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Changes in expression of PACAP in rat sensory neurons in response to sciatic nerve compression

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Abstract

In the present study, expression of pituitary adenylate cyclase-activating polypeptide (PACAP) in rat dorsal root ganglion (DRG) neurons and sciatic nerve following experimental sciatic nerve compression was studied with the use of quantitative immunohistochemistry and *in situ* hybridization. Previously, we have investigated changes in PACAP expression after nerve transection and, here, the far more frequently encountered condition of nerve compression injury is examined. Nerve compression was performed unilaterally on the rat sciatic nerve, at mid-thigh level, by application of a narrow silicone tube around the nerve for 3, 7, 14 or 28 days, respectively. We detect a statistically significant upregulation in the number and density of PACAP mRNA expression in both small and large DRG neurons in response to nerve compression. An increased number of PACAP-immunoreactive neurons is also found in the ipsilateral DRG. In addition, PACAP immunoreactivity is observed in the compressed sciatic nerve segment and adjacent nerve tissue after nerve compression. The present findings can be compared with previous studies where we have shown that PACAP expression is upregulated in DRG; in response to peripheral inflammation (primarily in small–medium neurons), and after axotomy (dramatic upregulation in medium–large neurons). In view of the recent findings of an increased PACAP expression in DRG after nerve compression, as well as the previous findings of a modulation of PACAP expression in response to axotomy and inflammation, it is likely that PACAP is also involved in the modulation of the response to peripheral nerve compression.

Introduction

The neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) is normally expressed in small–medium dorsal root ganglion (DRG) neurons (Moller *et al*., 1993; Zhang *et al*., 1995, 1996b; Dun *et al*., 1996), whereas expression of the PACAP-preferring receptor, PAC_1, has not been found in DRG neurons, but rather in dorsal horn neurons (Jongsma *et al*., 2000; Vaudry *et al*., 2000). The expression of PACAP is very plastic, and is modified in response to both inflammation (Zhang *et al*., 1998; Jongsma Wallin *et al*., 2003) and nerve transection (Zhang *et al*., 1995, 1996b; Jongsma Wallin *et al*., 2001), where small–medium and medium–large neurons, respectively, are responding. Therefore, a modulatory role for PACAP is suggested during these conditions. Further, PACAP has been implicated in the modulation of pain, both inhibition (Zhang *et al*., 1993b, 1996a; Yamamoto & Tatsuno, 1995) and potentiation (Narita *et al*., 1996; Xu & Wiesenfeld-Hallin, 1996; Jongsma *et al*., 2001).

Nerve compression is a far more common condition than nerve transection, and the prevalence of, e.g., carpal tunnel syndrome (compression of the median nerve in the narrow carpal tunnel between the carpal bones and the relatively rigid flexor retinaculum at wrist level) has been estimated to be approximately 4% of an adult Swedish human population, predominantly women (Atroshi *et al*., 1999). The aetiology of compression injuries is diverse and in most cases the cause is unknown. The condition is initially characterized by numbness, and a tingling and/or burning sensation in the area of innervation. If left untreated, the condition can lead to severe problems, e.g. permanent loss of sensation and atrophy of the innervated muscles (Rempel *et al*., 1999; Mackinnon, 2002). Pain can be a prominent feature, especially during severe compression, and we therefore wanted to investigate whether there is a change in PACAP expression also in response to nerve compression. Some patients experience pain or allodynia during compression and/or after decompression of the affected nerve. In the present study we used a rat model, which mimics the clinical situation, to study nerve compression (Weisl & Osborne, 1964; Dahlin & Kanje, 1992). With the use of a narrow silicone tube, which was applied around the sciatic nerve, the nerve was compressed for various time periods. Expression of PACAP was investigated with regards to PACAP mRNA content and PACAP immunoreactivity in rat sciatic nerve, DRG and spinal cord. Further, the expression and distribution of the PACAP receptor, PAC_1, in DRG and spinal cord was examined with *in situ* hybridization and ligand binding.

Materials and methods

Experimental animals and tissue processing

All animal procedures were conducted in accordance with the European Communities Council Directive (86/609/EEC) and approved by the Malmö/Lund Animal Ethics Committee on Animal Experiments. All efforts were made to minimize the number of animals used. The left sciatic nerve of adult, female Sprague–Dawley

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rats (approximately 200 g; Møllegaard, Denmark) was exposed under pentobarbital (19 mg/kg)/diazepam (3 mg/kg)/physiological saline anaesthesia (ip). A 10-mm-long silicone tube (internal diameter 0.76 mm) was incised longitudinally and wrapped around the nerve at mid-thigh level. Thereafter, two 9/0 Ethilon sutures were applied around the silicone tube, closing it to a constant diameter (Fig. 1). The wound was sealed by sutures and the rats were allowed to recover in heated cages to maintain body temperature. Animals were left with the compressed nerve for 3 ($n = 3$), 7 ($n = 3$), 14 ($n = 3$) or 28 ($n = 4$) days, respectively, and checked daily. All animals were killed by heart puncture after administration of an overdose of pentobarbital (60 mg/mL, ip) anaesthesia, and spinal cord (lumbar segment), DRGs lumbar level (L4–5) and sciatic nerves (a 20 mm segment of the nerve beneath, proximal and distal to the silicone tube, plus the corresponding part of the contralateral nerve) were dissected from the rats. The tissues from the contralateral side were used as controls. Tissue specimens were mounted in O.C.T. Compound (Tissue-Tek®, Histolab products AB, Göteborg, Sweden), fresh-frozen in isopentane at −30 °C immediately after dissection, and stored at −80 °C for *in situ* hybridization and ligand binding. For immunohistochemistry, the tissues were fixed in Stefanini's fixative [2% paraformaldehyde and 15% of a saturated aqueous picric acid solution, in 0.1 M Sörensens phosphate-buffer, pH 7.2] overnight, followed by repeated rinsing in sucrose-enriched (10%) Tyrode's solution. Specimens were mounted in Tissue-Tek®, frozen on dry ice and stored at −80 °C. Tissue specimens were sectioned (6 μm, adjacent sections for co-localization and quantification of mRNA; 10 μm for immunohistochemistry; 20 μm for ligand binding) in a cryostat and thaw-mounted on to Super Frost® Plus slides (Menzel-Gläser, Germany). Sciatic nerves were sectioned longitudinally to visualize the compressed and surrounding areas in the same sections.

All sections were analysed in an Olympus BX-60 microscope connected to an Olympus DP-50 digital camera. Photomicrographs were captured using the Viewfinder Lite software, version 1.0 (Pixera Corporation, Berkshire, UK) and stored in TIF format. Image editing software, Adobe Photoshop® 5.0, was used to adjust size, brightness and contrast.

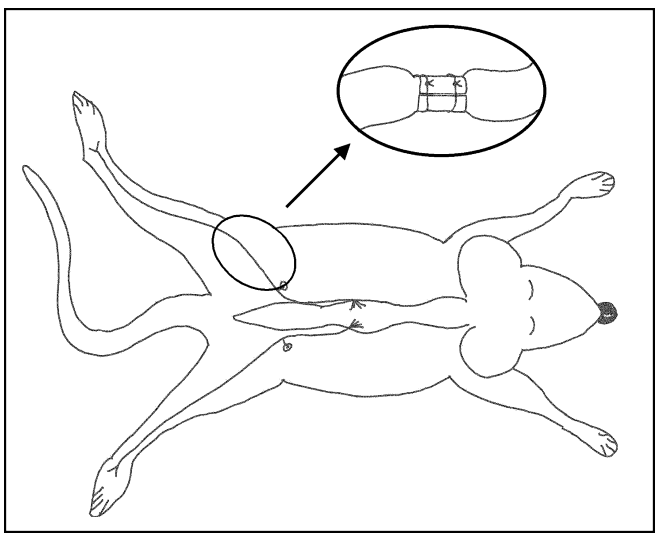

FIG. 1. Schematic drawing of the experimental compression model. A silicone tube (inner diameter 0.76 mm) was incised longitudinally and wrapped around the left sciatic nerve at mid-thigh level. Sutures were applied around the tube closing it (see inset). The wound was sutured and animals were left with the compressed nerve for 3, 7, 14 or 28 days, respectively.

Immunohistochemistry

The antibodies used in this study have been characterized previously, and both the polyclonal and the monoclonal antibodies recognize PACAP 1–27 and PACAP 1–38 (Koves *et al*., 1990; Hannibal *et al*., 1995). Both antibodies have been shown to give a similar pattern of immunoreactivity in the spinal cord dorsal horn, whereas only the monoclonal antibody gave specific staining of PACAP immunoreactivity appearing in ventral horn neurons after nerve injury (Pettersson *et al*., 2004). It has previously been demonstrated, using radioimmunoassay, that PACAP-38 is the predominant form in spinal cord, DRG and sciatic nerve (Zhang *et al*., 1996b). The protocols for immunohistochemistry have been described in detail (Pettersson *et al*., 2004). The procedures are described in brief in the following.

Polyclonal antibody against PACAP

Sections were incubated in moist chambers with primary antibody against synthetic PACAP 1–27 (1 : 1280; code 88121–4, kind gift from Professor Akira Arimura, Tulane University, Hebert Center, US-Japan Biomedical Research Laboratories, New Orleans, LA, USA) for 18–24 h. Excess antibody was washed away and sections were incubated with fluorescein isothiocyanate-conjugated swine anti-rabbit IgG (DAKO, Copenhagen, Denmark). Slides were mounted in PBS/glycerin (1 : 1).

To establish the specificity of the immunostaining, additional slides were incubated with antibody absorbed overnight with the antigen (synthetic PACAP 1–27, Sigma-Aldrich, St. Louis, MO, USA) in excess, and processed as above.

Monoclonal antibody against PACAP

Endogenous peroxidase activity was quenched with the treatment of 1% hydrogen peroxide in PBS, unspecific binding was blocked by treatment with 5% bovine serum albumin in PBS, and slides were incubated in moist chambers with monoclonal antibody against synthetic PACAP 1–38 (1 : 10, code JHH 1, kind gift from Professor Jan Fahrenkrug, Bispebjerg Hospital, Copenhagen, Denmark) for 18–24 h. Following incubation with biotinylated rabbit anti-mouse IgG (DAKO), slides were incubated with streptavidin conjugated to horse radish peroxidase (TSA™ Biotin System, Perkin Elmer), and treated with biotinyl tyramide (TSA Indirect TSA™ Biotin System, Perkin Elmer). Finally, slides were incubated with avidin conjugated to Oregon Greene (Molecular Probes, Eugene, OR, USA) and mounted in PBS/glycerin (1 : 1).

To establish the specificity of the immunostaining, additional slides were incubated with antibody absorbed overnight with the antigen (synthetic PACAP 1–38; A1439, Sigma-Aldrich) in excess, and processed as above.

In situ *hybridization*

Radiolabelled (^{35}S)-labelled oligoDNA probes for detection of PACAP mRNA was used for *in situ* hybridization. OligoDNA probes complementary to and selective for the PACAP mRNA were synthesized; PACAP nucleotides 701–747 (Hurley *et al*., 1995) and PACAP receptor; PAC_1 mRNA, a mix of probes complementary to nucleotides 380–412, 629–661, 1421–1453 and 1673– 1705 (Hashimoto *et al*., 1993), were synthesized and purified at the University of Calgary DNA services, Alberta, Canada, respectively, at the Biomedical Resource Facility, Lund University, Sweden. All used cDNA regions were checked against the GenBank database (NIH, at the Internet site http://www.ncbi.nlm.

nih.gov, October 2003); no greater than 60% homology was found to sequences other than the cognate transcript.

Labelling of the probes and the complete protocol for *in situ* hybridization has been described in detail (Pettersson *et al.*, 2002). To prevent degradation of RNA by external RNases, all steps prior to hybridization were carried out under RNase-free conditions, and all dilutions were performed in autoclaved double-distilled water. Probes were labelled with ^{35}S-dATP (Perkin Elmer), and the specific activity, approximately 1.1×10^9 cpm/μg and 0.64×10^9 cpm/μg for PACAP and PACAP receptor probes, respectively, was measured in a liquid scintillator. All probes were stored at −18 °C until use.

The slides containing double, adjacent pairs of left (compressed) and right (uninjured) DRG sections were air dried, and hybridized with radiolabelled probes (final activity of 10^7 cpm/mL) in a hybridization solution, without any additional pretreatment. Hybridization, with 50–100 μL hybridization solution/slide, was conducted overnight at 37 °C. Control slides were incubated with the addition of corresponding, unlabelled probe in 100-fold molar excess.

Following hybridization, the slides were dipped in 1 × saline sodium citrate, the parafilm was removed and the remaining washes were performed in 1 × saline sodium citrate. The sections were dehydrated in ethanol, air dried and immersed in Kodak NTB2 photoemulsion (diluted 1 : 1 in distilled water) and stored with desiccant at 4 °C in light-sealed containers. After 3.5 weeks the slides were developed in Kodak D19, fixed in Kodak polymax fix, counterstained with toluidine blue and mounted with Pertex (Histolab, Göteborg, Sweden). All sections/slides were hybridized and processed under the same conditions at the same time, to ensure similar treatment and enable comparisons between the ipsi- and contralateral side DRGs.

Quantification and image analysis

To evaluate the effect of nerve compression on the expression of PACAP mRNA in DRG neurons, all slides were first analysed qualitatively. Relative changes in hybridization signal were noted for all sections mounted on the same slide. Sections that were to be compared were mounted on the same slide to avoid variance in overall signal between the different slides (each slide containing double, adjacent pairs of left and right DRGs). Changes in hybridization signal were examined for all sections, and the quality of each section was noted. Representative slides (where all DRG sections mounted on the same slide contain similar numbers of neurons, and the sections reflect the overall labelling for DRGs exposed to the same conditions) were selected for quantitative analysis. Each individual neuron was examined and the relative changes in hybridization signal were determined, for the ipsi- and contralateral DRGs and for the different time periods, using computer-assisted image analysis. Image analysis was performed on adjacent DRG sections processed for *in situ* hybridization to detect PACAP mRNA ($n = 4$ animals, one rat per time point analysed, eight DRG sections where approximately 1900 neuronal DRG L5 profiles were examined). Montages of photomicrographs (500 ×) were prepared from eight pairs of adjacent sections of left or right DRGs from animals with 3, 7, 14 or 28 days of unilateral sciatic nerve compression. Individual neurons with a visible nucleus in at least one of the two adjacent sections were identified and numbered (approximately 240 neurons/montage). Light microscopy images (60 ×) were collected from each individual DRG L5 neuron using an Olympus DP-50 digital camera. Cross-sectional areas of each neuronal profile and the percentage of the cytoplasmic area that was covered by silver grains were measured in all the ganglia, using the public domain NIH Image program (written by Wayne Rasband at the US National Institute of Health, and available from the internet by anonymous FTP from zippy.nimh.nih.gov). Individual cell diameters were calculated from the cross-sectional areas on the assumption that the neurons were spherical. Neurons were defined as small when the diameter was < 35 μm, and large when the diameter was > 35 μm (Giuffrida & Rustioni, 1992). The density threshold was adjusted interactively for each image, so that the area per grain was constant for all neurons analysed on a given slide. Neurons were considered labelled for PACAP mRNA if they contained more than five times background labelling of silver grains. The background was determined from measurements of the silver grain density over five defined areas of the neuropil devoid of positively labelled cell bodies. Background was measured and determined specifically for each quantified section.

After the quantification of DRG L5 neurons we performed a somewhat less extensive examination of the DRG L4 neurons (which also support the sciatic nerve) to compare the PACAP mRNA distribution with the ganglia at the L5 level. All sections were examined and selected according to the criteria above, and this time four new animals were quantified. Montages were made ($n = 4$ animals, one animal per time point analysed, eight DRG sections and approximately 2300 DRG L4 profiles), neurons defined and numbered, whereafter each neuron (approximately 290 neurons/montage) was examined directly in the microscope (60 ×). The total percentage of PACAP mRNA-labelled neurons was determined on the compressed and intact sides, respectively, but no measurements of neuronal diameter were made, i.e. no distinction of the distribution in relation to neuronal size was made for the DRG L4 neurons, and changes in silver grain density/neuron were not calculated.

Furthermore, the effect of nerve compression on peptide expression was evaluated by quantification of the proportion of PACAP-immunoreactive cell bodies in the ganglia. Contra- and ipsilateral DRG sections were analysed 3, 7, 14 and 28 days after nerve compression injury. In addition, DRG sections from two intact animals were analysed. All slides were first examined qualitatively in the microscope and PACAP-immunoreactive cells were counted on representative slides (see above for *in situ* hybridization). Montages of photomicrographs (270 ×) were prepared ($n = 7$; uninjured animals = 2, nerve compression injury = one rat per time point analysed, except for 28 days after injury when two rats were analysed). The total amount of neuronal cell bodies was counted in each selected ganglia (approximately 4300 neuronal profiles were examined) and the number of PACAP-immunoreactive cells was related to the total number of cells in the ganglia.

The differences in the proportions of neurons expressing PACAP or PACAP mRNA between the ipsi- and contralateral side DRGs were analysed using the chi-square test, and $P < 0.05$ was considered significant.

[^{125}I]-PACAP 27 binding

[^{125}I]-PACAP 27 binding was used to locate binding of PACAP to its receptor. The procedure for ligand binding has been described in detail (Pettersson *et al.*, 2002). In brief, sections were preincubated in a Tris buffer, incubated with 150 pM [^{125}I]-PACAP 27 (Perkin Elmer) at 20 °C for 1 h. Control sections were incubated with the addition of 1.5 μM synthetic PACAP 1–27 (Sigma-Aldrich) to check for non-specific binding. Slides were washed and, when dry, covered with Hyperfilm MP (Amersham, Solna, Sweden), stored at 4 °C in the dark for 1 week, after which the film was developed in Kodak D19, fixed in Kodak polymax and examined in the microscope.

Tissue morphology

To examine if changes in soma morphology of DRG neurons or degenerative changes of the compressed sciatic nerves took place, additional animals were exposed to sciatic nerve compression ($n = 4$) as described above, and dissected tissues were embedded in plastic and evaluated. In detail, after 3, 7, 14 or 28 days of compression, animals were anaesthetized (see above) and perfused via the left heart ventricle with physiological saline solution to clear away the blood, and 2.5% glutaraldehyde in sodium-caccodylate buffer for tissue fixation. Dissected tissues; ipsi- and contralateral DRGs (L4, L5) and sciatic nerves (a segment beneath and 5 mm distal to the silicone tube, plus a corresponding segment of the contralateral nerve) were further fixed by immersion in 2.5% glutaraldehyde in sodium-caccodylate buffer overnight, and then repeatedly rinsed in sodium-caccodylate buffer. The specimens were then postfixed in 2% osmium tetroxide in sodium-caccodylate buffer for 2 h and rinsed in the buffer overnight, prior to dehydration in graded series of ethanol and propylene oxide. After dehydration, tissues were immersed in propylene oxide and Agar 100 resin 1 : 1 for 5 hours, embedded in Agar 100 resin and left at 4 °C for 6 hours before polymerization at 60 °C for 3 days. Transverse sections of the embedded tissue specimens (1 μm) were counterstained with 1% Azur II and 1% methylene blue (1 : 1) and examined in the light microscope.

Results

Tissue morphology

No obvious signs of chromatolysis (disintegration of Nissl substance) and no indications of eccentricity of the nuclei were found in DRGs from the ipsi- or contralateral sides (Fig. 2A and B). The ipsilateral sciatic nerve showed a heterogeneous appearance, ranging from areas with normal morphology to areas greatly affected by the compression injury with signs of oedema and nerve fibres in various stages of degeneration and possible regeneration and areas with loss of axons (Fig. 2D). Also, an enlargement of the epineurium was observed along the length of the compressed segment of the nerve about 7 days after surgery, and persisted during the time period studied (not shown). No obvious differences in tissue morphology were found between the compressed nerve segment and the segment slightly distal to the compressed area. The contralateral nerve showed normal morphology (Fig. 2C).

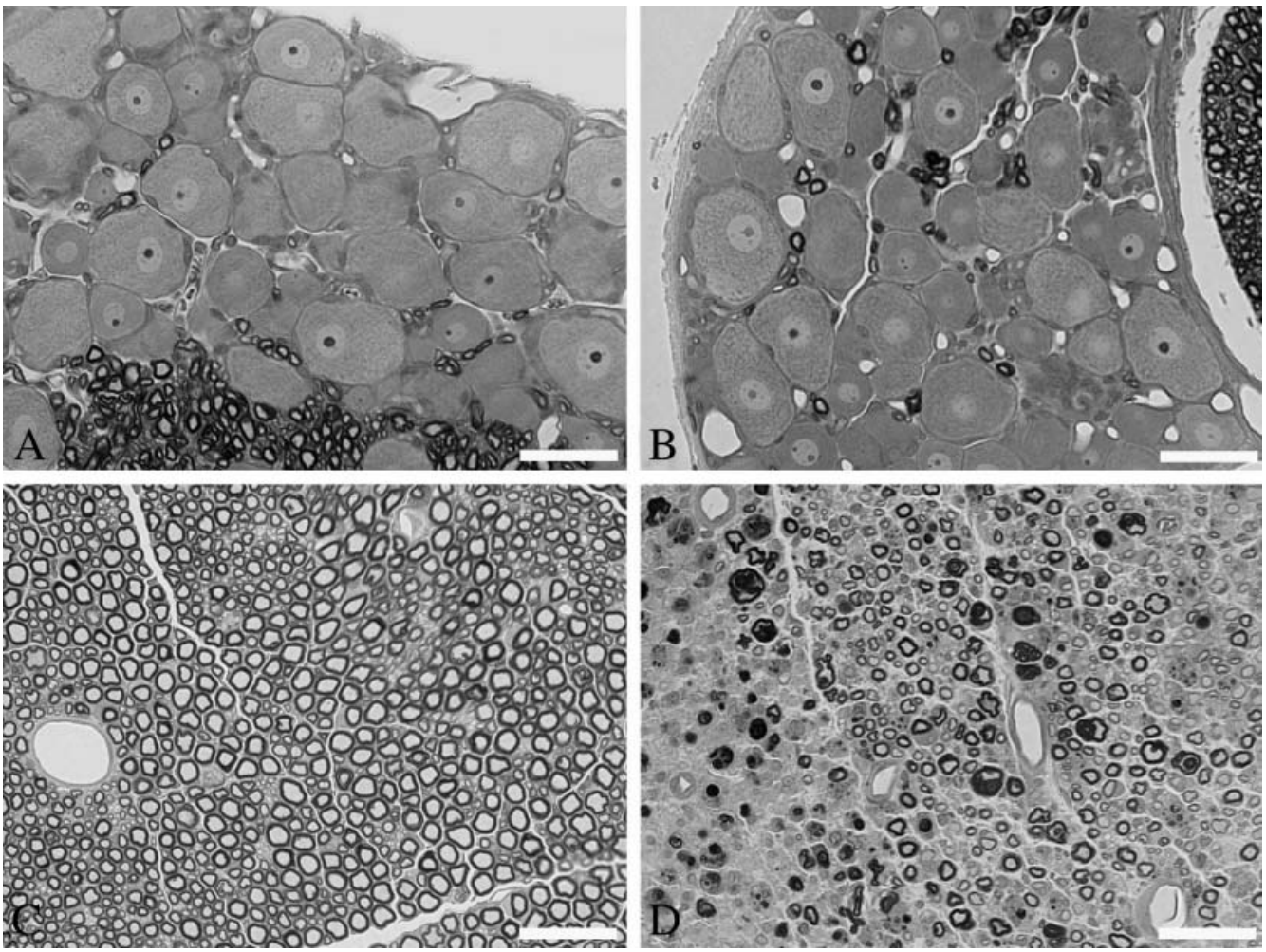

FIG. 2. Plastic-embedded sections of contralateral (A) and ipsilateral (B) DRG and contralateral (C) and ipsilateral (D) sciatic nerve after compression injury. No obvious signs of eccentricity of the nuclei are detected in nerve cell bodies on either side (A and B). The contralateral nerve shows an appearance characteristic of an intact nerve (C), whereas signs of Wallerian degeneration, possible regeneration, oedema and loss of axons are observed on the ipsilateral side (D). Scale bar (A–D), 50 μm.

Immunohistochemistry

The two PACAP antibodies gave similar patterns of immunoreactivity in the spinal cord and in the sciatic nerve. The monoclonal antibody was preferably used for staining of PACAP immunoreactivity in DRG neurons, as it was used in conjunction with a signal amplification protocol to increase the level of detection. Scarce immunoreactivity for PACAP was observed in a few fibres in the intact sciatic nerves, whereas PACAP-immunoreactive staining in the compressed nerves was observed in nerve fibres in the sciatic nerve segment beneath and bordering the compressed area, especially accumulated at the nodal regions (Fig. 3A and B). The increase in PACAP immunoreactivity in the compressed nerve was most prominent 3, 7 and 14 days after injury, although some increased immunoreactivity was still visible 28 days post-injury (examined qualitatively). Inflammatory cells, resembling macrophages and mast cells (based on their general histology), were found in the endoneurium, primarily around the distal border of the compressed nerve segment. Further, an increased PACAP immunoreactivity (number of neurons, quantitative examination; and staining density, qualitative examination) was noted in the ipsilateral DRGs when compared with the contralateral side (Fig. 4A and B). On the contralateral side the proportion of PACAP-immunoreactive neuronal cell bodies ranged from 14.7 to 18.6% of the total number of cells, and the corresponding distributions in the intact animals were 15.9% and 16.1%. On the ipsilateral side the proportion of PACAP-immunoreactive neuronal cell bodies was greater at all time points: 3 days after compression injury 33.8% of the neuronal cell bodies were stained for PACAP, 7 days 28.9%, 14 days 27.0% and 28 days after nerve compression 35.1% of the neuronal cell somas showed PACAP immunoreactivity. The differences between the ipsi- and contralateral sides were statistically significant at all time points, whereas no statistical differences were detected when contralateral DRGs and DRGs from intact animals were compared. No pronounced changes in PACAP immunoreactivity were found in the spinal cord at any of the time points examined (not shown).

In situ *hybridization*

The signal for PACAP mRNA in DRG (L5) was increased on the ipsilateral side at 3, 7, 14 and 28 days after unilateral sciatic nerve compression (Figs 4C–F and 5; Table 1). The upregulation of PACAP mRNA expression on the ipsilateral side (number of neurons) was statistically significant at all time points studied, ranging from about two to almost four times the contralateral expression depending on the time point investigated. The greatest proportion of PACAP mRNA-expressing neurons (3.9 times the contralateral expression) was detected 28 days post-surgery (Table 1). On the intact side, 14–21% of the DRG neurons (small size) express PACAP mRNA, whereas 40–55% of the neurons express PACAP mRNA on the compressed side. The upregulation in PACAP mRNA expression after nerve compression is found in both small (< 35 μm) and large (> 35 μm) size DRG neurons (Fig. 5, Table 1). The quantitative examinations of the PACAP mRNA expression in L4 DRG neurons confirmed our data from the L5 level, showing very similar levels of expression in response to nerve compression, except for 3 days post-surgery when a very slight variance in the proportion of PACAP mRNA-positive neurons was observed (Table 1). Also, on the contralateral side the percentage of PACAP mRNA-expressing neurons was very consistent in the L4 and L5 DRG neurons and between the different injury durations, except for 7 days after surgery when a small increase in the number of PACAP mRNA-expressing neurons (L4 and L5) was found, that was not present at the other time points (Table 1). When the expression in heavily labelled populations (> 32 × background) was compared between both sides (ipsilateral vs. contralateral proportions in parentheses), a significant difference in the number of heavily expressing neurons was found 3 (12% vs. 4.0%), 14 (10% vs. 3.0%) and 28 (11.5% vs. 1.8%) days after compression, but not 7 (12% vs. 7.9%) days after compression. On the contralateral side, heavy expression was observed exclusively in neurons with small diameter, whereas after compression (3, 14 and 28 days) a significant increase in expression in both small- and large-diameter neurons was observed. Also, the proportion of neurons expressing PACAP mRNA at a very high level was similar in ipsilateral DRG neurons at all time points investigated.

The qualitative examinations of the DRG sections hybridized for detection of PAC_1 mRNA did not indicate that any changes in PAC_1 mRNA expression had taken place during nerve compression and, therefore, no further quantifications of these sections were made. Further, no obvious differences in the distribution pattern of either PACAP mRNA or PAC_1 mRNA were observed when the ipsi- and contralateral spinal cords were examined (not shown).

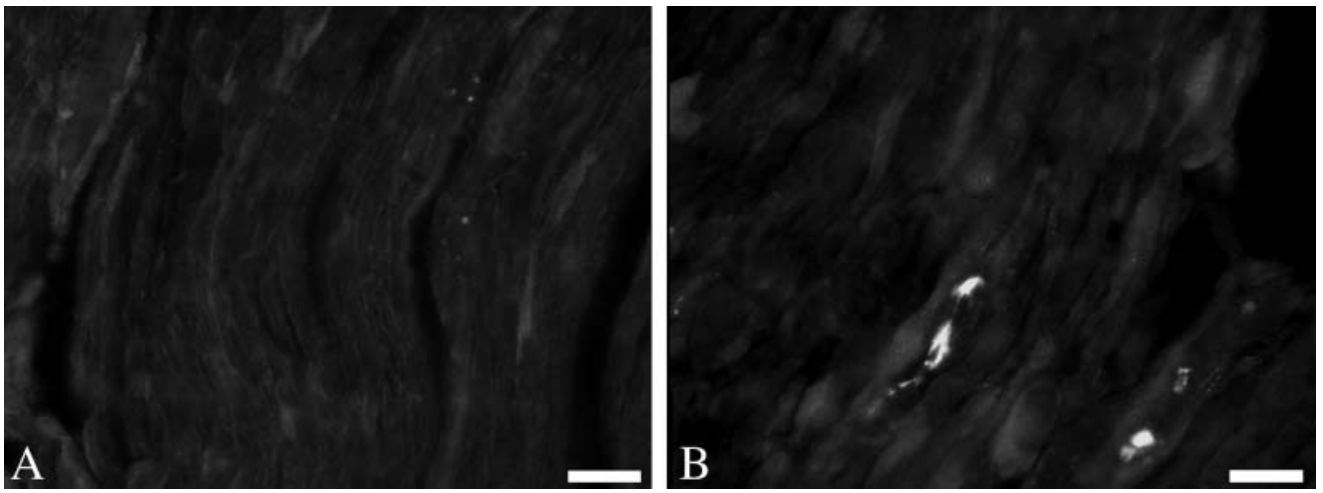

FIG. 3. Longitudinally sectioned sciatic nerves 7 days after nerve compression, contra- (A) and ipsilateral (B) sides, stained for PACAP immunoreactivity (polyclonal antibody). An induction of PACAP immunoreactivity in fibres, especially accumulated at the nodal regions, was detected in the compressed nerve segment and in the surrounding areas (B). In the intact nerve, only scarce and weak immunostaining was observed (A). Scale bar (A and B), 25 μm.

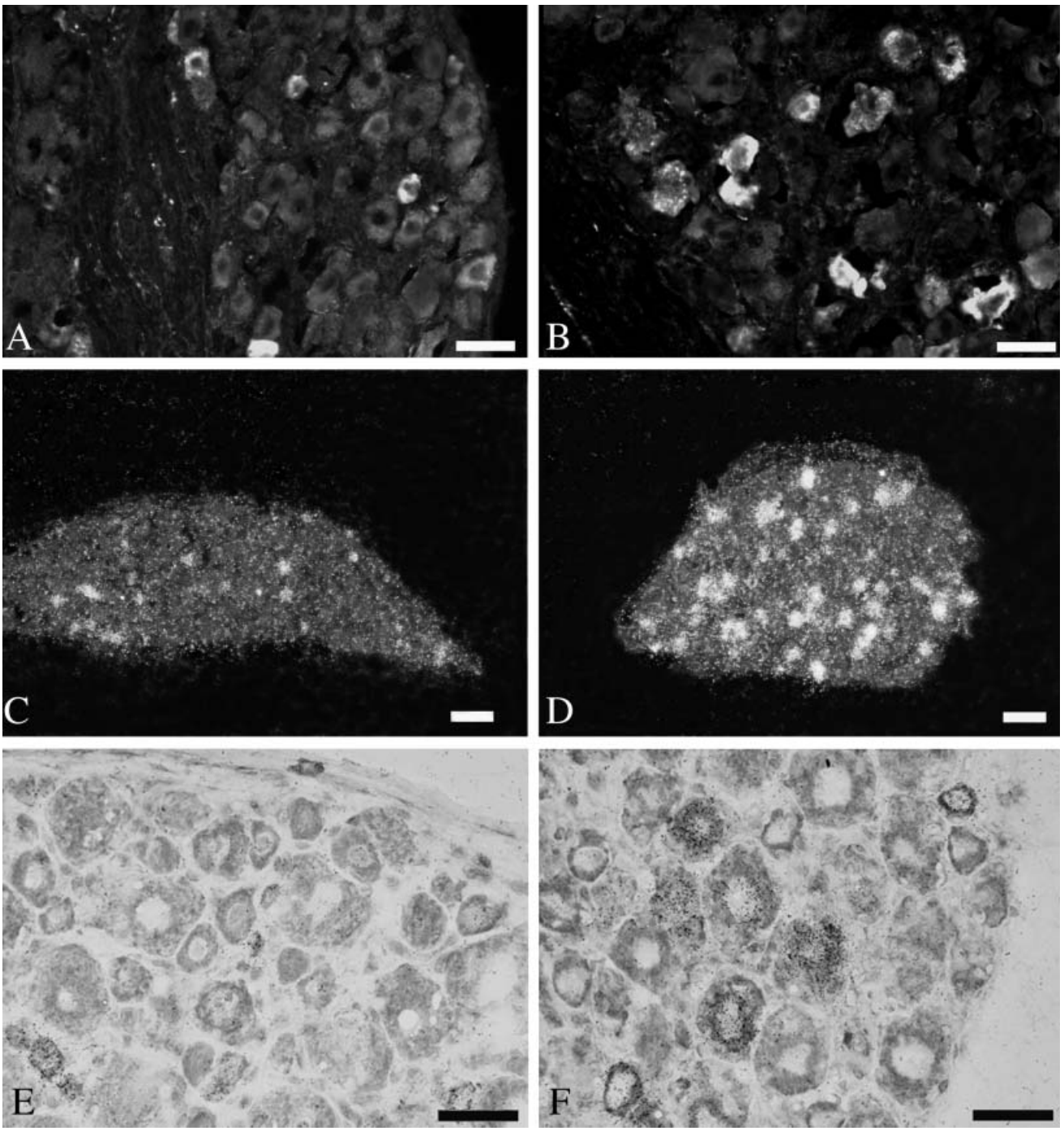

FIG. 4. Immunohistochemical staining of PACAP immunoreactivity (monoclonal antibody) (A and B) and *in situ* hybridization signal for PACAP mRNA (C–F) in rat DRG after 3 (C–F) and 7 (A and B) days of unilateral sciatic nerve compression. An increased PACAP immunoreactivity was noted in the ipsilateral ganglia after nerve compression (B), as compared with the contralateral side (A). Dark-field, low-magnification pictures visualizing the PACAP mRNA expression on the contralateral, uninjured side (C) and on the ipsilateral side (D) where it is upregulated in both small and large DRG neurons in response to the nerve compression. High-magnification, light-field pictures visualizing the silver grain labelling of individual neurons shown in (C and D), where (E) is a close up of (C) and (F) is a close up of (D). Scale bars, 50 μm (A and B); 100 μm (C and D); 50 μm (E and F).

[^{125}I]-PACAP 27 binding

No obvious changes in [^{125}I]-PACAP 27 binding between ipsi- and contralateral sides of the spinal cord were observed at any of the time points investigated after nerve compression (Fig. 6), i.e. the distribution pattern of [^{125}I]-PACAP 27 binding was essentially similar to that in earlier studies of intact rat spinal cords (Jongsma *et al*., 2000). Further, no induction of PACAP binding to its receptor was observed in the DRGs (not shown).

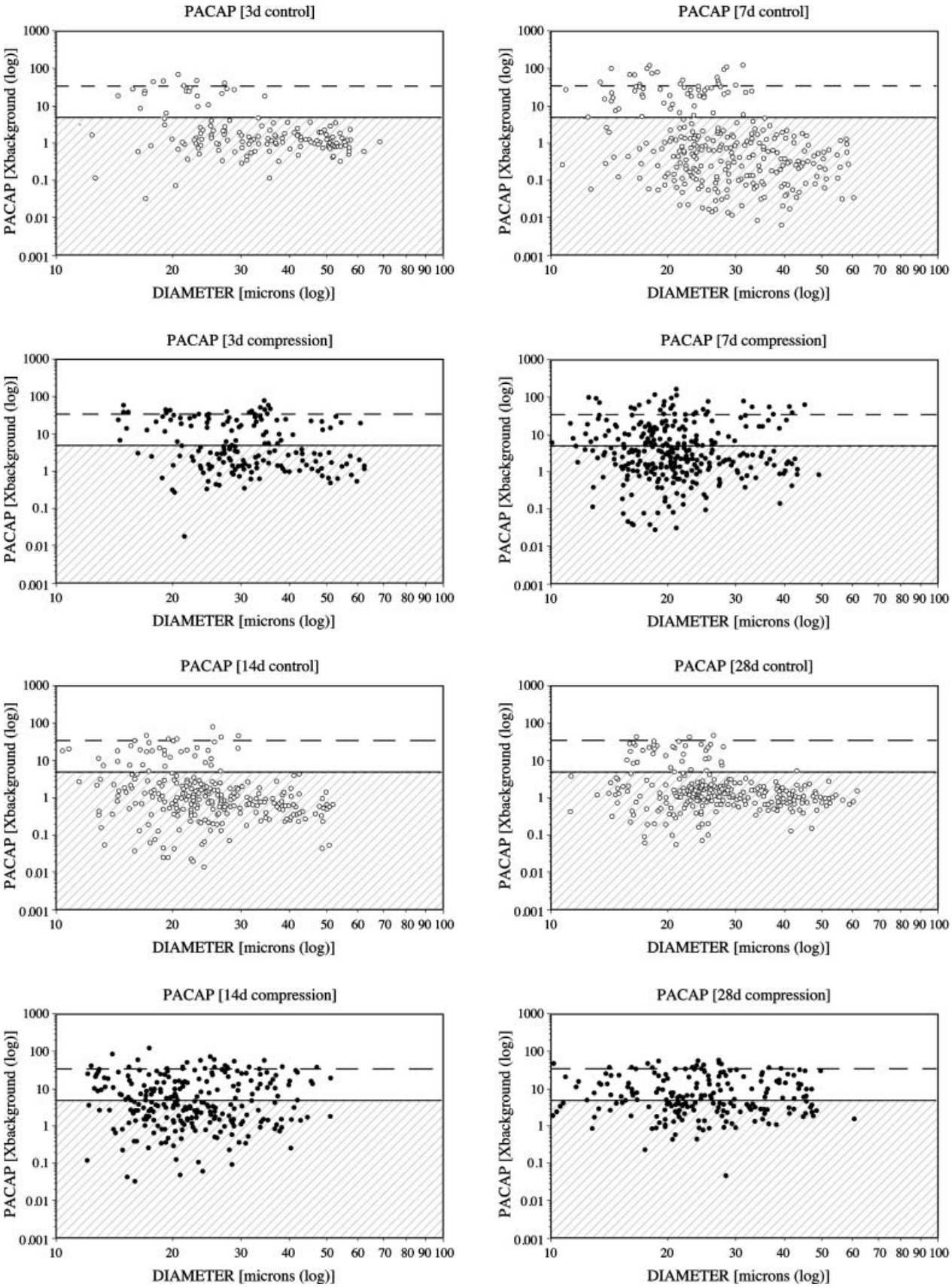
PACAP [3d control]
PACAP [7d control]
PACAP [3d compression]
PACAP [7d compression]
PACAP [14d control]
PACAP [28d control]
PACAP [14d compression]
PACAP [28d compression]
PACAP [Xbackground (log)]
DIAMETER [microns (log)]
1000
100
10
1
0.1
0.01
0.001
10
20
30
40
50
60
70
80
90
100

TABLE 1. Summary of the data presented in Fig. 5 (DRG L5) and addition data from L4 DRG neurons

Sciatic nerve compression	Experimental state	Numbers of neurons expressing PACAP mRNA as proportions (and percentages)		
		All cells	Small cells (< 35 μm)	Large cells (≥ 35 μm)
3 days	Control (L5)	22/149 (15)	22/149 (15)	0/149 (0)
	Control (L4)	43/266 (16)	–	–
	Compressed (L5)	72/167 (43)	55/167 (33)	17/167 (10)
	Compressed (L4)	138/285 (48)	–	–
7 days	Control (L5)	62/291 (21)	61/291 (21)	1/291 (0)
	Control (L4)	59/317 (19)	–	–
	Compressed (L5)	121/305 (40)	112/305 (37)	9/305 (3)
	Compressed (L4)	122/294 (41)	–	–
14 days	Control (L5)	38/268 (14)	38/268 (14)	0/268 (0)
	Control (L4)	43/298 (14)	–	–
	Compressed (L5)	118/257 (46)	101/257 (39)	17/257 (7)
	Compressed (L4)	139/300 (43)	–	–
28 days	Control (L5)	38/278 (14)	37/278 (14)	1/278 (0)
	Control (L4)	45/269 (17)	–	–
	Compressed (L5)	114/208 (55)	89/208 (43)	25/208 (12)
	Compressed (L4)	142/273 (52)	–	–

Total number of neurons (i.e. neuronal profiles) positive for PACAP mRNA expression (> 5 × background) is noted in relation to the total number of neurons quantified in each ganglia (with percentages in parentheses). For the L5 ganglia, PACAP mRNA-expressing neurons were classified as small or large. At all time points, a statistically significant increase in the number of neurons (both L4 and L5) expressing PACAP mRNA was found on the compressed side compared with the control side (chi-square test, $P < 0.05$).

Discussion

The present study shows that experimental nerve compression induces an upregulation of PACAP expression in the number and density (expression level/neuron) of both small and large sensory neuronal cell bodies in the DRG, as well as an induction of immunoreactivity for PACAP in the compressed nerve segment. However, no change in expression was observed in the spinal cord. Alterations in PACAP expression have previously been shown in response to both complete sciatic nerve transection (Zhang *et al*., 1995, 1996b; Jongsma Wallin *et al*., 2001) and peripheral inflammation (Zhang *et al*., 1998; Jongsma Wallin *et al*., 2003). Nerve transection induces an increase in PACAP expression (number of neurons and expression level/neuron) in medium–large DRG neurons (Jongsma Wallin *et al*., 2001). In contrast, the upregulation (number of neurons and expression level/neuron) associated with inflammation takes place primarily in small–medium neurons (Zhang *et al*., 1998; Jongsma Wallin *et al*., 2003). The present and previous studies indicate that there is an injury-related expression of PACAP in cell bodies of differential sizes, which is important information when discussing neuropeptide expression after various trauma.

Nerve compression is a frequently encountered condition, affecting about 4% of the Swedish adult, human population (Atroshi *et al*., 1999), and this syndrome is sometimes associated with pain. The consequences of compression, on the nerve, are related to the magnitude and duration of the applied pressure (Rempel *et al*., 1999). Initially, compression induces an impaired intraneural microvascular circulation and formation of oedema. An impediment or a complete block of the energy-dependent axonal transport of, e.g., structural proteins synthesized in the cell soma (anterograde transport) or trophic substances from the periphery (retrograde transport) occur in the compressed nerve (Dahlin & McLean, 1986; Dahlin *et al*., 1986b). Later, structural changes such as local myelin damage and axonal disintegration are induced (Rydevik *et al*., 1981; Lundborg & Dahlin, 1992). Biomechanical principles explain why larger fibres are more adversely affected by compression (large myelinated fibres) and ischaemia (thinner myelinated fibre than are smaller fibres (e.g. non-myelinated C-fibres), as are fibres in superficial fascicles compared with fibres in the more deeply located fascicles. Furthermore, fascicles embedded in small amounts of connective tissue in the epineurium are more affected by compression than fascicles surrounded by a larger amount of insulating connective tissue (MacGregor *et al*., 1975; Battista & Alban, 1983; Dahlin *et al*., 1989; Lundborg & Dahlin, 1992).

The inhibition of axonal transport, caused by ischaemia and/or mechanical injury during experimental nerve compression, may elicit pronounced cell body reactions (Dahlin & McLean, 1986; Dahlin *et al*., 1986a, 1987). Changes in neuropeptide expression in the nerve cell bodies have been observed. An increased immunoreactivity for both C-terminal flanking peptide of neuropeptide Y (CPON; primarily large neurons) and galanin (primarily small neurons) was found in DRGs following chronic nerve compression (Bergmark *et al*., 2001; Dahlin *et al*., 2003). In the present study we show that there is an increase in the number of both small and large DRG neurons expressing PACAP mRNA after nerve compression. The number of small neurons that express PACAP mRNA in the ipsilateral ganglia is approximately 1.8–3.3 times the expression in the contralateral ganglia. The total number of large neurons induced to express PACAP mRNA is not very high; however, the increase in expression in these neurons is quite impressive when compared with their normal PACAP mRNA expression (∼ 0.1%). After nerve compression approximately 3–12% of the larger DRG neurons express PACAP mRNA on the ipsilateral side. Furthermore, the proportion of PACAP-immunoreactive DRG neurons is increased (∼ twice) on the ipsilateral side, when compared with the contralateral side or with ganglia from uninjured animals, which supports the findings of an increased PACAP mRNA expression after nerve compression.

Cell death, changes in cell body size and eccentricity of the nucleus in DRG neurons have been reported in conjunction with nerve injuries (Lieberman, 1971, 1974). These events have to be considered because they would affect the proportions of labelled neurons (total number as well as the proportions of small vs. large neurons) that are quantified. The level of injury seems to be a crucial factor, and cell death in response to proximal nerve transection is rather insignificant in rat at durations shorter than 15 days, as shown with stereological methods (Vestergaard *et al*., 1997), whereas a distal transection (mid-thigh) did not induce any significant cell death in rat until after 8 weeks (Tandrup *et al*., 2000). Changes in mean perikaryal volume were seen 4 days after proximal nerve transection, and 15 days after proximal nerve crush (Vestergaard *et al*., 1997; Degn *et al*., 1999). The effects of nerve compression were studied after different time periods, ranging

FIG. 5. Scatterplot diagrams of the *in situ* hybridization labelling for PACAP mRNA density in relation to neuronal size in individual neuronal profiles in the ipsi- and contralateral L5 DRGs; 3, 7, 14 and 28 days after nerve compression. Each dot represents a quantified neuron, and neurons are considered labelled when the ratio of silver grain density over the neuronal cytoplasm is > 5 × the grain density over areas of the neuropil devoid of nerve cell bodies. Solid lines divide the plots into presumed labelled (> 5 × background labelling) and unlabelled (shaded) populations, whereas dashed lines separate heavily labelled (> 32 × background) from lightly and moderately labelled neuronal populations.

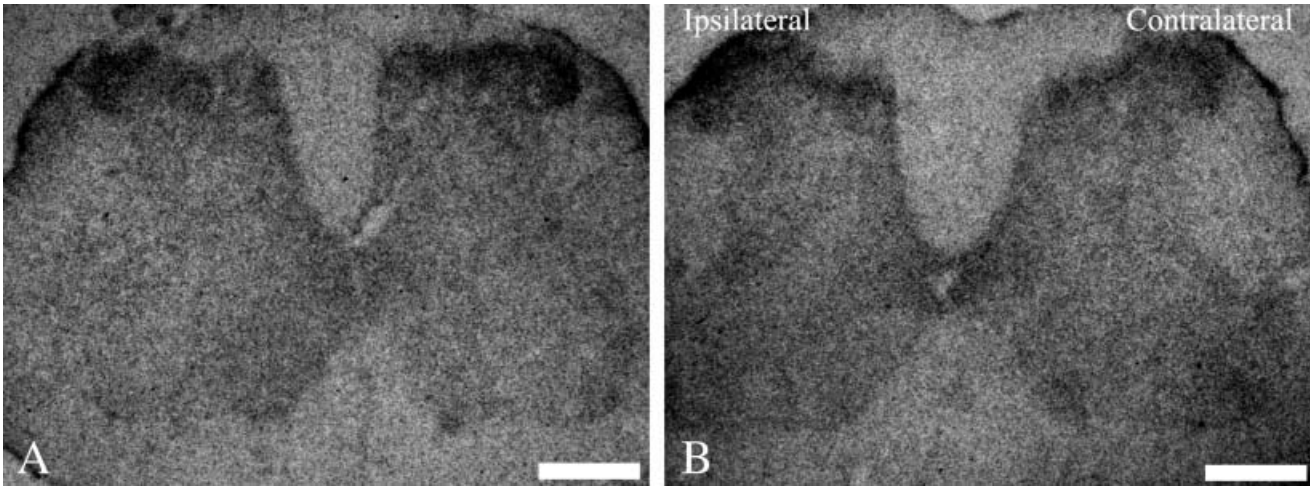

FIG. 6. Autoradiographic distribution of [^{125}I]-PACAP 27 in rat spinal cord, intact (A) and 28 days after sciatic nerve compression (B). Binding of radioactive ligand is scattered throughout the spinal cord grey matter with the greatest labelling density in the superficial layers of the dorsal horn (A and B). No obvious change in PACAP receptor distribution is observed after this type of injury when compared with spinal cords from intact animals. Dorsal horns upwards in figure. Scale bar (A and B), 500 μm.

from 3 to 28 days, but the decrease in cell volume observed after nerve crush (Degn *et al*., 1999) was limited to ~2% of cells with a diameter range around the border of 35 μm (which we use as a cut off to separate small and large cells). This indicates that cell volume shrinkage does not influence our results to any great extent. Besides, we employed a less severe injury model according to the definitions described by Sunderland (1978) and, furthermore, the compression injury was inflicted at a more distal level (mid-thigh) than in the study by Degn *et al*. (1999). Eccentricity of the nucleus would interfere with the proportions of small vs. large neurons in our quantifications as they are based on the criteria that neurons with a visible nucleus in at least one of two adjacent sections are quantified, and that the cell body is spherical. If eccentricity of the nucleus was to take place, this would make us underestimate the size of some of the neurons if sectioned at the margin of the neuron, but still showing a visible (eccentric) nucleus. Because we did not detect any signs of eccentricity of the nuclei in the DRG neurons after nerve compression in any of the sections (3, 7, 14 or 28 days; fresh-frozen or plastic-embedded tissues), this was not regarded as a major issue.

The greatest number of neurons expressing PACAP mRNA was detected 28 days after surgery, indicating that there is still a pronounced upregulation at that time point, and suggesting that longer durations of nerve compression might lead to even greater changes in expression. In contrast, the increase in the number of sensory neurons immunoreactive for CPON during a 28-day period of chronic nerve compression was transient (Bergmark *et al*., 2001). The intensity and number of PACAP-immunoreactive neurons are also increased in DRGs, in a pattern similar to that of the PACAP mRNA expression, indicating that the PACAP mRNA is translated into functional peptide in these neurons. No change in receptor mRNA expression or ligand binding in the ipsi- and contralateral DRGs was observed, indicating that there was no induction of receptor synthesis after compression. Furthermore, PAC_1 mRNA expression, as well as ligand binding to the spinal cord, was virtually the same after compression (ipsi- and contralateral sides) as in intact spinal cords, suggesting that the increased amounts of PACAP expressed in the DRGs after compression execute their effect on PAC_1 receptors already present in the intact spinal cord.

Compression injuries may in some patients, although not all, be associated with pain, especially during severe nerve compression. PACAP has been suggested to be involved in the modulation of pain (Zhang *et al*., 1993a, 1996a; Yamamoto & Tatsuno, 1995; Narita *et al*., 1996; Xu & Wiesenfeld-Hallin, 1996; Jongsma *et al*., 2001), and it is not unlikely that the increased PACAP expression in response to compression injury might affect the regulation of pain, particularly as the level of PAC_1 expression was unchanged in the spinal cord dorsal horn during the compression period. This is in contrast to the reduction in ligand binding observed in the dorsal horn after nerve transection (Jongsma *et al*., 2000). The PACAP mRNA expression increased in relation to the duration of the compression, except for 7 days after surgery when there was a small transient dip in upregulation. Furthermore, there is a slight inconsistency in the proportions of PACAP mRNA-expressing neurons between DRG L5 and L4 (3 days of compression). Taken together, this might suggest that there is a period of transition somewhere between 3 and 7 days after application of the compression tube, where animals are more or less affected by the surgery and tube application per se. These procedures may affect the expression and lead to a superimposed increase in PACAP mRNA expression initially, as well as a transient contralateral effect (see day 7), which in turn can partly mask an increased expression on the ipsilateral side when expression on both sides is compared. The increased contralateral expression at 7 days conceals the increased expression density/neuron when heavily labelled neuronal populations are compared at this time point. It seems that the dip in the number of PACAP mRNA-expressing neurons (at 7 days compared with 3 days) is an indirect effect of an initially pronounced upregulation in the large neurons (3 days of compression), which then falls to a more modest expression (7 days of compression) and again slowly increases with time. As discussed above, large axons are most severely influenced by compression as a result of their greater diameter (Lundborg & Dahlin, 1992), and it might be this effect that is manifested initially after the surgery. The PACAP mRNA expression in small-diameter neurons is steadily increased over time. Nerve compression is a complex condition where the nerve is exposed to both ischaemia and a certain degree of direct mechanical injury to the nerve fibres, depending on the pressure applied and its duration. In addition, an inflammatory component may be induced, which might promote an increased PACAP mRNA expression in smaller neurons. This mixed condition may help to explain the dual effects we saw after the experimental nerve

compression, i.e. an increased PACAP mRNA expression in both small and larger DRG neurons. The severe compression lesion inflicted in the present study induced Wallerian degeneration in the compressed nerve segment and in the nerve segment distal to the compression injury. This elicits an inflammatory reaction with invasion of macrophages, giving evidence that there is an inflammatory component present with the possibility to contribute to the upregulation.

Previously, we have shown that PACAP mRNA expression is at least partly regulated by the neurotrophins, nerve growth factor (NGF), neurotrophin 3 (NT-3) and brain-derived neurotrophic factor (BDNF; Jongsma Wallin *et al*., 2001, 2003; Pettersson *et al*., 2003). NT-3 negatively regulates PACAP in intact DRG neurons, and also mitigates the increased PACAP mRNA expression in large neurons after axotomy, whereas NGF appears to promote an upregulation of PACAP mRNA expression in intact and inflamed small DRG neurons. In addition, BDNF contributes to the upregulation in PACAP mRNA expression in response to axotomy (Pettersson *et al*., 2003). Nerve compression results in an impaired retrograde axonal transport (see above) and is therefore likely to induce changes in the expression of neuropeptides in DRG neurons, possibly via impairment of transport of, e.g., trophic factors. In this study we have observed an upregulation in PACAP mRNA expression in both small and large DRG neurons, which may be brought on by an interruption in axonal transport of NT-3 from cells in the periphery possibly leading to an upregulation of PACAP mRNA expression in large neurons, and a locally increased production of NGF and/or BDNF in response to the inflammation that may result in an increased expression of PACAP mRNA in small DRG neurons. An increased NGF and BDNF synthesis in the sciatic nerve and DRG, respectively, has been observed after induction of peripheral tissue inflammation (Donnerer *et al*., 1992; Cho *et al*., 1997).

We observed a scarce PACAP immunoreactivity in fibres in the intact sciatic nerve. However, an increased immunostaining in the compressed segment and in the parts of the nerve bordering that area suggests that there is an inhibition of axonally transported PACAP and/or an increased transport of PACAP from the DRG to the periphery. An increased PACAP immunoreactivity in the sciatic nerve has also been observed in response to sciatic nerve transection (Zhang *et al*., 1995, 1996b). However, the role of PACAP in the compressed nerve segment and in the periphery has yet to be clarified. It has been hypothesized that PACAP can act as a survival factor for axotomized neurons, and that this action in some cases might differ from that of the classical growth factors (Waschek, 2002). Furthermore, PACAP has been shown to exert an anti-inflammatory effect via inhibition of production of pro-inflammatory agents, e.g. cytokines and nitric oxide (Martinez *et al*., 1998; Delgado *et al*., 1999a,c), and stimulation of production of anti-inflammatory cytokines (Delgado *et al*., 1999b) by activated macrophages.

Taken together, our findings implicate that the neuropeptide PACAP is involved in the neuronal response to a peripheral nerve compression injury, and that the PACAP expression in sensory neurons of different size is dependent on the type of injury. Pain can be a prominent feature among patients with nerve compression lesions, and the findings of an increased PACAP expression in sensory neurons in response to nerve compression contribute to the understanding of the pathophysiology of these common lesions.

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Abbreviations

BDNF, brain-derived neurotrophic factor; CPON, C-terminal flanking peptide of neuropeptide Y; DRG, dorsal root ganglion; NGF, nerve growth factor; NT-3, neurotrophin 3; PACAP, pituitary adenylate cyclase-activating polypeptide; PBS, phosphate-buffered saline.

References

Atroshi, I., Gummesson, C., Johnsson, R., Ornstein, E., Ranstam, J. & Rosen, I. (1999) Prevalence of carpal tunnel syndrome in a general population. *JAMA*, **282**, 153–158.

Battista, A.F. & Alban, E. (1983) Effect of graded ligature compression on nerve conduction. *Exp. Neurol.*, **80**, 186–194.

Bergmark, M., Kanje, M., Widerberg, A. & Dahlin, L.B. (2001) Experimental nerve compression and upregulation of CPON in DRG. *Neuroreport*, **12**, 3783–3786.

Cho, H.J., Kim, J.K., Zhou, X.F. & Rush, R.A. (1997) Increased brain-derived neurotrophic factor immunoreactivity in rat dorsal root ganglia and spinal cord following peripheral inflammation. *Brain Res.*, **764**, 269–272.

Dahlin, L.B. & Kanje, M. (1992) Conditioning effect induced by chronic nerve compression. An experimental study of the sciatic and tibial nerves of rats. *Scand. J. Plast. Reconstr. Surg. Hand Surg.*, **26**, 37–41.

Dahlin, L.B. & McLean, W.G. (1986) Effects of graded experimental compression on slow and fast axonal transport in rabbit vagus nerve. *J. Neurol. Sci.*, **72**, 19–30.

Dahlin, L.B., Meiri, K.F., McLean, W.G., Rydevik, B. & Sjostrand, J. (1986a) Effects of nerve compression on fast axonal transport in streptozotocin-induced diabetes mellitus. An experimental study in the sciatic nerve of rats. *Diabetologia*, **29**, 181–185.

Dahlin, L.B., Nordborg, C. & Lundborg, G. (1987) Morphologic changes in nerve cell bodies induced by experimental graded nerve compression. *Exp. Neurol.*, **95**, 611–621.

Dahlin, L.B., Shyu, B.C., Danielsen, N. & Andersson, S.A. (1989) Effects of nerve compression or ischaemia on conduction properties of myelinated and non-myelinated nerve fibres. An experimental study in the rabbit common peroneal nerve. *Acta Physiol. Scand.*, **136**, 97–105.

Dahlin, L.B., Sjostrand, J. & McLean, W.G. (1986b) Graded inhibition of retrograde axonal transport by compression of rabbit vagus nerve. *J. Neurol. Sci.*, **76**, 221–230.

Dahlin, L.B., Stenberg, L. & Kanje, M. (2003) Galanin expression in sensory neurons after nerve compression or transection. *Neuroreport*, **14**, 359–362.

Degn, J., Tandrup, T. & Jakobsen, J. (1999) Effect of nerve crush on perikaryal number and volume of neurons in adult rat dorsal root ganglion. *J. Comp. Neurol.*, **412**, 186–192.

Delgado, M., Martinez, C., Pozo, D., Calvo, J.R., Leceta, J., Ganea, D. & Gomariz, R.P. (1999a) Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activation polypeptide (PACAP) protect mice from lethal endotoxemia through the inhibition of TNF-alpha and IL-6. *J. Immunol.*, **162**, 1200–1205.

Delgado, M., Munoz-Elias, E.J., Gomariz, R.P. & Ganea, D. (1999b) Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide enhance IL-10 production by murine macrophages: in vitro and in vivo studies. *J. Immunol.*, **162**, 1707–1716.

Delgado, M., Munoz-Elias, E.J., Gomariz, R.P. & Ganea, D. (1999c) Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide prevent inducible nitric oxide synthase transcription in macrophages by inhibiting NF-kappa B and IFN regulatory factor 1 activation. *J. Immunol.*, **162**, 4685–4696.

Donnerer, J., Schuligoi, R. & Stein, C. (1992) Increased content and transport of substance P and calcitonin gene-related peptide in sensory nerves innervating inflamed tissue: evidence for a regulatory function of nerve growth factor in vivo. *Neuroscience*, **49**, 693–698.

Dun, N.J., Miyazaki, T., Tang, H. & Dun, E.C. (1996) Pituitary adenylate cyclase activating polypeptide immunoreactivity in the rat spinal cord and

medulla: implication of sensory and autonomic functions. *Neuroscience*, **73**, 677–686.

Giuffrida, R. & Rustioni, A. (1992) Dorsal root ganglion neurons projecting to the dorsal column nuclei of rats. *J. Comp. Neurol.*, **316**, 206–220.

Hannibal, J., Mikkelsen, J.D., Clausen, H., Holst, J.J., Wulff, B.S. & Fahrenkrug, J. (1995) Gene expression of pituitary adenylate cyclase activating polypeptide (PACAP) in the rat hypothalamus. *Regul. Pept.*, **55**, 133–148.

Hashimoto, H., Ishihara, T., Shigemoto, R., Mori, K. & Nagata, S. (1993) Molecular cloning and tissue distribution of a receptor for pituitary adenylate cyclase-activating polypeptide. *Neuron*, **11**, 333–342.

Hurley, J.D., Gardiner, J.V., Jones, P.M. & Bloom, S.R. (1995) Cloning and molecular characterization of complementary deoxyribonucleic acid corresponding to a novel form of pituitary adenylate cyclase-activating polypeptide messenger ribonucleic acid in the rat testis. *Endocrinology*, **136**, 550–557.

Jongsma Wallin, H., Danielsen, N., Johnston, J.M., Gratto, K.A., Karchewski, L.A. & Verge, V.M. (2001) Exogenous NT-3 and NGF differentially modulate PACAP expression in adult sensory neurons, suggesting distinct roles in injury and inflammation. *Eur. J. Neurosci.*, **14**, 267–282.

Jongsma Wallin, H., Pettersson, L.M., Verge, V.M. & Danielsen, N. (2003) Effect of anti-nerve growth factor treatment on pituitary adenylate cyclase activating polypeptide expression in adult sensory neurons exposed to adjuvant induced inflammation. *Neuroscience*, **120**, 325–331.

Jongsma, H., Danielsen, N., Sundler, F. & Kanje, M. (2000) Alteration of PACAP distribution and PACAP receptor binding in the rat sensory nervous system following sciatic nerve transection. *Brain Res.*, **853**, 186–196.

Jongsma, H., Pettersson, L.M., Zhang, Y., Reimer, M.K., Kanje, M., Waldenstrom, A., Sundler, F. & Danielsen, N. (2001) Markedly reduced chronic nociceptive response in mice lacking the PAC_1 receptor. *Neuroreport*, **12**, 2215–2219.

Koves, K., Arimura, A., Somogyvari-Vigh, A., Vigh, S. & Miller, J. (1990) Immunohistochemical demonstration of a novel hypothalamic peptide, pituitary adenylate cyclase-activating polypeptide, in the ovine hypothalamus. *Endocrinology*, **127**, 264–271.

Lieberman, A.R. (1971) The axon reaction: a review of the principal features of perikaryal responses to axon injury. *Int. Rev. Neurobiol.*, **14**, 49–124.

Lieberman, A.R. (1974) Some factors affecting retrograde neuronal responses to axonal lesions. In Bellairs, R. & Gray, E.G. (Eds), *Essays on the Nervous System*. Clarendon Press, Oxford, pp. 71–105.

Lundborg, G. & Dahlin, L.B. (1992) The pathophysiology of nerve compression. *Hand Clin.*, **8**, 215–227.

MacGregor, R.J., Sharpless, S.K. & Luttges, M.W. (1975) A pressure vessel model for nerve compression. *J. Neurol. Sci.*, **24**, 299–304.

Mackinnon, S.E. (2002) Pathophysiology of nerve compression. *Hand Clin.*, **18**, 231–241.

Martinez, C., Delgado, M., Pozo, D., Leceta, J., Calvo, J.R., Ganea, D. & Gomariz, R.P. (1998) Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide modulate endotoxin-induced IL-6 production by murine peritoneal macrophages. *J. Leukoc. Biol.*, **63**, 591–601.

Moller, K., Zhang, Y.Z., Hakanson, R., Luts, A., Sjolund, B., Uddman, R. & Sundler, F. (1993) Pituitary adenylate cyclase activating peptide is a sensory neuropeptide: immunocytochemical and immunochemical evidence. *Neuroscience*, **57**, 725–732.

Narita, M., Dun, S.L., Dun, N.J. & Tseng, L.F. (1996) Hyperalgesia induced by pituitary adenylate cyclase-activating polypeptide in the mouse spinal cord. *Eur. J. Pharmacol.*, **311**, 121–126.

Pettersson, L.M., Geremia, N.M., Verge, V.M. & Danielsen, N. (2003) BDNF regulates the expression of PACAP mRNA in rat DRG neurons after nerve injury. *Soc. Neurosci. Abstr.* [Society for Neuroscience, Program no. 38.3. Abstract Viewer/Itinerary Planner, Washington.]

Pettersson, L.M., Heine, T., Verge, V.M., Sundler, F. & Danielsen, N. (2004) PACAP mRNA is expressed in rat spinal cord neurons. *J. Comp. Neurol.*, **471**, 85–96.

Pettersson, L.M., Sundler, F. & Danielsen, N. (2002) Expression of orphanin FQ/nociceptin and its receptor in rat peripheral ganglia and spinal cord. *Brain Res.*, **945**, 266–275.

Rempel, D., Dahlin, L. & Lundborg, G. (1999) Pathophysiology of nerve compression syndromes: response of peripheral nerves to loading. *J. Bone Joint Surg. Am.*, **81**, 1600–1610.

Rydevik, B., Lundborg, G. & Bagge, U. (1981) Effects of graded compression on intraneural blood flow. An in vivo study on rabbit tibial nerve. *J. Hand Surg. [Am.]*, **6**, 3–12.

Sunderland, S. (1978) *Nerves and Nerve Injuries*, 2nd Edn. Churchill Livingstone, Edinburgh.

Tandrup, T., Woolf, C.J. & Coggeshall, R.E. (2000) Delayed loss of small dorsal root ganglion cells after transection of the rat sciatic nerve. *J. Comp. Neurol.*, **422**, 172–180.

Vaudry, D., Gonzalez, B.J., Basille, M., Yon, L., Fournier, A. & Vaudry, H. (2000) Pituitary adenylate cyclase-activating polypeptide and its receptors: from structure to functions. *Pharmacol. Rev.*, **52**, 269–324.

Vestergaard, S., Tandrup, T. & Jakobsen, J. (1997) Effect of permanent axotomy on number and volume of dorsal root ganglion cell bodies. *J. Comp. Neurol.*, **388**, 307–312.

Waschek, J.A. (2002) Multiple actions of pituitary adenylyl cyclase activating peptide in nervous system development and regeneration. *Dev. Neurosci.*, **24**, 14–23.

Weisl, H. & Osborne, G. (1964) The pathological changes in rats' nerves subject to moderate compression. *J. Bone Joint Surg.*, **46B**, 297–306.

Xu, X.J. & Wiesenfeld-Hallin, Z. (1996) Intrathecal pituitary adenylate cyclase activating polypeptide facilitates the spinal nociceptive flexor reflex in the rat. *Neuroscience*, **72**, 801–804.

Yamamoto, T. & Tatsuno, I. (1995) Antinociceptive effect of intrathecally administered pituitary adenylate cyclase activating polypeptide (PACAP) on the rat formalin test. *Neurosci. Lett.*, **184**, 32–35.

Zhang, Y., Danielsen, N., Sundler, F. & Mulder, H. (1998) Pituitary adenylate cyclase-activating peptide is upregulated in sensory neurons by inflammation. *Neuroreport*, **9**, 2833–2836.

Zhang, Y.Z., Hannibal, J., Zhao, Q., Moller, K., Danielsen, N., Fahrenkrug, J. & Sundler, F. (1996b) Pituitary adenylate cyclase activating peptide expression in the rat dorsal root ganglia: up-regulation after peripheral nerve injury. *Neuroscience*, **74**, 1099–1110.

Zhang, Y., Malmberg, A.B., Sjolund, B. & Yaksh, T.L. (1996a) The effect of pituitary adenylate cyclase activating peptide (PACAP) on the nociceptive formalin test. *Neurosci. Lett.*, **207**, 187–190.

Zhang, Q., Shi, T.J., Ji, R.R., Zhang, Y.Z., Sundler, F., Hannibal, J., Fahrenkrug, J., Hokfelt, T. & Zhang, Y. (1995) Expression of pituitary adenylate cyclase-activating polypeptide in dorsal root ganglia following axotomy: time course and coexistence. *Brain Res.*, **705**, 149–158.

Zhang, Y.Z., Sjolund, B., Moller, K., Hakanson, R. & Sundler, F. (1993b) Pituitary adenylate cyclase activating peptide produces a marked and long-lasting depression of a C-fibre-evoked flexion reflex. *Neuroscience*, **57**, 733–737.

Zhang, X., Verge, V.M., Wiesenfeld-Hallin, Z., Piehl, F. & Hokfelt, T. (1993a) Expression of neuropeptides and neuropeptide mRNAs in spinal cord after axotomy in the rat, with special reference to motoneurons and galanin. *Exp. Brain Res.*, **93**, 450–461.