamen, and internal and external parts of the globus pallidus, while the immunoreactivity remained unchanged in the cortex of MPTP-treated parkinsonian monkey brains. This decrease was attenuated in some brain areas in monkeys treated with L-DOPA. The strong, specific decrease of the PACAP receptor immunosignal in the basal ganglia of parkinsonian macaque monkey brains suggests that the PACAP/Pac1R system may play an important role in the development/progression of the disease.

Keywords Parkinson's disease · PACAP · Caudate · Putamen · Pallidum · Cortex

Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a well-known neuropeptide with strong neurotrophic and neuroprotective effects (Vaudry et al. 2009). The first described action of the peptide was its stimulatory effect on adenylate cyclase in the pituitary, but later dozens of other physiological effects have been attributed to the peptide. PACAP is phylogenetically conserved indicating that it plays a role in basic biological processes. One such action is its strong neuroprotective effect, which has been shown in a wide range of species, from invertebrates to lower vertebrates and humans (Horvath et al. 2010; Kasica et al. 2016; Pirger et al. 2014; Racz et al. 2010; Shioda and Nakamachi 2015; Somogyvari-Vigh and Reglodi 2004, Szabadfi et al. 2016). The peptide has protective effects in vitro in various neuronal cell lines and in vivo, in different injuries, such as toxic, hypoxic/ischemic, or traumatic injuries of different parts of the central nervous system (Reglodi et al. 2011; Tamas et al. 2012; Shioda and Nakamachi 2015; Lee and Seo 2014). PACAP exerts its protective actions via three G protein-coupled receptors: the specific Pac1 receptor (Pac1R) and the Vpac1/Vpac2 receptors, which also bind vasoactive intestinal peptide (VIP), the peptide with closest structural similarity to PACAP. Although VIP

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is also known to exert several neuroprotective effects, including actions in Parkinson's disease (Yelkenli et al. 2016), several lines of evidence have shown that the neuroprotective effects are mainly mediated by Pac1R, through which PACAP initiates antiapoptotic signaling (Vaudry et al. 2009; Vaczy et al. 2016).

PACAP and its receptors occur in the mammalian brain, including regions associated with Parkinson's disease. PACAP and its Pac1R have been detected in the basal ganglia of rodent, monkey, and human brains (Jolivel et al. 2009; Joo et al. 2004; Palkovits et al. 1995; Vaudry et al. 2009). The protective role of PACAP in models of Parkinson's disease and other neurodegenerative diseases is now well-established (Reglodi et al. 2017; Lee and Seo 2014; Yang et al. 2015). In vitro studies have shown that PACAP protects mesencephalic dopaminergic neurons and dopaminergic cells of other origin against several stimuli, such as 6-hydroxydopamine (6-OHDA) (Takei et al. 1998), salsolinol (Brown et al. 2013), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Deguil et al. 2007; Lamine et al. 2016), inflammatory environment (Brown et al. 2014), ethanol (Manavalan et al. 2017), nicotine (Manavalan et al. 2017), and rotenone (Wang et al. 2005). Similarly, in vivo studies have also confirmed the efficacy of PACAP in different models of Parkinson's disease. Our research group was the first to show this effect in a 6-OHDA rat model of Parkinson's disease (Reglodi et al. 2004, 2006a, b). Subsequent studies have confirmed this action on other models, such as MPTP-induced model of Parkinson's disease in mice (Wang et al. 2008; Deguil et al. 2010; Lamine et al. 2016), in addition to other mouse models of the disease induced by methamphetamine and inflammation (Shivers et al. 2014; Guillot et al. 2008).

Endogenous PACAP has also been suggested to have protective effects in neurodegenerative diseases. It is well-known that dopaminergic neurons in the substantia nigra pars compacta are more vulnerable than those in the ventral tegmental area. Low level of endogenous PACAP has been suggested as one of the possible factors for this vulnerability, since gene expression for PACAP is markedly higher in the human ventral tegmental area (Chung et al. 2005). Experimental evidence also shows that mice lacking PACAP have higher vulnerability to different harmful stimuli (Reglodi et al. 2012), including toxic agents causing neurodegeneration. For example, PACAP knockout mice react with increased sensitivity to low levels of paraquat leading to no toxic damage in wild-type mice, while causing dopaminergic neuronal loss in PACAP knockout mice (Watson et al. 2013). The increased sensitivity of the aging brain has also been associated with PACAP signaling, as expression of PACAP declines in aging brain (Banki et al. 2015; Tripathy et al. 2012), and more so in chronic pathological conditions, like Alzheimer's disease (Han et al. 2014). Although there are only sporadic studies on PACAP and Pac1R expression in the aging brain, some results point to the possibility of a compensatory Pac1R upregulation in aging rat brain (Lee et al. 2010) and under challenged conditions, like ischemia and mild cognitive impairment (Gillardon et al. 1998; Han et al. 2015).

In Parkinson's disease, we have no information about Pac1R expression. The model closest to the human Parkinson's disease is the MPTP-induced macaque model (Barcia et al. 2013; Segura-Aguilar and Kostrzewa 2015). Therefore, in order to increase the translational value of the experimental data supporting the role of PACAP in neurodegenerative diseases, the aim of the present study was to evaluate changes in Pac1R expression in basal ganglia related to Parkinson's disease in a parkinsonian macaque model. Furthermore, the effects of L-DOPA treatment on Pac1R expression were also tested in an experimental paradigm known to ameliorate the biochemical and behavioral consequences of MPTP treatment (Herrero et al. 1995, 1996a, b).

Materials and Methods

Animals

Experiments were performed with 12 adult male cynomolgus monkeys (*Macaca fascicularis*, 3.8–4.5 kg) (purchased from R.C. Hartelust BV, The Netherlands). Three monkeys remained as control subjects, and the other nine were rendered parkinsonian by MPTP-hydrochloride intoxication (Sigma, 0.3–0.4 mg/kg i.v. for 6 months, 1 injection every 2 weeks) as previously described (Barcia et al. 2005). After reaching a stable parkinsonism, six monkeys were treated daily with Madopar® (Roche, 100 mg/kg levo-DOPA (L-DOPA) and 25 mg/kg benserazide; ratio 4:1) (termed L-DOPA hereafter) for 4 months until they developed stable and moderate–severe L-DOPA-induced dyskinesias (Kastner et al. 1994). Monkeys were supervised by veterinarians and technicians skilled in the health care and maintenance of non-human primates. The animals were housed in primate cages under controlled conditions of humidity, light, and temperature (Masuri primate diet; Scientific Dietary Services, UK); fresh fruit and water were available ad libitum. All studies were approved by the Ethical Committee of the University of Murcia and carried out in accordance with the guidelines of the European Convention for the protection of Vertebrate Animals used for Experimental and other scientific purposes of the Council of Europe (no. 123, June 15th, 2006) and the European Communities Council Directive 2010/63/ECC.

Behavioral analysis was performed in order to ascertain that histological analysis is performed on animals with developed disease. The level of parkinsonism was assessed with a previously described motor scale which evaluates the following symptoms (Faucheux et al. 1995; Herrero et al. 1993): akinesia/bradykinesia, freezing, tremor, self-feeding, posture,

and spontaneous activity (maximum disability score, 25). Parkinsonian disability was assessed at the end of each session in order not to interfere with the assessment of levels of general activity. The degree of disability in the monkeys increased with every new injection and remained stable for months. All MPTP-treated monkeys reached similar stable parkinsonism levels displaying the typical parkinsonian posture. The intensity of dyskinesia was rated for each body segment (face, neck, trunk, arms, and legs) every 30 min using a Dyskinesia Disability Scale (maximal score of 21 points, Herrero et al. 1996a, b). The animals were placed in special observation cages for filming, and the dyskinetic score was evaluated blindly. In all MPTP-treated monkeys, the intoxication provoked rigidity, akinesia, forward-flexed posture, postural tremor, and reduced spontaneous activity (alertness, locomotion, and climbing were infrequent), but all animals could eat alone and reacted when stimulated by the examiners. The average disability score was around 15/25 corresponding to stage III of Hoehn and Yahr's disability scale for humans (Herrero et al. 1993). The effect of L-DOPA was similar in all six L-DOPA-treated monkeys, whose disability motor scores compared with their stable parkinsonian state improved.

Fixation, Tissue Collection, Sectioning

The monkeys were euthanized by a lethal injection of pentobarbital after a pre-anesthesia with an intramuscular injection of ketamine (8 mg/kg bodyweight). Brains were quickly removed, dissected, and fixed for 3 days in 4% freshly depolymerized paraformaldehyde that was dissolved in 0.1 M phosphate buffer. Tissue blocks containing the striatum and globus pallidus as well as the substantia nigra were sectioned into 40-μm-thick coronal sections at a sliding microtome (Microm, HM400). Series of sections that were regularly spaced at intervals of 1440 μm were collected. One series of sections of three monkeys per experimental group was processed for semi-quantitative Pac1R-NeuN double labeling to prove the expected neuronal identity of Pac1R immunoreactive (ir) cells. A second series was used to assess the coexistence of tyrosine–hydroxylase and Pac1R immune signals.

Semi-Quantitative Double-Label Immunofluorescence for Pac1R and NeuN

Four coronal sections per animal containing the substantia nigra, caudate nucleus, putamen, globus pallidus externus, globus pallidus internus, and the adjacent cortical areas were stained. After 4 × 15-min 0.1 M phosphate-buffered saline (PBS) washes, sections were permeabilized by 0.5% Triton X-100 (Sigma-Aldrich Kft Budapest, Hungary) in PBS and subsequently treated with 2% normal donkey serum (NDS, Jackson ImmunoResearch, Suffolk, UK) diluted in PBS for 60 min. The specimens were incubated overnight at room temperature in the cocktail of the primary antisera diluted in PBS containing 2% NDS. Pac1R antibodies were raised in rabbit against a synthetic peptide corresponding to an 18-amino acid peptide from the C-terminus of human Pac1R (Sigma, SAB2900693; lot # 42183, dilution: 1:500). To prove the neuronal identity of Pac1R-ir cells in the basal ganglia and neocortex, the well-trusted neuronal marker (Mullen et al. 1992), monoclonal mouse anti-NeuN (MAB 377, Chemicon, Temecula, CA, USA; dilution 1:250), was also used. After 2 × 15-min washes in PBS, the sections were placed into a mixture of secondary antibodies containing Cy3-conjugated donkey anti-rabbit (1:500; Jackson) and Alexa-488-labeled donkey anti-mouse (1:400, Jackson) sera for 3 h in PBS. Then, after rinses with PBS for 2 × 15 min, sections were mounted on gelatin-coated slides, air-dried, and covered with glycerol–PBS (1:1) solution.

Double-Label Immunofluorescence for Pac1R and Tyrosine–Hydroxylase

To assess the efficacy of MPTP treatment, a second series of sections described above was labeled for Pac1R and tyrosine–hydroxylase (TH). This labeling was performed as described for Pac1R and NeuN staining except that the primary antiserum cocktail contained the mouse monoclonal anti-TH antibody (1:1000, Sigma-Aldrich, Hungary, T2928, Lot#013K4838) instead of anti-NeuN.

Controls for Immunolabelings

Omission of primary or secondary antisera or their replacement by respective normal sera abolished the labeling in all cases (images not shown). The specificity of Pac1R antibody was verified by Western blot according to the homepage of the supplier: http://www.sigmaaldrich.com/catalog/product/sigma/sab2107479. Our Pac1R antiserum (Sigma-Aldrich, Hungary) used in this study gave a clear cytoplasmic signal which was clearly comparable with results of an immunolabeling performed on randomly selected sections of the same brain sample by a well-trusted "reference" rabbit antibody for Pac1R (a generous gift from Prof. S. Shioda, Showa University School of Medicine, Tokyo, Japan, Nakamachi et al. 2016; Suzuki et al. 2003).

Our monoclonal mouse NeuN antiserum (Chemicon) is a widely used marker for neurons which has been tested in multiple species (Mullen et al. 1992) including human (Richter et al. 2016) and non-human primates (Halene et al. 2016). The sensitivity and specificity of our monoclonal mouse anti-TH antibody directed against the N-terminal 40–152 amino acid fragment of rat TH has been tested earlier in Western blot analyses including monkey brain tissue (http://www.sigmaaldrich.com/catalog/product/sigma/t2928).

Microscopy and Image Analysis

Sections were digitalized using an Olympus FLUOVIEW 1000 confocal laser scanning microscope. To perform the semi-quantitation of the fluorescent signal, photon count mode was used with a confocal aperture of 80 μm. A 488- and 542-nm laser beam with 100% intensity was applied to excite Alexa 488 and Cy3, respectively. One pixel was exposed to the laser beam for 10 μs. Representative, 0.36-mm^2 areas were sequentially scanned with a ×20 lens (numeric aperture 0.75) by a 1024 × 1024 pixel resolution from the following regions: substantia nigra pars compacta, caudate nucleus, putamen, external globus pallidus, internal globus pallidus, and the internal granular and pyramidal layers of the frontal neocortex found adjacently to the basal ganglia. The fluorescent signals in our double-labeled samples were digitalized. The artificial colors red and green were chosen. Images of both channels were individually digitally stored, and after their automatic superimposition, their merged picture was also saved.

Simple manual cell counting of TH, NeuN, and Pac1R-ir perikarya was performed on four non-edited digital images of the above listed brain regions of each animal. The morphometry was performed by a skilled colleague who was blinded to the identity of sections. The Pac1R immunofluorescent signal in all examined regions and the TH immunoreactivity in the striatum were also semi-quantified by measurement of the signal density corrected for the background yielding the specific signal density (SSD) expressed in arbitrary units (Gaszner et al. 2009). For the determination of neuronal Pac1R immunoreactivity, the fluorescent signal was measured in ten selected and manually marked perikaryal regions in each non-edited confocal image. In the substantia nigra, we found in some cases less than ten Pac1R-ir perikarya in one image. In these cases, the SSD of all Pac1R-ir cells was registered and averaged, but at least two nerve cells were examined. The SSD of TH immunoreactivity was also semi-quantified in the striatum. For this purpose, in each image three rectangles of 50 × 50-pixel size were selected outside neuronal perikarya and the signal density was measured. This value was corrected for the background density measured in three manually selected areas of TH immunonegative neuronal perikarya. The signal measurement was performed by ImageJ software (version 1.37, NIH Bethesda, CA) exactly as published earlier (Kormos et al. 2016). For qualitative purposes, images were contrasted using Adobe Photoshop 7.0.1 software. For the printed version of the publication, images were grayscaled.

Statistical Analysis

Results were presented as the average of each experimental group ± standard error of the mean (SEM) for all variables. For assessment of data, one-way analysis of variance (ANOVA) was performed, after testing the normality and homogeneity of data. Post hoc comparisons were performed by Fisher's test. Statistics were performed by Statistica 8.0, (StatSoft, Tulsa, CO, USA), and the statistical threshold was set to alpha = 5% in all cases.

Results

General Considerations

The immunofluorescent labeling on the substantia nigra pars compacta, striatal areas, and the divisions of the globus pallidus as well as the adjacent neocortical areas was successful as we found clear Pac1R immunolabeled somata in all examined areas. To prove the neuronal identity of Pac1R immunopositive cells, we used the nerve cell marker NeuN. Our double labeling unequivocally supported that the Pac1R immunopositive perikarya in all the examined areas belong to neuronal somata (Figs. 3, 4, 5, 6, and 7).

The quantitation of NeuN-labeled perikarya revealed no significant change of the count of neuronal cell bodies in the caudate nucleus (one-way ANOVA, main effect of treatment: $F_{2,9} = 1.62$; $p = 0.25$), putamen ($F_{2,9} = 2.19$; $p = 0.16$), globus pallidus externus (ANOVA: $F_{2,9} = 1.01$; $p = 0.41$), globus pallidus internus ($F_{2,9} = 3.30$; $p = 0.09$), and neocortex (ANOVA: $F_{2,9} = 1.14$; $p = 0.36$) as demonstrated by histograms "a," in Figs. 3, 4, 5, 6, and 7, respectively.

The efficacy of our MPTP treatment was validated by the quantification of TH neurons in the substantia nigra pars compacta (Fig. 1). We detected a 68.48 ± 18.12% reduction of TH neuron count in parkinsonian brains (control vs. MPTP treatment, $p < 0.01$) which was not affected by L-DOPA administration (vs. MPTP treatment, $p = 0.33$) (Fig. 1a, ANOVA: $F_{2,9} = 20.61$, $p < 0.001$). Our double labeling allowed us to quantify the proportion of TH neurons which co-express Pac1R. Here we found that only 3.64 ± 2.30% of TH neurons carry the Pac1R in intact monkeys (Fig. 1). The comparison with MPTP- and MPTP + L-DOPA-administered groups revealed that these treatments do not affect the proportion of TH cells which co-express Pac1R (data not shown, ANOVA: $F_{2,9} = 0.69$, $p = 0.52$).

The validity of our MPTP model was further supported by our TH labeling in the striatum (Fig. 2), as we detected a 42.50 ± 0.85% ($p < 0.02$) reduction of TH immunosignal upon MPTP treatment compared to controls (ANOVA: $F_{2,9} = 9.86$, $p < 0.01$). The L-DOPA administration did not reverse the effect of MPTP ($p = 0.34$). The double labeling allowed us to show that the TH-ir nerve fibers are

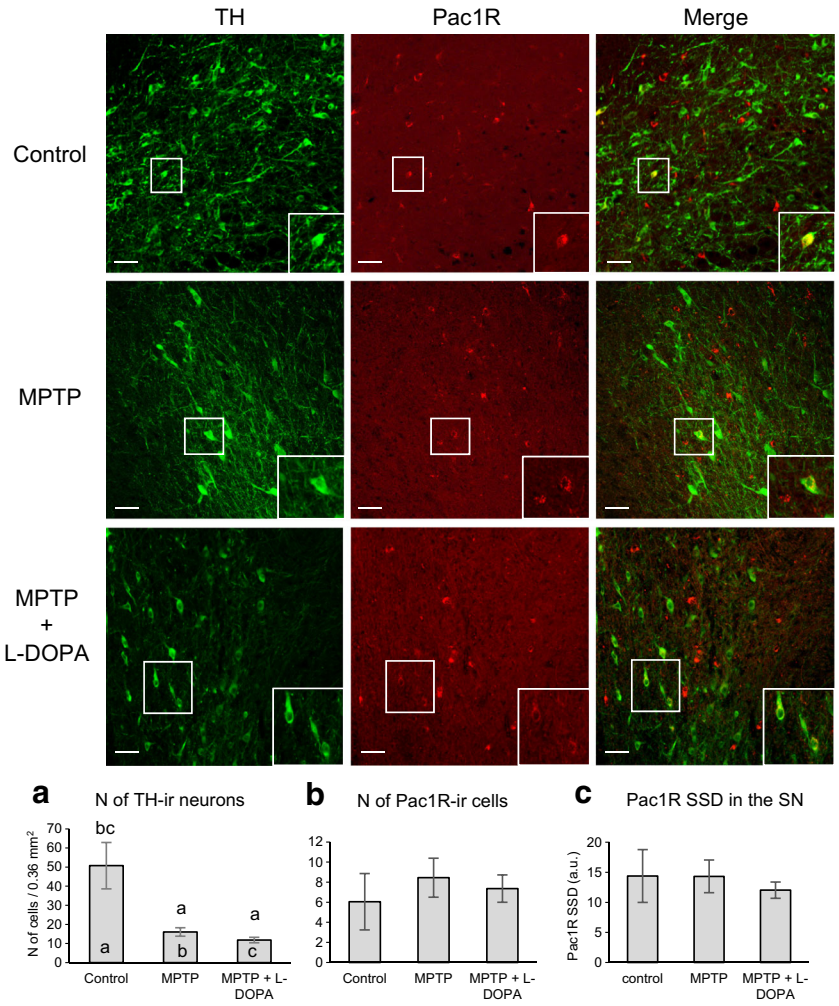

Fig. 1 Representative images of tyrosine–hydroxylase (TH), Pac1R immunoreactivities, and their overlay in the substantia nigra (SN) pars compacta of control macaque monkeys in comparison to MPTP-treated parkinsonian subjects and MPTP-administered animals which received levo-DOPA (L-DOPA) medication also. Note that the TH (green) immunosignal is reduced in MPTP- and MPTP + L-DOPA-treated animals. The Pac1R immunoreactivity (red) was observed in a low proportion of TH neurons as indicated by white boxes and the corresponding higher-magnification insets in the right-bottom corner of the respective images. **a** The number of TH neurons in the photographed area. Lettering at the bottom and top of bars in the histograms shows the significant differences between pairs of groups according to Fisher's post hoc test upon one-way analysis of variance ($p < 0.05$). **b** The number (N) of Pac1R receptor immunoreactive (ir) cells per image in the SN. **c** A comparison of Pac1R-specific signal density (SSD) in the SN expressed in arbitrary units (a.u.). Bars 50 μm (color figure online)

juxtaposed to the Pac1R-ir neurons suggesting possible synaptic connectivity between dopaminergic fibers and Pac1R-ir cells (see inset representing the control group in Fig. 2).

Both simple manual cell counting and the measurement of Pac1R SSD was performed to test whether MPTP treatment with or without L-DOPA co-administration affects the occurrence of Pac1R with following results.

Caudate Nucleus

Neuronal perikarya in the head of the caudate nucleus were found to express Pac1R (Fig. 3). The comparison of the number of Pac1R-expressing NeuN cell bodies revealed that the effect of treatment did not reach statistical significance (one-way ANOVA: $F_{2,9} = 3.64$; $p = 0.07$, Fig. 3b).

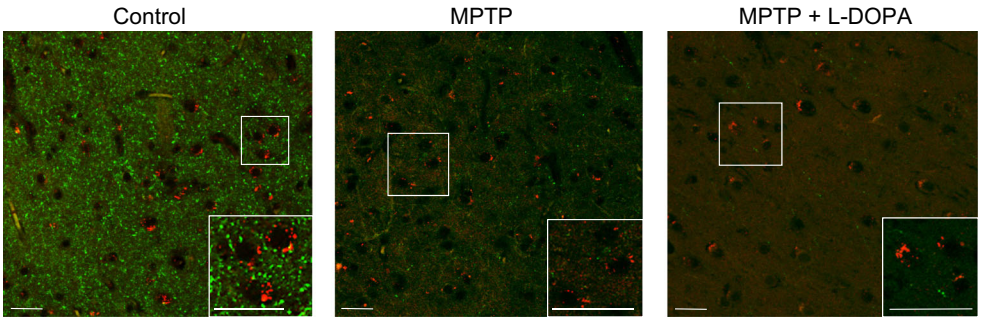

Fig. 2 Representative images of tyrosine–hydroxylase (TH) and Pac1R immunoreactivity overlay in the striatum of control macaque monkeys in comparison to MPTP-treated parkinsonian subjects and MPTP-administered animals which received levo-DOPA (L-DOPA) medication also. Note that the TH immunoreactivity (green) is markedly reduced in MPTP- and MPTP + L-DOPA-treated animals. The juxtaposition of TH nerve fibers and Pac1R immunoreactive (ir) perikarya (red) can be observed in control samples (see also inset). Importantly, the loss of TH immunoreactive nerve fibers is accompanied by markedly reduced Pac1R expression. Bars 50 μm (color figure online)

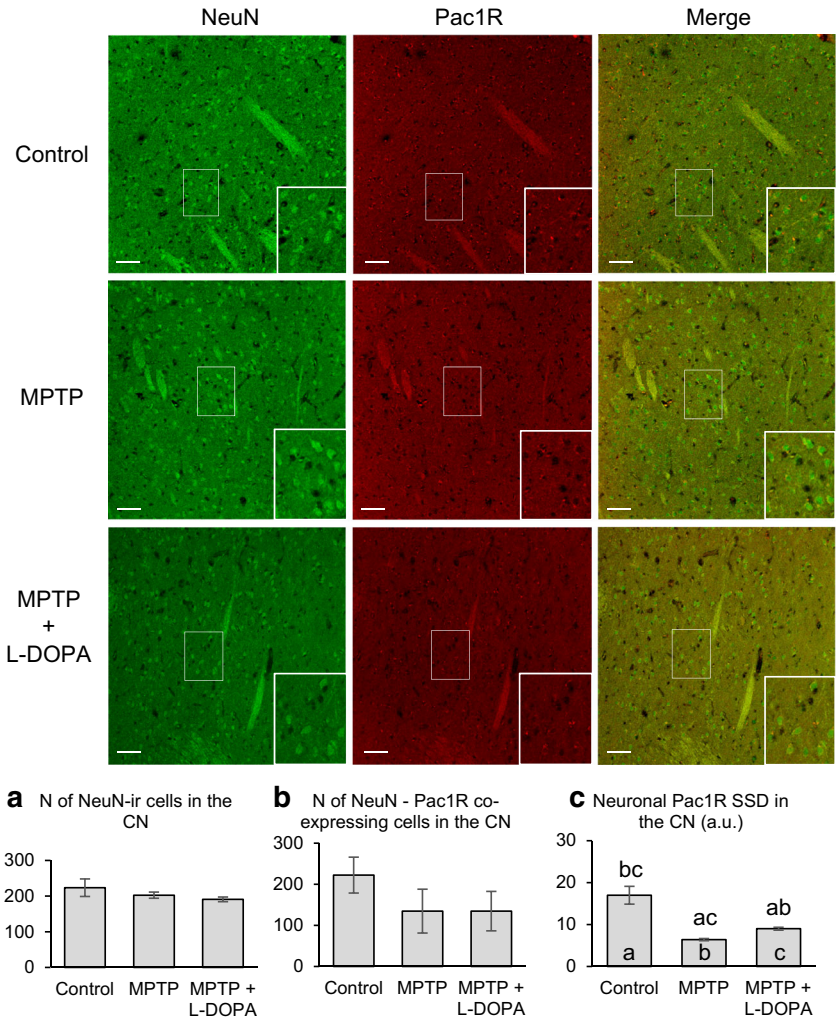

Fig. 3 Pac1R immunoreactivity in the caudate nucleus (CN) of control and MPTP- and MPTP + levo-DOPA (L-DOPA)-treated monkeys. Representative confocal laser scanning microscopic images show a 0.36-mm^2 area of the CN. The neuronal marker NeuN (green), the Pac1R (red) immunoreactivities, and their overlay (merge) are demonstrated. Areas marked by white boxes are shown in high-magnification insets at the right bottom corner of the respective image. **a** Comparison on the number (*N*) of NeuN immunoreactive (ir) cells. **b** The number of Pac1R-ir cells with NeuN immunosignal in the photographed areas within the CN. **c** The result of the comparisons of Pac1R-specific signal density (SSD) in NeuN cells of the CN expressed in arbitrary units (a.u.). Lettering at the bottom and top of bars in **c** shows the significant differences between pairs of groups according to Fisher's post hoc test upon one-way analysis of variance ($p < 0.05$). (For printing, images were grayscaled.) Bars 50 μm (color figure online)

The semi-quantitation of Pac1R SSD in NeuN perikarya revealed that the treatment effectively influenced Pac1R immunoreactivity ($F_{2,9} = 34.38$; $p = 0.0001$). Monkeys both upon MPTP administration ($p = 0.0001$) and upon MPTP with L-DOPA ($p = 0.0001$) co-treatment showed strongly reduced Pac1R immunosignal density compared to controls. Co-treatment with L-DOPA caused some improvement in the Pac1R immunoreactivity, when compared to MPTP-treated animals ($p = 0.02$), but the magnitude of Pac1R immunosignal did not reach the control values (Fig. 3c).

Putamen

The Pac1R antibodies effectively bound to nerve cell bodies also in the putamen (Fig. 4). The number of Pac1R-ir neurons was significantly reduced by the treatment (Fig. 4b; $F_{2,9} = 9.48$; $p = 0.006$). The number of Pac1R-expressing NeuN cells was reduced by 34.05% ($p = 0.03$) upon MPTP treatment. L-DOPA co-administration did not reverse this effect ($p = 0.16$, Fig. 4b) Also a significant effect was detectable when the Pac1R SSDs of NeuN-ir neurons were compared ($F_{2,9} = 14.51$; $p = 0.001$), as MPTP caused a reduction by 56% ($p = 0.04$). The effect of MPTP on Pac1R SSD in the putamen was improved ($p = 0.01$) but was not completely reversed by L-DOPA administration (Fig. 4c; $p = 0.008$).

Globus Pallidus Externus

The external part of the globus pallidus contained Pac1R-ir nerve cell bodies (Fig. 5). Treatment exerted a significant effect on Pac1R-ir neuronal cell counts ($F_{2,9} = 12.28$; $p = 0.0036$). The magnitude of Pac1R-ir NeuN cell count reduction was significant upon MPTP treatment (Fig. 5b; $p = 0.006$). L-DOPA did not reverse this effect ($p = 0.47$). The comparison of Pac1R SSD values (Fig. 5c) in the globus pallidus externus revealed that the model exerted a significant effect ($F_{2,9} = 5.99$; $p = 0.03$). MPTP treatment resulted in a significant reduction of Pac1R SSD compared to controls ($p = 0.015$). L-DOPA treatment did not diminish this effect significantly ($p = 0.57$; Fig. 5c).

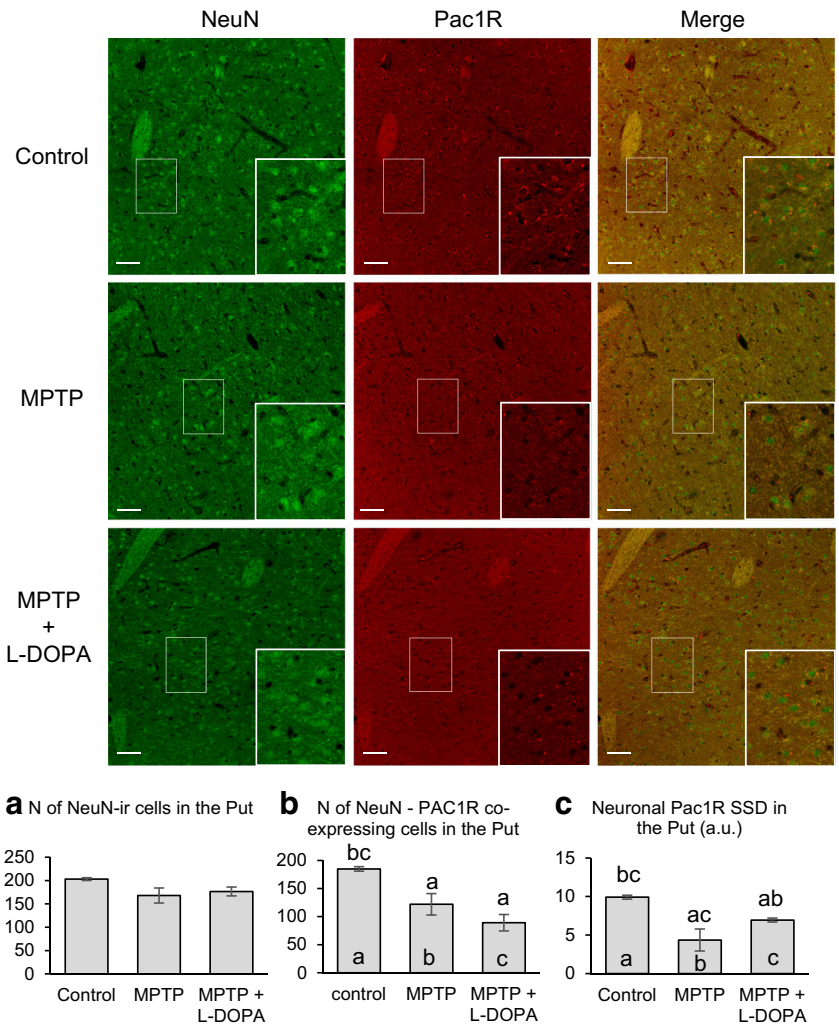

Fig. 4 Pac1R immunoreactivity in the putamen (Put) of control and MPTP- and MPTP + levo-DOPA (L-DOPA)-treated monkeys. Representative confocal laser scanning microscopic images show a 0.36-mm^2 area of the Put. The neuronal marker NeuN (green), the Pac1R (red) immunoreactivities, and their overlay (merge) are demonstrated. Areas marked by white boxes are shown in high-magnification insets at the right bottom corner of the respective image. **a** Comparison on the number (*N*) of NeuN immunoreactive (ir) cells. **b** The number of Pac1R-ir cells with NeuN immunosignal in the photographed areas within the Put. **c** The result of the comparisons of Pac1R-specific signal density (SSD) in NeuN cells of the Put expressed in arbitrary units (a.u.). Lettering at the bottom and top of bars in the histograms shows the significant differences between pairs of groups according to Fisher's post hoc test upon one-way analysis of variance ($p < 0.05$). (For printing, images were grayscaled.) Bars 50 μm (color figure online)

Globus Pallidus Internus

Pac1R immunoreactivity was also found in the neuronal perikarya of the internal part of the globus pallidus. The effect of drug administration affected the cell count of NeuN-ir neurons carrying Pac1R ($F_{2,9} = 6.79$; $p = 0.018$). MPTP treatment reduced the number of Pac1R-expressing NeuN cells by 49% ($p = 0.01$), the alteration of which was refractive to L-DOPA treatment ($p = 0.64$; Fig. 6b). When the Pac1R SSD (Fig. 6c) was evaluated ($F_{2,9} = 12.37$; $p = 0.003$), a strong decrease was detected both upon MPTP ($p = 0.001$) and upon MPTP + L-DOPA administration ($p = 0.024$). The latter treatment caused some improvement ($p = 0.23$), but did not reverse the effect of MPTP ($p = 0.24$).

Substantia Nigra Pars Compacta

Pac1R immunoreactivity was also present in the substantia nigra pars compacta, but in comparison to the other areas only few cells showed Pac1R immunopositivity (Fig. 1). As described above, Pac1R was only occasionally found in TH neurons. Neither the Pac1R cell counts ($F_{2,9} = 0.30$; $p = 0.74$; Fig. 1b) nor the Pac1R SSD ($F_{2,9} = 0.30$; $p = 0.74$; Fig. 1c) was affected by the treatment in this area.

Neocortex

The neocortical regions in the coronal sections containing the basal ganglia were also examined to test whether the effect of MPTP on Pac1R immunoreactivity was restricted to the basal ganglia or it also affected cortical regions. The neocortical Pac1R immunosignal was assessed in the internal granular and pyramidal layers. Although we found clearly recognizable neuronal perikaryal immunosignal here (Fig. 7), we did not see any changes caused by the treatment either on the number (Fig. 7b) of Pac1R-expressing cells ($F_{2,9} = 1.14$; $p = 0.36$) or on neocortical Pac1R SSD (Fig. 7c, $F_{2,9} = 0.49$; $p = 0.62$).

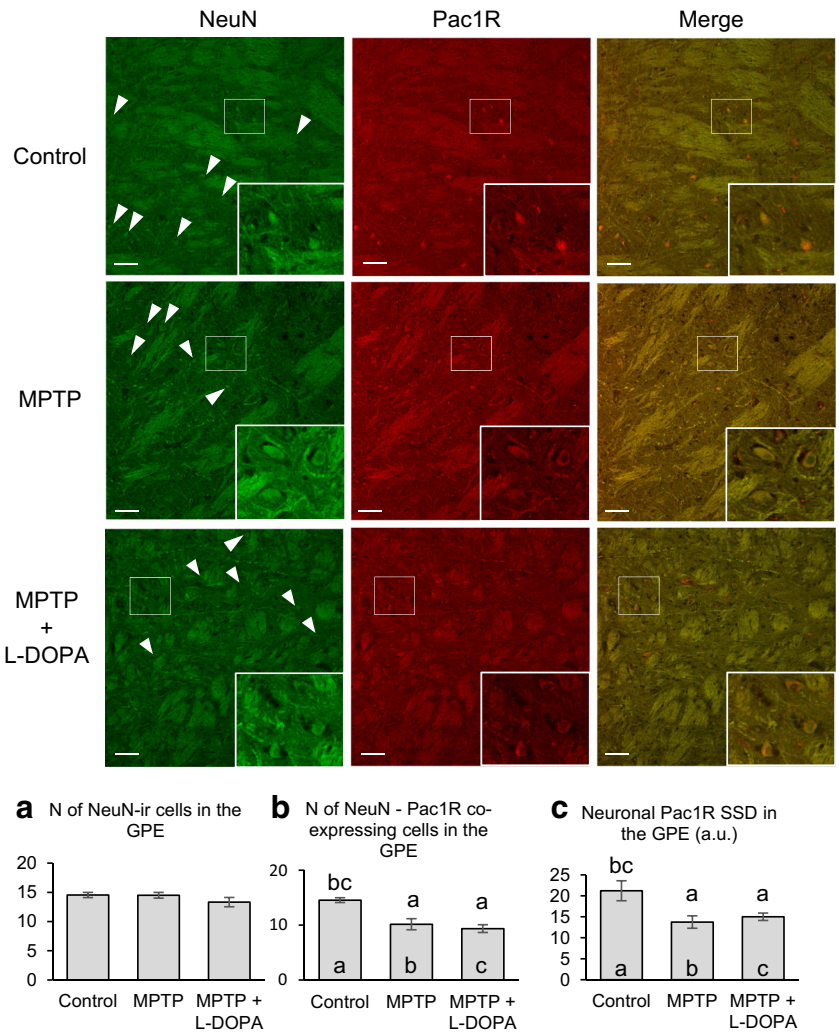

Fig. 5 Pac1R immunoreactivity in the globus pallidus externus (GPE) of control and MPTP- and MPTP + levo-DOPA (L-DOPA)-treated monkeys. Representative confocal laser scanning microscopic images show a 0.36-mm^2 area of the GPE. The neuronal marker NeuN (green), the Pac1R (red) immunoreactivities, and their overlay (merge) are demonstrated. Areas marked by white boxes are shown in high-magnification insets at the right bottom corner of the respective image. Some neuronal perikarya were highlighted by arrowheads in the images demonstrating the NeuN labeling. **a** Comparison on the number (*N*) of NeuN immunoreactive (ir) cells. **b** The number of Pac1R-ir cells with NeuN immunosignal in the photographed areas within the GPE. **c** The result of the comparisons of Pac1R-specific signal density (SSD) in NeuN cells of the GPE expressed in arbitrary units (a.u.). Lettering at the bottom and top of bars in the histograms shows the significant differences between pairs of groups according to Fisher's post hoc test upon one-way analysis of variance ($p < 0.05$). (For printing, images were grayscaled.) Bars 50 μm (color figure online)

Discussion

In the present study, we found that the immunoreactivity for a specific PACAP receptor, Pac1R, was strongly decreased in the basal ganglia, while the fluorescence intensity remained unchanged in the cortex and substantia nigra of MPTP-treated parkinsonian monkey brains. This decrease was slightly attenuated in some brain areas in monkeys treated with L-DOPA.

In spite of the close relationship and translational value of the monkey brain, little is known about the presence and effects of PACAP in the monkey central nervous system. PACAP binding sites have been previously demonstrated in the brain of macaque monkeys (Jolivel et al. 2009). Brain areas involved in the pathogenesis of Parkinson's disease have been shown to express PACAP receptors at moderate to strong levels. Ligand displacement measurements have revealed that these binding sites most probably respond to the specific Pac1R binding sites, since they could be displaced with PACAP, but not with VIP. Globus pallidus, caudate nucleus, and putamen have strong PACAP binding sites. In addition, several other areas show different levels of expression: cerebral cortex, hypothalamus, thalamus, and several brainstem nuclei (Jolivel et al. 2009). This distribution pattern corresponds well with findings in rodent brains (Vaudry et al. 2009; Joo et al. 2004). Moreover, it has been shown that treatment of monkey brain slices with PACAP could remarkably decrease caspase-3 activity. The authors argue that these results show the translational value of their findings, and that the well-known neuroprotective effects of PACAP in rodents (Somogyvari-Vigh and Reglodi 2004; Reglodi et al. 2011; Shioda and Nakamachi 2015) might be extrapolated to humans, since the same degree of neuroprotection was found in monkey brain slices as earlier in rodent brain slices (Jolivel et al. 2009). These findings also highlight the importance of our present observations regarding the Pac1R expression in parkinsonian macaque brains.

Our present results show that the number of Pac1R immunoreactive cells was significantly decreased in the caudate nucleus and in the globus pallidus externus and internus in parkinsonian brains. As shown by our NeuN results, this is

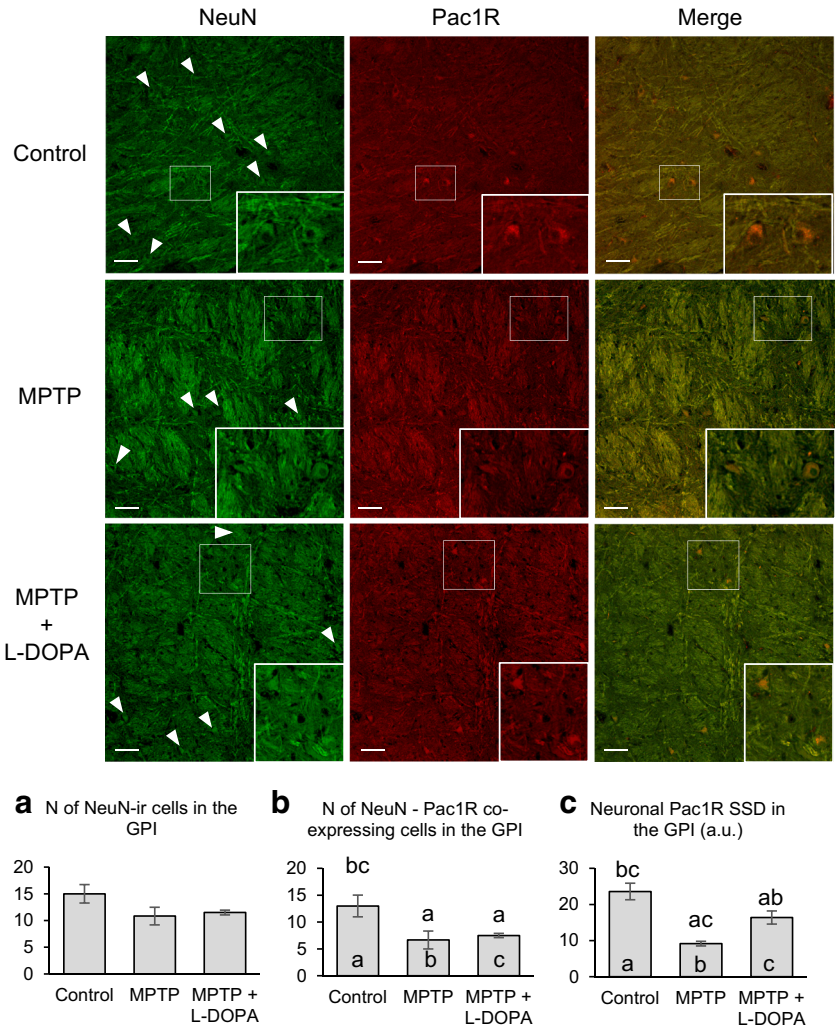

Fig. 6 Pac1R immunoreactivity in the globus pallidus internus (GPI) of control and MPTP- and MPTP + levo-DOPA (L-DOPA)-treated monkeys. Representative confocal laser scanning microscopic images show a 0.36-mm^2 area of the GPI. The neuronal marker NeuN (green), the Pac1R (red) immunoreactivities, and their overlay (merge) are demonstrated. Areas marked by white boxes are shown in high-magnification insets at the right bottom corner of the respective image. Some neuronal perikarya were highlighted by arrowheads in the images demonstrating the NeuN labeling. **a** Comparison on the number (*N*) of NeuN mmunoreactive (ir) cells. **b** The number of Pac1R-ir cells with NeuN immunosignal in the photographed areas within the GPE. **c** The result of the comparisons of Pac1R-specific signal density (SSD) in NeuN cells of the GPI expressed in arbitrary units (a.u.). Lettering at the bottom and top of bars in the histograms shows the significant differences between pairs of groups according to Fisher's post hoc test upon one-way analysis of variance ($p < 0.05$). (For printing, images were grayscaled.) Bars 50 μm (color figure online)

not related to neuronal loss, but it might be explained by the reduction of the Pac1R protein content of the cells. Indeed, the density of Pac1R immunoreactivity was strongly decreased in the neurons of both the external and internal globus pallidus, in the caudate nucleus, and in the putamen of the MPTP-induced parkinsonian macaque brains. These changes seem to be specifically related to Parkinson's disease, because receptor expression remained unchanged in the neocortex, which is not directly involved in this type of neurodegeneration. The substantia nigra pars compacta plays a key role in Parkinson's disease, and one would expect that besides the telencephalic basal ganglia, the Pac1R expression would also be reduced. Surprisingly, we could not support this hypothesis in our present experiment. It seems that the Pac1R expression in the substantia nigra is not influenced by the MPTP treatment in the monkey. Our TH-Pac1R double labeling showed that only an approximately 3% fraction of TH neurons co-expresses Pac1R in the monkey. As PACAP exerts neuroprotective effects in the substantia nigra dopaminergic cells also when given directly into the substantia nigra (Reglodi et al. 2004; Maasz et al. 2017), upregulation of its receptors by PACAP treatment or receptor-independent actions can account for the protective signaling mechanism (Somogyvari-Vigh and Reglodi 2004; Doan et al. 2012). Protective effects observed in models of Parkinson's disease through intracerebroventricular or systemic PACAP administration can have an action site other than the substantia nigra (Reglodi et al. 2011, 2017).

Numerous studies have shown that the main receptor for conveying the neuroprotective effects of PACAP is the specific Pac1R (Somogyvari-Vigh and Reglodi 2004; Vaczy et al. 2016; Vaudry et al. 2009). This explains why VIP shows similar neuroprotective effects only at much higher concentrations or is not effective at all in certain models (Somogyvari-Vigh and Reglodi 2004; Szabadfi et al. 2012). Furthermore, reports with maxadilan, the specific Pac1R agonist, also support the important role of Pac1R in neuroprotection (Guo et al. 2016; Vaczy et al. 2016). In spite of this acknowledged role of the Pac1R in the PACAP-induced neuroprotection and several other effects of the peptide, relatively little is known about

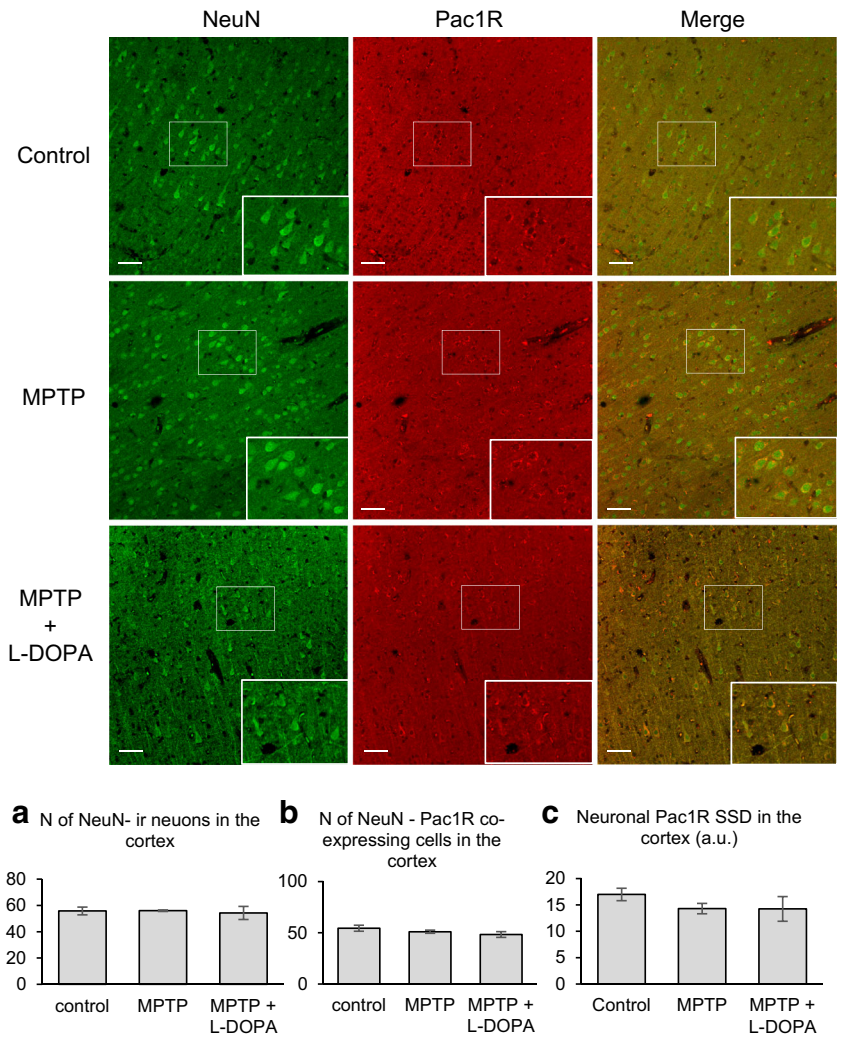

Fig. 7 Pac1R immunoreactivity in the neocortex of control and MPTP- and MPTP + levo-DOPA (L-DOPA)-treated monkeys. Representative confocal laser scanning microscopic images show a 0.36-mm^2 area of the cortex. The neuronal marker NeuN (green), the Pac1R (red) immunoreactivities, and their overlay (merge) are demonstrated. Areas marked by white boxes are shown in high-magnification insets at the right bottom corner of the respective image. **a** Comparison on the number (*N*) of NeuN immunoreactive (ir) cells. **b** The number of Pac1R-ir cells with NeuN immunosignal in the photographed cortical areas in the internal granular and internal pyramidal layers. **c** The result of the comparisons of Pac1R-specific signal density (SSD) in NeuN cells of the cortex expressed in arbitrary units (a.u.). (For printing, images were grayscaled.) Bars 50 μm (color figure online)

endogenous changes of Pac1R or the endogenous roles based on gene knockout studies. Some studies have reported Pac1R polymorphism in association with diseases, like post-traumatic stress disorder (Ressler et al. 2011). Pac1R-deficient mice have been described to show cognitive deficits (Hagino 2008), central rhythm alterations (Hannibal et al. 2016), differential expression of glutamate transporters (Zink et al. 2004), altered stress reactions (Kormos and Gaszner 2013; Mustafa et al. 2015), and decreased granule cell survival in the cerebellum (Falluel-Morel et al. 2008).

PACAP receptor upregulation or downregulation has been reported in several injury models or human diseases (Somogyvari-Vigh and Reglodi 2004). Pac1R upregulation was found in the superior frontal gyrus, middle temporal gyrus, and primary visual cortex of patients suffering in mild cognitive impairment, but not in Alzheimer's disease (Han et al. 2015). This transient upregulation of the receptor expression suggests a compensatory mechanism in mild cognitive impairment, which precedes severe dementias, including Alzheimer's disease (Han et al. 2015). This is in accordance with Pac1R upregulation of several brain areas in aged rats (Joo et al. 2004; Lee et al. 2010). The lack of upregulation in definitive Alzheimer's disease suggests that patients may lose this compensatory mechanism as the disease progresses, which might be associated with the severity of the disease symptoms (Han et al. 2014, 2015). In rats, several acute injuries lead to upregulation of the Pac1R, while receptor expression is either unchanged or downregulated in some chronic insults. For example, in diabetic retinopathy, the initial upregulation of PACAP receptors is followed by a downregulation 3 weeks later (Giunta et al. 2012). Acute hypoxic conditions are also known to induce Pac1R expression (Lam et al. 2012; Lin et al. 2015), but no change has also been reported in other hypoxic conditions, like in middle cerebral artery occlusion-induced cerebral ischemia (Stumm et al. 2007). In addition, Pac1R expression has been described to be influenced by not only its own ligand, PACAP, but also other trophic factors, like nerve growth factor, epidermal growth factor, and insulin-like growth factor (Jamen et al. 2000, 2002). No data are known about injuries and Pac1R relationship in the monkey

brain, but a most recent study has found a decrease of PACAP peptide in the aging macaque brain (Han et al. 2017).

Little is known about the relationship between Pac1R and the dopaminergic system. It has been reported in the hippocampus, where genetic blockade of the dopamine D3 receptor leads to enhanced hippocampal Pac1R expression and to altered distribution in the cortex (Marzagalli et al. 2016). However, no data are available on striatal Pac1R expression alterations in Parkinson's disease. Our present findings suggest that changes induced in the parkinsonian monkey model might be related to the severity of the disease, based on the known neuroprotective effects of PACAP in animal models of different neurodegenerative diseases. This is first supported by the observation that TH immunoreactive nerve terminals are juxtaposed to Pac1R-carrying striatal neurons in intact monkeys, which disappear upon MPTP treatment. Further experiments are required to test whether striatal Pac1R-positive cells also carry dopamine receptors. Second, L-DOPA treatment had a tendency to increase Pac1R immunoreactivity, although in some cases results were not significant. Several morphological and biochemical changes observed in parkinsonian animals can be counteracted by L-DOPA treatment, while several others cannot (Faucheux et al. 1995; Gołembiowska et al. 2009; Herrero et al. 1996a, b; Solis et al. 2016), and L-DOPA also has numerous side effects (Bortolanza et al. 2016; Ndlovu et al. 2016). As this study provides evidence at protein level that Pac1R content of the basal ganglia is decreased in parkinsonian brains, the underlying hypothetic downregulation of Pac1R mRNA transcripts requires future confirmation. Besides this, further studies are required to explore the exact role of the PACAP/Pac1R system in human Parkinson's disease, but the strong, specific decrease of the PACAP receptor immunoreactivity in the basal ganglia of parkinsonian macaque monkey brains suggests that it may play an important role in the development/progression of the disease.

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Compliance with Ethical Standards All studies were approved by the Ethical Committee of the University of Murcia and carried out in accordance with the guidelines of the European Convention for the protection of Vertebrate Animals used for Experimental and other scientific purposes of the Council of Europe (no. 123, June 15th, 2006) and the European Communities Council Directive 2010/63/ECC.

References

Banki E, Sosnowska D, Tucsek Z, Gautam T, Toth P, Tarantini S, Tamas A, Helyes Z, Reglodi D, Sonntag WE, Csiszar A, Ungvari Z (2015) Age-related decline of autocrine pituitary adenylate cyclase-activating polypeptide impairs angiogenic capacity of rat cerebromicrovascular endothelial cells. J Gerontol A Biol Sci Med Sci 70(6):665–674. https://doi.org/10.1093/gerona/glu116

Barcia C, de Pablos V, Bautista-Hernández V, Sánchez-Bahillo A, Bernal I, Fernández-Villalba E, Martín J, Bañón R, Fernández-Barreiro A, Herrero MT (2005) Increased plasma levels of TNF-alpha but not of IL1-beta in MPTP-treated monkeys one year after the MPTP administration. Parkinsonism Relat Disord 11(7):435–439. https://doi.org/10.1016/j.parkreldis.2005.05.006

Barcia C, Ros CM, Ros-Bernal F, Gomez A, Annese V, Carrillo-de Sauvage MA, Yuste JE, Campuzano CM, de Pablos V, Fernández-Villalba E, Herrero MT (2013) Persistent phagocytic characteristics of microglia in the substantia nigra of long-term parkinsonian macaques. J Neuroimmunol 261(1-2):60–66. https://doi.org/10.1016/j.jneuroim.2013.05.001

Bortolanza M, Bariotto-Dos-Santos KD, Dos-Santos-Pereira M, da Silva CA, Del-Bel E (2016) Antidyskinetic effect of 7-nitroindazole and sodium nitroprusside associated with amantadine in a rat model of Parkinson's disease. Neurotox Res 30(1):88–100. https://doi.org/10.1007/s12640-016-9618-4

Brown D, Tamas A, Reglodi D, Tizabi Y (2013) PACAP protects against salsolinol-induced toxicity in dopaminergic SH-SY5Y cells: implication for Parkinson's disease. J Mol Neurosci 50(3):600–607. https://doi.org/10.1007/s12031-013-0015-7

Brown D, Tamas A, Reglodi D, Tizabi Y (2014) PACAP protects against inflammatory-mediated toxicity in dopaminergic SH-SY5Y cells: implication for Parkinson's disease. Neurotox Res 26(3):230–239. https://doi.org/10.1007/s12640-014-9468-x

Chung YC, Seo H, Sonntag KC, Brooks A, Lin L, Isacson O (2005) Cell type-specific gene expression of midbrain dopaminergic neurons reveals molecules involved in their vulnerability and protection. Hum Mol Genet 14(13):1709–1725. https://doi.org/10.1093/hmg/ddi178

Deguil J, Jailloux D, Page G, Fauconneau B, Houeto JL, Philippe M, Muller JM, Pain S (2007) Neuroprotective effects of pituitary adenylate cyclase-activating polypeptide (PACAP) in MPP+-induced alteration of translational control in neuro-2a neuroblastoma cells. J Neurosci Res 85(9):2017–2025. https://doi.org/10.1002/jnr.21318

Deguil J, Chavant F, Lafay-Chebassier C, Pérault-Pochat MC, Fauconneau B, Pain S (2010) Neuroprotective effect of PACAP on translational control alteration and cognitive decline in MPTP parkinsonian mice. Neurotox Res 17(2):142–155. https://doi.org/10.1007/s12640-009-9091-4

Doan ND, Chatenet D, Létourneau M, Vaudry H, Vaudry D, Fournier A (2012) Receptor-independent cellular uptake of pituitary adenylate cyclase-activating polypeptide. Biochim Biophys Acta 1823(4):940–949. https://doi.org/10.1016/j.bbamcr.2012.02.001

Falluel-Morel A, Tascau LI, Sokolowksi K, Braet P, DiCicco-Bloom E (2008) Granule cell survival is deficient in PAC1−/− mutant cerebellum. J Mol Neurosci 36(1-3):38–44. https://doi.org/10.1007/s12031-008-9066-6

Faucheux BA, Herrero MT, Villares J, Levy R, Javoy-Agid F, Obeso JA, Hauw JJ, Agid Y, Hirsch EC (1995) Autoradiographic localization and density of [125I]ferrotransferrin binding sites in the basal

ganglia of control subjects, patients with Parkinson's disease and MPTP-lesioned monkeys. Brain Res 691(1-2):115–124. https://doi.org/10.1016/0006-8993(95)00629-5

Gaszner B, Van Wijk DC, Korosi A, Jozsa R, Roubos EW, Kozicz T (2009) Diurnal expression of period 2 and urocortin 1 in neurones of the non-preganglionic Edinger-Westphal nucleus in the rat. Stress 12(2):115–124. https://doi.org/10.1080/10253890802057221

Gillardon F, Hata R, Hossmann KA (1998) Delayed up-regulation of Zac1 and PACAP type I receptor after transient focal cerebral ischemia in mice. Mol Brain Res 61(1-2):207–210. https://doi.org/10.1016/S0169-328X(98)00202-2

Giunta S, Castorina A, Bucolo C, Magro G, Drago F, D'Agata V (2012) Early changes in pituitary adenylate cyclase activating peptide, vasoactive intestinal peptide and related receptors expression in retina of streptozotocin-induced diabetic rats. Peptides 37(1):32–39. https://doi.org/10.1016/j.peptides.2012.06.004

Gołembiowska K, Dziubina A, Kowalska M, Kamińska K (2009) Effect of adenosine A(2A) receptor antagonists on L-DOPA-induced hydroxyl radical formation in rat striatum. Neurotox Res 15(2):155–166. https://doi.org/10.1007/s12640-009-9016-2

Guillot TS, Richardson JR, Wang MZ, Li YJ, Taylor TN, Ciliax BJ, Zachrisson O, Mercer A, Miller GW (2008) PACAP38 increases vesicular monoamine transporter 2 (VMAT2) expression and attenuates methamphetamine toxicity. Neuropeptides 42(4):423–434. https://doi.org/10.1016/j.npep.2008.04.003

Guo X, Yu R, Xu Y, Lian R, Yu Y, Cui Z, Ji Q, Chen J, Li Z, Liu H, Chen J (2016) PAC1R agonist maxadilan enhances hADSC viability and neural differentiation potential. J Cell Mol Med 20(5):874–890. https://doi.org/10.1111/jcmm.12772

Hagino N (2008) Performance of PAC1-R heterozygous mice in memory tasks-II. J Mol Neurosci 36(1-3):208–219. https://doi.org/10.1007/s12031-008-9101-7

Halene TB, Kozlenkov A, Jiang Y, Mitchell AC, Javidfar B, Dincer A, Park R, Wiseman J, Croxson PL, Giannaris EL, Hof PR, Roussos P, Dracheva S, Hemby SE, Akbarian S (2016) NeuN+ neuronal nuclei in non-human primate prefrontal cortex and subcortical white matter after clozapine exposure. Schizophr Res 170(2-3):235–244. https://doi.org/10.1016/j.schres.2015.12.016

Han P, Liang W, Baxter LC, Yin J, Tang Z, Beach TG, Caselli RJ, Reiman EM, Shi J (2014) Pituitary adenylate cyclase-activating polypeptide is reduced in Alzheimer disease. Neurology 82(19):1724–1728. https://doi.org/10.1212/WNL.0000000000000417

Han P, Caselli RJ, Baxter L, Serrano G, Yin J, Beach TG, Reiman EM, Shi J (2015) Association of pituitary adenylate cyclase-activating polypeptide with cognitive decline in mild cognitive impairment due to Alzheimer disease. JAMA Neurol 72(3):333–339. https://doi.org/10.1001/jamaneurol.2014.3625

Han P, Nielsen M, Song M, Yin J, Permenter MR, Vogt JA, Engle JR, Dugger BN, Beach TG, Barnes CA, Shi J (2017) The impact of aging on brain pituitary adenylate cyclase activating polypeptide, pathology and cognition in mice and rhesus macaques. Front Aging Neurosci 9:180. https://doi.org/10.3389/fnagi.2017.00180

Hannibal J, Georg B, Fahrenkrug J (2016) Altered circadian food anticipatory activity rhythms in PACAP receptor 1 (PAC1) deficient mice. PLoS One 11(1):e0146981. https://doi.org/10.1371/journal.pone.0146981

Herrero MT, Perez-Otano I, Oset C, Kastner A, Hirsch EC, Agid Y, Luquin MR, Obeso JA, Del Rio J (1993) GM-1 ganglioside promotes the recovery of surviving mid-brain dopaminergic neurons in MPTP-treated monkeys. Neuroscience 56(4):965–972. https://doi.org/10.1016/0306-4522(93)90142-3

Herrero MT, Augood SJ, Hirsch EC, Javoy-Agid F, Luquin MR, Agid Y, Obeso JA, Emson PC (1995) Effects of L-DOPA on preproenkephalin and preprotachykinin gene expression in the MPTP-treated monkey striatum. Neuroscience 68(4):1189–1198. https://doi.org/10.1016/0306-4522(95)00120-8

Herrero MT, Levy R, Ruberg M, Luquin MR, Villares J, Guillen J, Faucheux B, Javoy-Agid F, Guridi J, Agid Y, Obeso JA, Hirsch EC (1996a) Consequence of nigrostriatal denervation and L-dopa therapy on the expression of glutamic acid decarboxylase messenger RNA in the pallidum. Neurology 47(1):219–224. https://doi.org/10.1212/WNL.47.1.219

Herrero MT, Augood SJ, Asensi H, Hirsch EC, Agid Y, Obeso JA, Emson PC (1996b) Effects of L-DOPA-therapy on dopamine D2 receptor mRNA expression in the striatum of MPTP-intoxicated parkinsonian monkeys. Brain Res Mol Brain Res 42(1):149–155. https://doi.org/10.1016/S0169-328X(96)00157-X

Horvath G, Reglodi D, Opper B, Brubel R, Tamas A, Kiss P, Toth G, Csernus V, Matkovits A, Racz B (2010) Effects of PACAP on the oxidative stress-induced cell death in chicken pinealocytes is influenced by the phase of the circadian clock. Neurosci Lett 484(2):148–152. https://doi.org/10.1016/j.neulet.2010.08.039

Jamen F, Laden JC, Bouschet T, Rodriguez-Henche N, Bockaert J, Brabet P (2000) Nerve growth factor upregulates the PA1 promoter by activating the MAP kinase pathway in rat PC12 cells. Ann N Y Acad Sci 921:390–394

Jamen F, Bouschet T, Laden JC, Bockaert J, Brabet P (2002) Up-regulation of the PACAP type-1 receptor (PAC1) promoter by neurotrophins in rat PC12 cells and mouse cerebellar granule cells via the Ras/mitogen-activated protein kinase cascade. J Neurochem 82(5):1199–1207

Jolivel V, Basille M, Aubert N, de Jouffrey S, Ancian P, Le Bigot JF, Noack P, Massonneau M, Fournier A, Vaudry H, Gonzalez BJ, Vaudry D (2009) Distribution and functional characterization of pituitary adenylate cyclase-activating polypeptide receptors in the brain of non-human primates. Neuroscience 160(2):434–451. https://doi.org/10.1016/j.neuroscience.2009.02.028

Joo KM, Chung YH, Kim MK, Nam RH, Lee BL, Lee KH, Cha CI (2004) Distribution of vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide receptors (VPAC1, VPAC2, and PAC1 receptor) in the rat brain. J Comp Neurol 476(4):388–413. https://doi.org/10.1002/cne.20231

Kasica N, Podlasz P, Sundvik M, Tamas A, Reglodi D, Kaleczyc J (2016) Protective effects of pituitary adenylate cyclase-activating polypeptide (PACAP) against oxidative stress in zebrafish hair cells. Neurotox Res 30(4):633–647. https://doi.org/10.1007/s12640-016-9659-8

Kastner A, Herrero MT, Hirsch EC, Guillen J, Luquin MR, Javoy-Agid F, Obeso JA, Agid Y (1994) Decreased tyrosine hydroxylase content in the dopaminergic neurons of MPTP-intoxicated monkeys: effect of levodopa and GM1 ganglioside therapy. Ann Neurol 36(2):206–214. https://doi.org/10.1002/ana.410360213

Kormos V, Gaszner B (2013) Role of neuropeptides in anxiety, stress, and depression: from animals to humans. Neuropeptides 47(6):401–419. https://doi.org/10.1016/j.npep.2013.10.014

Kormos V, Gaspar L, Kovacs LA, Farkas J, Gaszner T, Csernus V, Balogh A, Hashimoto H, Reglodi D, Helyes Z, Gaszner B (2016) Reduced response to chronic mild stress in PACAP mutant mice is associated with blunted FosB expression in limbic forebrain and brainstem centers. Neuroscience 330:335–358. https://doi.org/10.1016/j.neuroscience.2016.06.004

Lam SY, Liu Y, Liong EC, Tipoe GL, Fung ML (2012) Upregulation of pituitary adenylate cyclase activating polypeptide and its receptor expression in the rat carotid body in chronic and intermittent hypoxia. Adv Exp Med Biol 758:301–306. https://doi.org/10.1007/978-94-007-4584-1_41

Lamine A, Letourneau M, Doan ND, Maucotel J, Couvineau A, Vaudry H, Chatenet D, Vaudry D, Fournier A (2016) Characterizations of a synthetic pituitary adenylate cyclase-activating polypeptide analog displaying potent neuroprotective activity and reduced in vivo cardiovascular side effects in a Parkinson's disease model.

Neuropharmacology 108:440–450. https://doi.org/10.1016/j.neuropharm.2015.05.014

Lee EH, Seo SR (2014) Neuroprotective roles of pituitary adenylate cyclase-activating polypeptide in neurodegenerative diseases. BMB Rep 47(7):369–375. https://doi.org/10.5483/BMBRep.2014.47.7.086

Lee JC, Cho YJ, Kim J, Kim N, Kang BG, Cha CI, Joo KM (2010) Region-specific changes in the immunoreativity of vasoactive intestinal peptide and pituitary adenylate cyclase activating polypeptide reeptors VPAC2, and PAC1 receptor in the aged rats brains. Brain Res 1351:32–40. https://doi.org/10.1016/j.brainres.2010.06.048

Lin CH, Chiu L, Lee HT, Chiang CW, Liu SP, Hsu YH, Lin SZ, Hsu CY, Hsieh CH, Shyu WC (2015) PACAP38/PAC1 signaling induces bone marrow-derived cells homing to ishemic brain. Stem Cells 33(4):1153–1172. https://doi.org/10.1002/stem.1915

Maasz G, Zrinyi Z, Reglodi D, Petrovics D, Rivnyak A, Kiss T, Jungling A, Tamas A, Pirger Z (2017) Pituitary adenylate cyclase-activating polypeptide (PACAP) has neuroprotective function in dopamine-based neurodegeneration developed in rat and snail parkinsonian models. Dis Model Mech 10(2):127–139. https://doi.org/10.1242/dmm.027185

Manavalan S, Getachew B, Manaye KF, Khundmiri SJ, Csoka AB, McKinley R, Tamas A, Reglodi D, Tizabi Y (2017) PACAP protects against ethanol and nicotine toxicity in SH-SY5Y cells: implications for drinking-smoking co-morbidity. Neurotox Res 32(1):8–13. https://doi.org/10.1007/s12640-017-9727-8

Marzagalli R, Leggio GM, Bucolo C, Pricoco E, Keay KA, Cardile V, Castorina S, Salomone S, Drago F, Castorina A (2016) Genetic blockade of the dopamine D3 receptor enhancces hippocampal expression of PACAP and receptors and alters their cortical distribution. Neuroscience 316:279–295. https://doi.org/10.1016/j.neuroscience.2015.12.034

Mullen RJ, Buck CR, Smith AM (1992) NeuN, a neuronal specific nuclear protein in vertebrates. Development 116(1):201–211

Mustafa T, Jiang SZ, Eiden AM, Weihe E, Thislethwaite I, Eiden LE (2015) Impact of PACAP and PAC1 receptor deficiency on the neurochemical and ehavioral effects of acute and chronic restraint stress in male C57BL/6 mice. Stress 18(4):408–418. https://doi.org/10.3109/10253890.2015.1025044

Nakamachi T, Ohtaki H, Seki T, Yofu S, Kagami N, Hashimoto H, Shintani N, Baba A, Mark L, Lanekoff I, Kiss P, Farkas J, Reglodi D, Shioda S (2016) PACAP suppresses dry eye signs by stimulating tear secretion. Nat Commun 7:12034. https://doi.org/10.1038/ncomms12034

Ndlovu BC, Daniels WM, Mabandla MV (2016) Amelioration of L-dopa-associated dyskinesias with triterpenoic acid in a parkinsonian rat model. Neurotox Res 29(1):126–134. https://doi.org/10.1007/s12640-015-9567-3

Palkovits M, Somogyvari-Vigh A, Arimura A (1995) Concentrations of pituitary adenylate cyclase activating polypeptide (PACAP) in human brain nuclei. Brain Res 699(1):116–120. https://doi.org/10.1016/0006-8993(95)00869-R

Pirger Z, Naskar S, Laszlo Z, Kemenes G, Reglodi D, Kemenes I (2014) Reversal of age-related learning deficiency by the vertebrate PACAP and IGF-1 in a novel invertebrate model of aging: the pond snail (Lymnaea stagnalis). J Gerontol A Biol Sci Med Sci 69(11):1331–1338. https://doi.org/10.1093/gerona/glu068

Racz B, Horvath G, Reglodi D, Gasz B, Kiss P, Gallyas F Jr, Sumegi B, Toth G, Nemeth A, Lubics A, Tamas A (2010) PACAP ameliorates oxidative stress in the chicken inner ear: an in vitro study. Regul Pept 160(1-3):91–98. https://doi.org/10.1016/j.regpep.2009.12.003

Reglodi D, Lubics A, Tamas A, Szalontay L, Lengvari I (2004) Pituitary adenylate cyclase activating polypeptide protects dopaminergic neurons and improves behavioral deficits in a rat model of Parkinson's disease. Behav Brain Res 151(1-2):303–312. https://doi.org/10.1016/j.bbr.2003.09.007

Reglodi D, Lubics A, Kiss P, Lengvari I, Gaszner B, Toth G, Hegyi O, Tamas A (2006a) Effect of PACAP in 6-OHDA-induced injury of the substantia nigra in intact young and ovariectomized female rats. Neuropeptides 40(4):265–274. https://doi.org/10.1016/j.npep.2006.06.001

Reglodi D, Tamas A, Lengvari I, Toth G, Szalontay L, Lubics A (2006b) Comparative study on the effects of PACAP in young, aging, and castrated males in a rat model of Parkinson's disease. Ann N Y Acad Sci 1070(1):518–524. https://doi.org/10.1196/annals.1317.072

Reglodi D, Kiss P, Lubics A, Tamas A (2011) Review of the protective effects of PACAP in models of neurodegenerative diseases in vitro and in vivo. Curr Pharm Des 17(10):962–972. https://doi.org/10.2174/138161211795589355

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, Zs H, Welke L, Lakatos A, Tamas A (2012) PACAP is an endogenous protective factor—insights from PACAP deficient mice. J Mol Neurosci 48(3):482–492. https://doi.org/10.1007/s12031-012-9762-0

Reglodi D, Renaud J, Tamas A, Tizabi Y, Socías SB, Del-Bel E, Raisman-Vozari R (2017) Novel tactics for neuroprotection in Parkinson's disease: role of antibiotics, polyphenols and neuropeptides. Prog Neurobiol 155:120–148. https://doi.org/10.1016/j.pneurobio.2015.10.004

Ressler KJ, Mercer KB, Bradley B, Jovanovic T, Mahan A, Kerley K, Norrholm SD, Kilaru V, Smith AK, Myers AJ, Ramirez M, Engel A, Hammack SE, Toufexis D, Braas KM, Binder EB, May V (2011) Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. Nature 470(7335):492–497. https://doi.org/10.1038/nature09856

Richter Z, Janszky J, Sétáló G Jr, Horváth R, Horváth Z, Dóczi T, Seress L, Ábrahám H (2016) Characterization of neurons in the cortical white matter in human temporal lobe epilepsy. Neuroscience 333:140–150. https://doi.org/10.1016/j.neuroscience.2016.07.011

Segura-Aguilar J, Kostrzewa RM (2015) Neurotoxin mechanisms and processes relevant to Parkinson's disease: an update. Neurotox Res 27(3):328–354. https://doi.org/10.1007/s12640-015-9519-y

Shioda S, Nakamachi T (2015) PACAP as a neuroprotective factor in ischemic neuronal injuries. Peptides 72:202–207. https://doi.org/10.1016/j.peptides.2015.08.006

Shivers KY, Nikolopoulou A, Machlovi SI, Vallabhajosula S, Figueiredo-Pereira ME (2014) PACAP27 prevents Parkinson-like neuronal loss and motor deficits but not microglia activation induced by prostaglandin J2. Biochim Biophys Acta 1842(9):1707–1719. https://doi.org/10.1016/j.bbadis.2014.06.020

Solis O, García-Sanz P, Herranz AS, Asensio MJ, Moratalla R (2016) L-DOPA reverses the increased free amino acids tissue levels induced by dopamine depletion and rises GABA and tyrosine in the striatum. Neurotox Res 30(1):67–75. https://doi.org/10.1007/s12640-016-9612-x

Somogyvari-Vigh A, Reglodi D (2004) Pituitary adenylate cyclase activating polypeptide: a potential neuroprotective peptide. Review. Curr Pharm Des 10(23):2861–2889. https://doi.org/10.2174/1381612043383548

Stumm R, Kolodziej A, Prinz V, Endres M, Wu DF, Hollt V (2007) Pituitary adenylate cyclase activating polypeptide is up-regulated in cortical pyramidal cells after focal ischemia and protects neurons from hypoxia/ischemic damage. J Neurochem 103(4):1666–1681. https://doi.org/10.1111/j.1471-4159.2007.04895.x

Suzuki R, Arata S, Nakajo S, Ikenaka K, Kikuyama S, Shioda S (2003) Expression of the receptor for pituitary adenylate cyclase-activating polypeptide (PAC1-R) in reactive astrocytes. Brain Res Mol Brain Res 115(1):10–20. https://doi.org/10.1016/S0169-328X(03)00172-4

Szabadfi K, Atlasz T, Kiss P, Reglodi D, Szabo A, Kovacs K, Szalontai B, Setalo G Jr, Banki E, Csanaky K, Tamas A, Gabriel R (2012) Protective effects of the neuropeptide PACAP in diabetic

retinopathy. Cell Tissue Res 348(1):37–46. https://doi.org/10.1007/s00441-012-1349-0

Szabadfi K, Reglodi D, Szabo A, Szalontai B, Valasek A, Setalo G Jr, Kiss P, Tamas A, Wilhelm M, Gabriel R (2016) Pituitary adenylate cyclase activating polypeptide, a potential therapeutic agent for diabetic retinopathy in rats: focus on the vertical information processing pathway. Neurotox Res 29(3):432–446. https://doi.org/10.1007/s12640-015-9593-1

Takei N, Skoglosa Y, Lindholm D (1998) Neurotrophic and neuroprotective effects of pituitary adenylate cyclase activating polypeptide (PACAP) on mesencephalic dopaminergic neurons. J Neurosci Res 54(5):698–706. https://doi.org/10.1002/(SICI)1097-4547(19981201)54:5<698::AID-JNR15>3.0.CO;2-5

Tamas A, Reglodi D, Farkas O, Kovesdi E, Pal J, Povlishock JT, Schwarcz A, Czeiter E, Szanto Z, Doczi T, Buki A, Bukovics P (2012) Effects of PACAP in central and peripheral nerve injuries. Int J Mol Sci 13(12):8430–8448. https://doi.org/10.3390/ijms13078430

Tripathy D, Sanchez A, Yin X, Martinez J, Grammas P (2012) Age-related decrease in cerebrovascular-derived neuroprotective proteins: effect of acetaminophen. Microvasc Res 84(3):278–285. https://doi.org/10.1016/j.mvr.2012.08.004

Vaczy A, Reglodi D, Somoskeoy T, Kovacs K, Lokos E, Szabo E, Tamas A, Atlasz T (2016) The protective role of PAC1-receptor agonist maxadilan in BCCAO-induced retinal degeneration. J Mol Neurosci 60(2):186–194. https://doi.org/10.1007/s12031-016-0818-4

Vaudry D, Falluel-Morel A, Bourgault S, Basille M, Burel D, Wurtz O, Fournier A, Chow BK, Hashimoto H, Galas L, Vaudry H (2009) Pituitary adenylate cyclase-activating polypeptide and its receptors: 20 years after the discovery. Pharmacol Rev 61(3):283–357. https://doi.org/10.1124/pr.109.001370

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(18):4005–4011. https://doi.org/10.1016/j.febslet.2005.06.013

Wang G, Pan J, Tan YY, Sun XK, Zhang YF, Zhou HY, Ren RJ, Wang XJ, Chen SD (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(3):267–276. https://doi.org/10.1016/j.npep.2008.03.002

Watson MB, Nobuta H, Abad C, Lee SK, Bala N, Zhu C, Richter F, Chesselet MF, Waschek JA (2013) PACAP deficiency sensitizes nigrostriatal dopaminergic neurons to paraquat-induced damage and modulates central and peripheral inflammatory activation in mice. Neuroscience 240:277–286. https://doi.org/10.1016/j.neuroscience.2013.03.002

Yang R, Jiang X, Ji R, Meng L, Liu F, Chen X, Xin Y (2015) Therapeutic potential of PACAP for neurodegenerative diseases. Cell Mol Biol Lett 20(2):265–278. https://doi.org/10.1515/cmble-2015-0008

Yelkenli İH, Ulupinar E, Korkmaz OT, Şener E, Kuş G, Filiz Z, Tunçel N (2016) Modulation of corpus striatal neurochemistry by astrocytes and vasoactive intestinal peptide (VIP) in parkinsonian rats. J Mol Neurosci 59(2):280–289. https://doi.org/10.1007/s12031-016-0757-0

Zink M, Schmitt A, Henn FA, Gass P (2004) Differential expression of glutamate transporters EAAT1 and EAAT2 in mice deficient for PACAP-type I receptor. J Neural Transm (Vienna) 111(12):1537–1542. https://doi.org/10.1007/s00702-004-0175-2