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Neuropeptide Y inhibits axonal transport of particles in neurites of cultured adult mouse dorsal root ganglion cells

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Neuropeptide Y (NPY) plays a modulatory role in processing nociceptive information. The present study investigated the effects of NPY on axonal transport of particles in neurites of cultured adult dorsal root ganglion (DRG) cells using video-enhanced microscopy. Application of NPY decreased the number of particles transported in both the anterograde and retrograde directions. This effect was persistently observed during NPY application and was reversed after washout. The inhibitory effect of NPY was concentration dependent between 10^{-9} M and 10^{-6} M. The instantaneous velocity of individual particles moving in anterograde and retrograde directions was also reduced by NPY. Both the NPY Y_1 receptor agonist [Leu^{31},Pro^{34}]-NPY and NPY Y_2 receptor agonist NPY_{13-36} mimicked the effect of NPY on the number of transported particles. An immunocytochemical study using an antiserum against the NPY Y_1 receptor protein revealed that the Y_1 receptor was expressed in the majority (85.9 %) of cultured adult mouse DRG cells. Pre-treatment of cells with pertussis toxin, a GTP-binding protein (G protein) inhibitor, completely blocked the inhibitory effect of NPY. Each application of SQ-22536, an adenylate cyclase inhibitor, and H-89, a protein kinase A inhibitor, mimicked and occluded the effect of NPY. In contrast, dibutyryl cAMP (dbcAMP), a membrane permeable cAMP analogue, and forskolin, an activator of adenylate cyclase, produced a transient increase in axonal transport. The application of dbcAMP and forskolin in combination with NPY negated the effect of NPY alone. These results suggest that NPY, acting at Y_1 and Y_2 receptors, inhibits axonal transport of particles in sensory neurones. The effect seems to be mediated by a pertussis toxin-sensitive G protein, adenylate cyclase, and protein kinase A pathway. Therefore, NPY may be a modulatory factor for axonal transport in sensory neurones.

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Neuropeptide Y (NPY), a 36-amino acid peptide, is widely distributed in the central and peripheral nervous systems to control a variety of biological events. In the somatosensory system, NPY has been suggested to play an important role in the modulation of nociceptive information. Receptors for NPY are expressed in cell bodies and fibres in dorsal root ganglion (DRG) neurones (Mantyh *et al.* 1994; Zhang *et al.* 1994*a*, 1994*b*, 1995, 1997; Marchand *et al.* 1999). This peptide produces antinociceptive effects (Duggan *et al.* 1991; Hua *et al.* 1991; Broqua *et al.* 1996; Naveilhan *et al.* 2001) by inhibiting the release of neurotransmitters from central terminals of primary afferent neurones (Duggan *et al.* 1991) and by directly inhibiting primary afferent nociceptors (Mantyh *et al.* 1994).

Intracellular transport systems, e.g. axonal transport, are essential for expression and maintenance of neuronal cell function. Sensory neuropeptides such as substance P and calcitonin gene-related peptide (CGRP), transmitting nociceptive information, are conveyed from the cell body to axon terminals by an axonal transport system (Brimijoin *et al.* 1980; Harmar & Keen, 1982; Keen *et al.* 1982; Kashihara *et al.* 1989, Fernandez & Hodges-Savola, 1994). Receptors expressed in DRG neurones are also transported within axons (Zarbin *et al.* 1990; Hökfelt *et al.* 1998). Thus, axonal transport is important for the regulation of sensory nervous system function. However, it is not known how the regulatory peptide NPY affects axonal transport in sensory neurones. The object of the present study was to examine the effect of NPY on axonal transport in neurites of cultured mouse DRG cells. We used video-enhanced microscopy, which can detect a quick response to stimuli, to observe in real-time the particles moving within neurites.

METHODS

Cell culture

The experimental protocol was approved by the Animal Experimentation and Ethics Committee of Kitasato University School of Medicine. Adult male C57BL/6 mice (8 weeks old) were killed with ether and the dorsal root ganglia were removed. The ganglia were immersed immediately in Ham's F-12 culture medium (Gibco BRL, Grand Island, NY, USA) and incubated for

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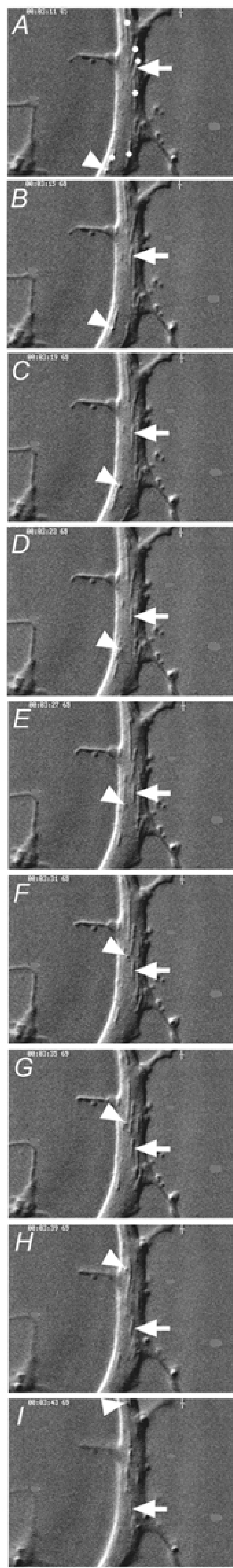

90 min at 37 °C in Ham's F-12 medium containing 2 mg ml^{-1} collagenase (Worthington Biochemical, Freehold, NJ, USA). The ganglia were then incubated for 15 min at 37 °C in Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution containing 2.5 mg ml^{-1} trypsin (Sigma Chemical Co., St Louis, MO, USA). Immediately after incubation, trypsin activity was inhibited by the addition of 0.125 mg ml^{-1} trypsin inhibitor (Sigma). After a rinse with enzyme-free Ham's F-12 medium, the ganglia were triturated using fire-polished pipettes (inner diameter: 0.2–0.5 mm). The cells were plated onto polylysine-coated coverslips and cultured for 48 h at 37 °C under 5 % CO_2 (pH 7.4) in Ham's F-12 medium containing 10 % fetal bovine serum and penicillin (100 units ml^{-1}) – streptomycin (100 μg ml^{-1}).

Experimental cell preparation

The coverslip on which cells were cultured was attached with waterproof tape to the underside of a 0.5 mm-thick stainless-steel chamber (50 × 80 mm) with a lozenge-shaped hole (25 × 35 mm). The topside of the stainless-steel chamber was covered with another coverslip, leaving a small opening on each side to perfuse solutions. The volume of the chamber was about 0.45 ml. The culture medium was then replaced with Hepes-buffered salt solution (135 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Hepes and 5.5 mM glucose, pH 7.4, 37 °C). The chamber was mounted onto the stage of an inverted Zeiss Axiomat microscope equipped with an oil-immersed planapochromat × 64 objective (Carl Zeiss, Oberkochen, Germany). The stage was maintained at 37 °C by a thermocontroller. Experiments commenced 30 min after changing the extracellular medium from culture medium to Hepes-buffered solution. The drug-containing solution (3 ml) was injected into one side opening using a Pasteur pipette, and the solution spilling from the other opening was removed by an infusion pump.

Drugs

Neuropeptide Y (NPY, Sigma), NPY receptor agonists ([Leu^{31},Pro^{34}]-NPY and NPY$_{13-36}$; both from Sigma), the guanosine triphosphate (GTP)-binding protein (G protein) inhibitor pertussis toxin (List Biological Laboratories, Inc., Campbell, CA, USA), and the membrane permeable cAMP analogue dibutyryl cAMP (Sigma) were dissolved directly in Hepes-buffered salt solution. The adenylate cyclase inhibitor SQ-22536 (Biomol Research Labs, Inc., Plymouth Meeting, PA, USA), the adenylate cyclase activator forskolin (RBI, Natick, MA, USA), and the protein kinase A inhibitor H-89 (Biomol) were each dissolved in dimethyl sulfoxide (DMSO, Wako Pure Chemical, Osaka, Japan) and then diluted with aqueous solution. The DMSO concentration was 0.01 % and at this concentration DMSO had no effect on axonal transport. Pertussis toxin was applied to cultured cells in the medium 4 h prior to the start of recording, and the cells were incubated again at 37 °C. Other drugs were injected into the chamber (37 °C) during video-enhanced microscopic recordings.

Figure 1. Particles moving within a neurite of the cultured adult mouse dorsal root ganglion (DRG) cell, visualised by video-enhanced microscopy

Serial images obtained at 4 s intervals indicate particles moving in anterograde (arrow) and retrograde (arrowhead) directions. Mitochondria (dots in *A*) were identified by their long-shape, large-size and saltatory movement. Scale bar, 2 μm.

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Video-enhanced microscopic recording

Nomarski images obtained by an inverted microscope were transformed into video signals by a video camera (Harpicon, Hamamatsu Photonics, Hamamatsu, Japan) and a camera controller (C2741, Hamamatsu Photonics). The signals were processed to digital video images with enhanced contrast by a video image enhancement system (DVS-20, Hamamatsu Photonics). Video images were displayed in real-time on a video monitor (C1864, Hamamatsu Photonics), and stored on a video recorder (PVW-2800, Sony, Tokyo, Japan). This processing provided a 10 000-fold final magnification on the video monitor.

Analysis of axonal transport

Axonal transport was analysed from the video replay. The number and instantaneous velocity of particles (diameter > 50 nm) moving toward the axon terminal (anterograde) and back to the cell body (retrograde) were measured. The number of particles was counted for 2 min at 3 min intervals before, during and, when necessary, after application of the drug. Averaged data are expressed as means (± S.D.) percentage of the control value that was obtained before the drug application. Analysis of variance (ANOVA) was used to evaluate the statistical significance of fluctuations over time. Differences between the control and test conditions were examined for statistical significance using Student's paired t test. Instantaneous velocity of individual moving particles was analysed as follows. Serial video images were digitised at 5 ms intervals to a Macintosh computer (Power Macintosh, 7600/200) equipped with an LG-3 video capture board (Scion Co., Frederick, MD, USA) using Scion image software (Scion). The instantaneous velocity was determined using the formula: velocity = distance/time.

Immunocytochemistry

Mouse DRG cells cultured for 48 h on coverslips were fixed with 4 % paraformaldehyde for 5 min at room temperature. After fixation, they were washed for 3 min with 0.025 M phosphate-buffered saline (PBS) containing 0.3 % Triton X-100 (PBST), and were treated for 10 min with protein blocking agent (Immunon, Pittsburgh, PA, USA) at room temperature to block non-specific protein sites. The cells were incubated overnight at 4 °C with rabbit anti-NPY Y_1 receptor serum (1:500, DiaSorin Inc, Stillwater, MN, USA). The antiserum was diluted with 0.2 % bovine serum albumin, 1 % normal goat serum, and 0.1 % sodium azide in PBST. After washing with PBS, the cells were incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (1:100, Organon Teknika Corp., Durham, NC, USA) for 30 min at room temperature. The immunostained cells were examined with an inverted Zeiss Axiomat microscope equipped with a 485 nm excitation filter and a 520 ± 15 nm emission filter (Carl Zeiss, Oberkochen, Germany). The number of immunoreactive neurones was determined under microscopic observation and also by examining microphotographs.

RESULTS

Isolated and cultured DRG neurones that made no contact with other cells were used for the experiment. Video-enhanced microscopy displayed the movement of particles in anterograde and retrograde directions (Fig. 1). Some of moving particles appeared to be mitochondria according to their microscopic morphology (Forman *et al.* 1987; Takenaka *et al.* 1990; Okada *et al.* 1995; Fig. 1). In the control extracellular medium (Hepes-buffered salt solution, pH 7.4, 37 °C), the mean number of particles (min^{-1}) transported in anterograde and retrograde directions were 71.3 ± 17.4 (mean ± S.D., $n = 75$) and 71.9 ± 16.5 ($n = 75$), respectively. The mean instantaneous velocities of moving particles in anterograde and retrograde directions under the control condition were 0.66 ± 0.42 $\mu m\ s^{-1}$ ($n = 178$) and 0.63 ± 0.39 $\mu m\ s^{-1}$ ($n = 159$), respectively.

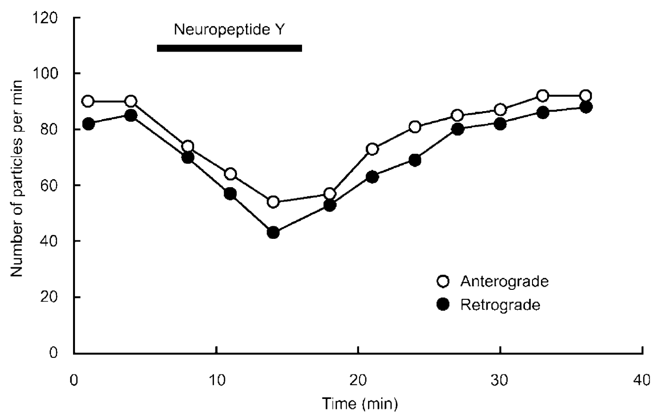

Figure 2. Effects of a brief application of neuropeptide Y (NPY) on the number of particles transported in neurites of cultured DRG cells

Changes in the number of transported particles in both anterograde (○) and retrograde (●) directions before, during, and after a brief (10 min) application of 100 nM NPY.

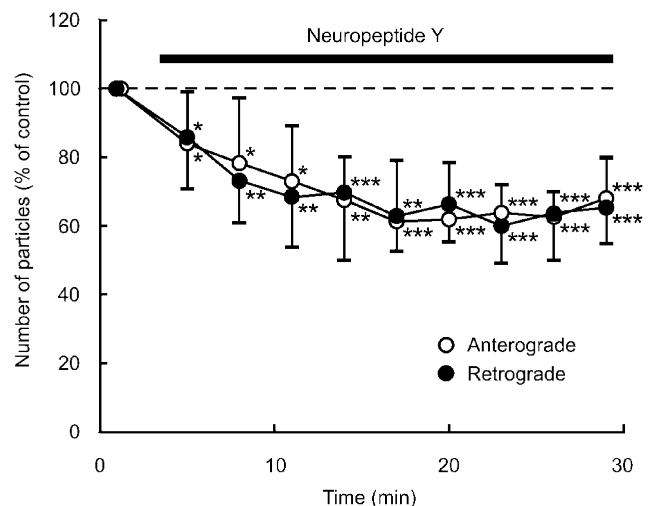

Figure 3. Effects of a prolonged application of NPY on the number of particles transported in neurites of cultured DRG cells

Percentage changes in the number of transported particles of control (the value before application) in anterograde (○) and retrograde (●) directions induced by application of 1 μM NPY. Each point indicates the mean (± S.D.) of the values obtained from five DRG cells. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$ compared to the value before application.

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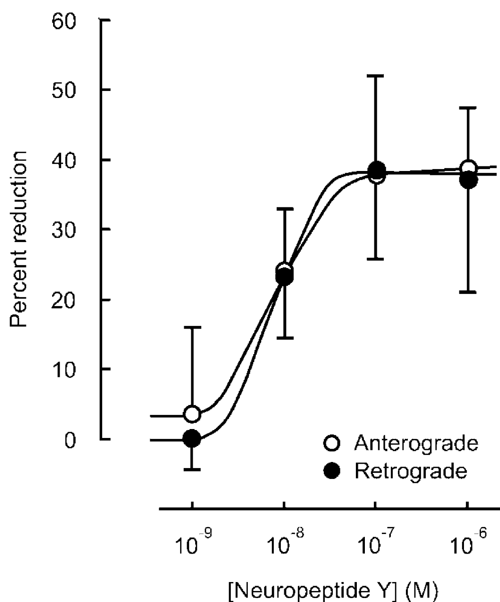

Figure 4. Concentration dependence of NPY-induced reduction in the number of particles transported in anterograde and retrograde directions

Percentage reduction in the number of transported particles at various concentrations of NPY. The value obtained 15 min after each application of NPY was compared to the value before the application (control), and is expressed as a percentage. Each point indicates the mean (± S.D.) of the values obtained from five DRG cells.

Effects of NPY on axonal transport

Application of NPY (100 nM) for 10 min resulted in a rapid but reversible decrease in the number of particles transported in both anterograde and retrograde directions (Fig. 2). Application of NPY (1 μM) for a longer period (26 min) resulted in a significant decrease in the number of transported particles during the application in each of five neurones tested (Fig. 3). Maximum inhibition of particle transport in both directions amounted to 60 % of control at 14 min after the start of application, reaching a

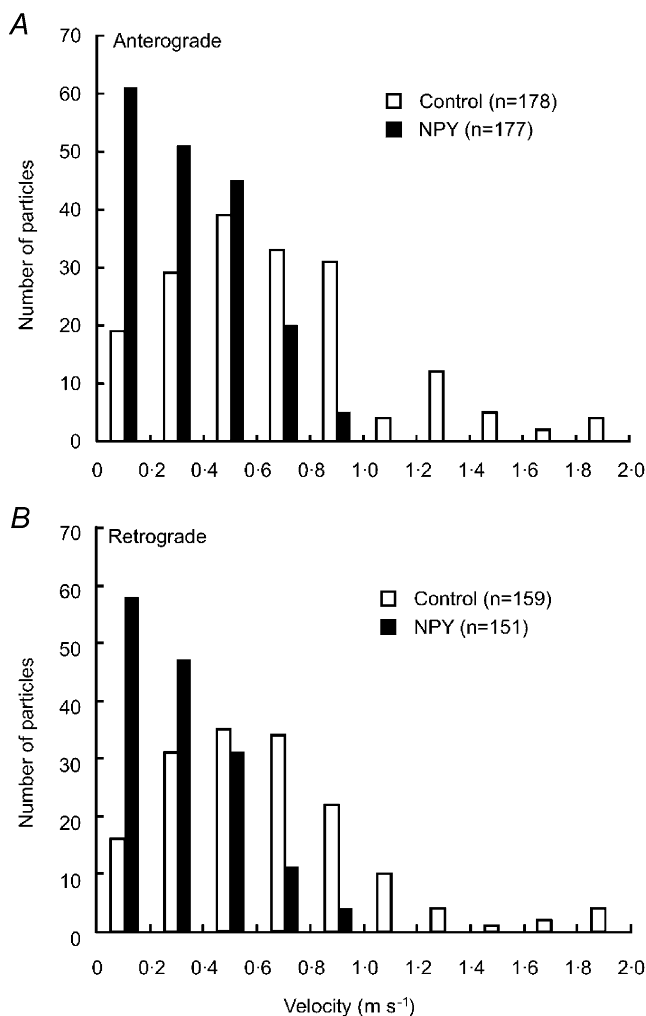

Figure 5. Histogram of instantaneous velocity of individual particles moving in anterograde (*A*) and retrograde (*B*) directions

Data were obtained before (control) and during application of 1 μM NPY.

plateau for the remaining period of the experiment (Fig. 3). Comparison of the values measured before and 15 min after each application indicated that NPY reduced the number of particles in a concentration-dependent manner between 10^{-9} M and 10^{-6} M (Fig. 4). The median inhibitory concentrations (IC_{50}) in anterograde and retrograde directions were 7.5×10^{-9} M and 9×10^{-9} M, respectively (Fig. 4). When the instantaneous velocities of particles were analysed in one of the tested neurones, NPY (1 μM) was found to reduce the instantaneous velocities of particles moving in both anterograde and retrograde directions (Fig. 5).

Effects of NPY receptor agonists

In DRG neurones, NPY Y_1 and Y_2 receptors and their mRNAs are expressed (Mantyh *et al.* 1994; Zhang *et al.* 1994*a*, 1995, 1997; Hökfelt *et al.* 1998; Naveilhan *et al.* 1998; Marchand *et al.* 1999; Zhang *et al.* 1999). We therefore investigated whether these receptor subtypes are involved in the inhibitory response to NPY. Application of the NPY Y_1 receptor agonist [Leu^{31},Pro^{34}]-NPY (1 μM) significantly reduced the number of particles in anterograde and retrograde axonal transport in a similar manner to NPY in each of five neurones tested (Fig. 6*A*). The number decreased to 45 % of control for both directions (Fig. 6*A*). The Y_2 receptor agonist fragment NPY_{13-36} (1 μM) also decreased the number of particles to 60 % of control in both anterograde and retrograde directions in each of the five neurones tested (Fig. 6*B*). These results suggest that both Y_1 and Y_2 receptors probably participate in mediating the NPY-induced inhibition of axonal transport.

Immunocytochemical staining of NPY receptors

To confirm the expression of NPY receptors in cultured DRG cells, an immunocytochemical study using an antiserum against NPY Y_1 receptor protein was performed. A Nomarski image of DRG neurones cultured for 48 h and their immunoreactivity for Y_1 receptors are shown in Fig. 7. Of a total 149 neurones tested, 128 neurones (85.9 %) were positive for Y_1 receptor immunoreactivity, while the remaining 21 neurones (14.1 %) were negative. The percentage of positive cells obtained in this study was higher than that reported by others on DRG neurones *in situ* (~20 %; Mantyh *et al.* 1994; Zhang *et al.* 1994*a*, 1994*b*). Therefore, to investigate the influence of culture on the number of Y_1 receptor immunoreactive

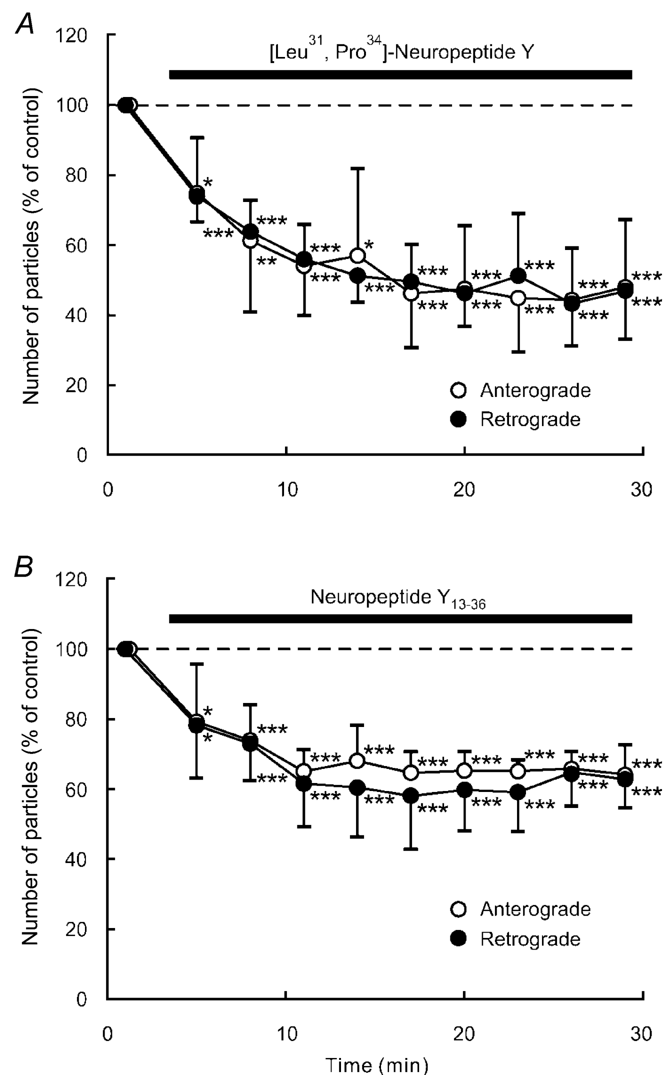

Figure 6. Effects of NPY Y_1 and Y_2 receptor agonists on axonal transport

Percentage changes in the number of transported particles of control (the value before the application) in anterograde (open circles) and retrograde (closed circles) directions induced by the Y_1 receptor agonist [Leu^{31},Pro^{34}]-NPY (1 μM) (*A*) and Y_2 receptor agonist NP_{13-36} (1 μM; *B*). Each data point represents the mean (± S.D.) of the values obtained from five DRG cells. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$ compared to the value before the application.

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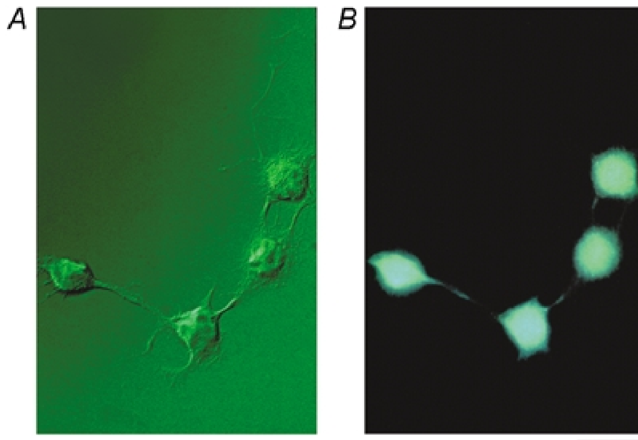

Figure 7. Immunofluorescence of NPY Y_1 receptors in cultured DRG cells

A, Nomarski image of cultured DRG cells. *B*, immunofluorescent image of the same cells using antibody for NPY Y_1 receptor visualised by FITC. Scale bar, 20 μm.

cells, we performed additional immunostaining of DRG cells before (0 h), 24 h, 48 h, and 7 days after the start of culture. The percentages of Y_1 receptor-positive cells were similar throughout the culture period, 78.2 % (241/308) at 0 h, 85.1 % (302/355) at 24 h, 85.4 % (311/364) at 48 h, and 76.7 % (260/339) at 7 days, indicating no influence of the culture period on the expression of Y_1 receptors. Since specific antibodies for NPY Y_2 receptors are not available, expression of Y_2 receptors in cultured DRG cells could not be examined.

Studies on signal transduction mechanisms for the NPY effect

Receptors for NPY are coupled to G proteins, which inhibit adenylate cyclase activity and thereby intracellular cAMP synthesis (Kassis *et al.* 1987; Westlind-Danielsson *et al.* 1987; Aakerlund *et al.* 1990; Michel *et al.* 1990; Michel 1991; Herzog *et al.* 1992; Larhammar *et al.* 1992; Shigeri & Fujimoto, 1992; Wan & Lau, 1995; Larhammar, 1996; Larhammar *et al.* 1998). It has been confirmed that Y_1 and Y_2 receptors are also coupled to these signal transduction pathway (Aakerlund *et al.* 1990; Wan & Lau, 1995; Larhammar, 1996; Larhammar *et al.* 1998). Previous studies on axonal transport have shown that elevation of intracellular cAMP and subsequent activation of protein kinase A activity potentiates axonal transport (Takenaka *et al.* 1994; Takenaka & Kawakami, 1996; Hiruma *et al.* 2000*a*). Thus, we hypothesised that the inhibitory response of axonal transport to NPY is mediated by activation of Gi proteins and by subsequent inhibition of the adenylate cyclase/protein kinase A system. We tested this hypothesis and obtained the following results.

Pre-treatment for 4 h with pertussis toxin (200 ng ml^{-1}), an inhibitor of Gi and Go proteins, completely blocked the inhibitory effect of NPY (1 μM) on anterograde and retrograde axonal transport in each of five DRG neurones tested (Fig. 8), suggesting that the inhibitory effect of NPY is mediated by pertussis toxin-sensitive Gi and/or Go proteins.

Application of SQ-22536 (100 μM), an adenylate cyclase inhibitor, decreased the number of particles in anterograde and retrograde directions in each of five neurones tested (Fig. 9*A*). This decrease reached approximately 50–60 % of control at 11–17 min after the

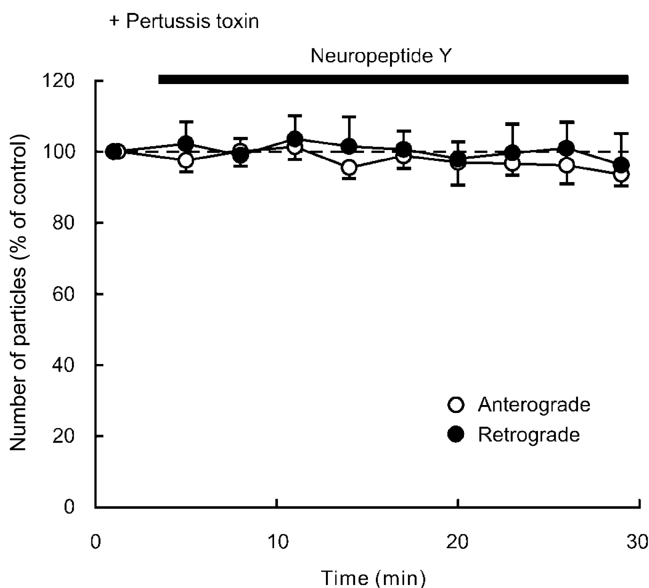

Figure 8. Effects of pre-treatment with pertussis toxin on the response to NPY

Data represent percentage changes in the number of transported particles. Neuropeptide Y (NPY, 1 μM) was applied after pre-treatment (for 4 h) with pertussis toxin (200 ng ml^{-1}), a G protein inhibitor. Note that pre-treatment with pertussis toxin blocked the inhibitory response to NPY.

start of the application, and this low level was sustained throughout the application. Treatment with H-89 (1 μM), a protein kinase A inhibitor, gave similar results to SQ-22536 ($n = 5$; Fig. 10*A*). Prior exposure of a cell to 100 μM SQ-22536 ($n = 5$) or 1 μM H-89 ($n = 5$) occluded the effect of 1 μM NPY (Figs 9*B* and 10*B*). Each application of 1 mM dbcAMP ($n = 5$), a membrane permeable cAMP analogue, and 1 μM forskolin ($n = 5$), an adenylate cyclase activator, resulted in a transient increase in the number of particles transported in both directions (Figs 11*A* and 12*A*). The number of particles peaked at 5–8 min after the start of each application of these drugs, and thereafter gradually declined. The peak values were approximately 140–150 % of control in both dbcAMP- and forskolin-treated cells. The application of 1 mM dbcAMP ($n = 5$) or 1 μM forskolin ($n = 5$) in combination with NPY negated the decreasing effect of NPY alone (Figs 11*B* and 12*B*). Thus, NPY probably inhibited endogenous adenylate cyclase activity, resulting in a decrease in endogenous cAMP levels. Then, the decrease in endogenous cAMP

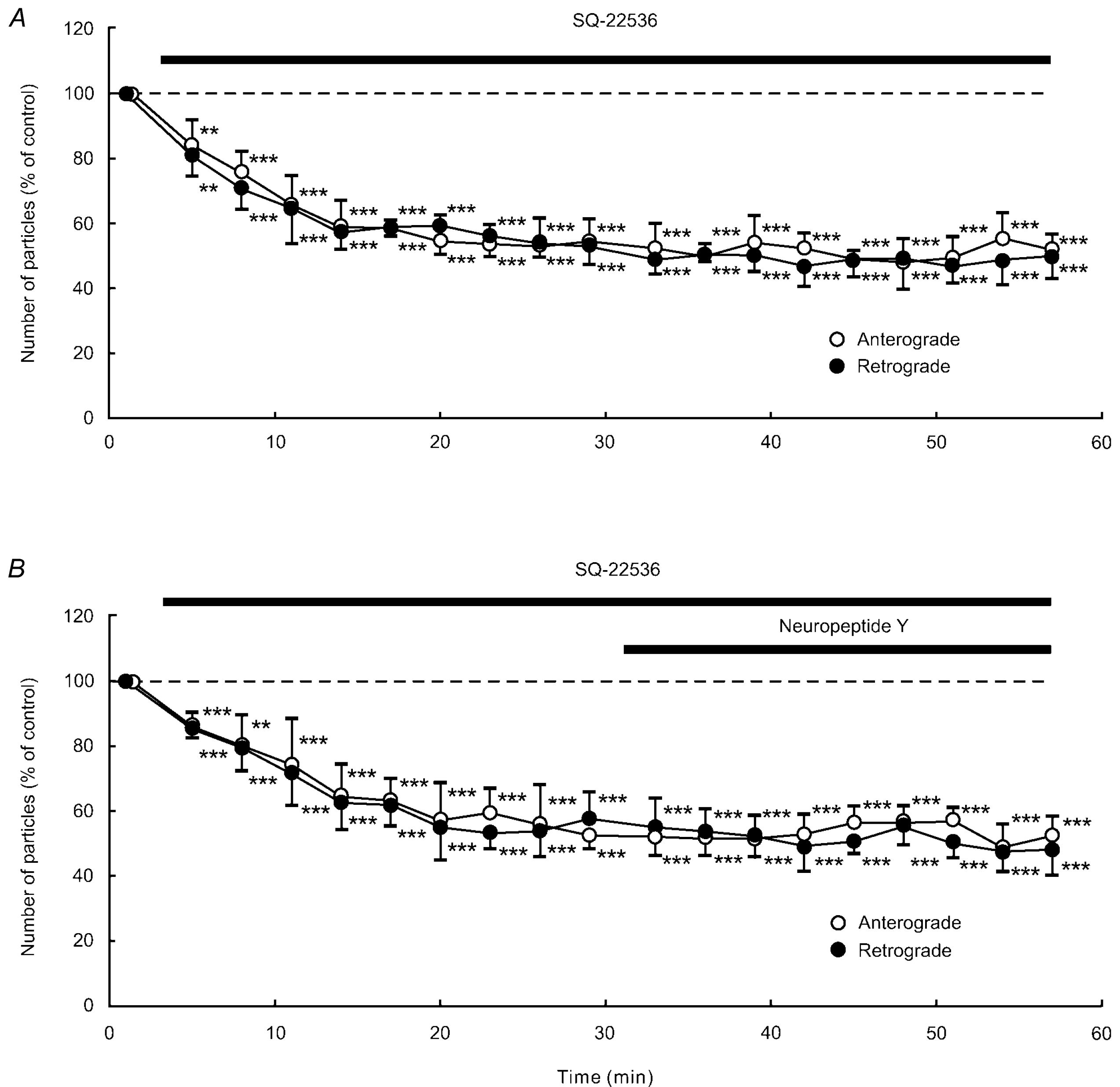

Figure 9. Effects of SQ-22536 (*A*) or NPY after SQ-22536 treatment (*B*)

Data represent percentage changes in the number of transported particles. Cells were treated with the adenylate cyclase inhibitor SQ-22536 (100 μM) alone (*A*) or NPY (1 μM) after SQ-22536 (100 μM) treatment (*B*). Note that SQ-22536 inhibited anterograde and retrograde axonal transport, but the subsequently applied NPY failed to produce a further decrease in axonal transport. Each data point indicates the mean (± S.D.) of the values obtained from five DRG cells. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$ compared to the value before the application of SQ-22536.

levels was counteracted by exogenous cAMP, dbcAMP. These results suggest that the inhibitory response to NPY is presumably mediated by suppression of adenylate cyclase and subsequent reduction in intracellular cAMP levels and protein kinase A activity. Furthermore, these results suggest that the involved G proteins are Gi proteins that inhibit adenylate cyclase activity.

DISCUSSION

Inhibitory effect of NPY on axonal transport

The present results provide direct evidence that NPY inhibits both anterograde and retrograde axonal transport in cultured adult mouse DRG neurones. This response was rapid and reversible, which could be detected by video-enhanced microscopy. The present study also revealed that the inhibitory response to NPY was concentration dependent. The median inhibitory concentrations (IC_{50}) for anterograde and retrograde axonal transport were similar, 7.5×10^{-9} M in an anterograde direction and 9×10^{-9} M in a retrograde direction. The instantaneous velocities in anterograde and retrograde directions were also reduced by NPY. Taking into account these results, NPY seems to act on mechanisms common to both anterograde and retrograde axonal transport. In this respect, the molecular target for the action of NPY on axonal transport is unknown. Since different types of motor proteins are responsible for each anterograde and

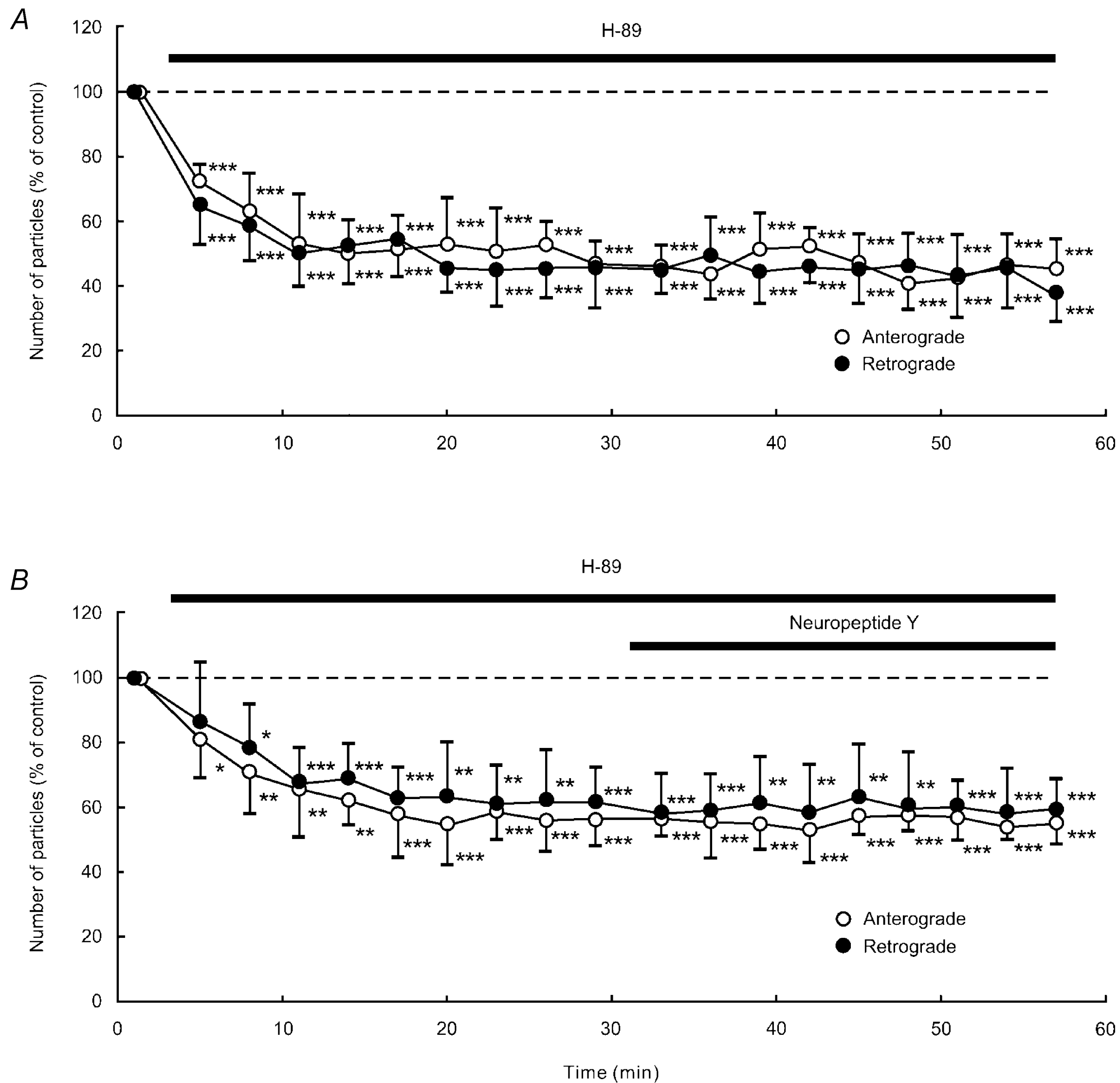

Figure 10. Effects of H-89 (*A*) or NPY after H-89 treatment (*B*)

Data represent percentage changes in the number of transported particles. Cells were treated with the protein kinase A inhibitor H-89 (1 μM) alone (*A*), or NPY (1 μM) after treatment with H-89 (1 μM; *B*). Note that H-89 inhibited anterograde and retrograde axonal transport, but the subsequently applied NPY failed to produce a further decrease in axonal transport. Each data point indicates the mean (± S.D.) of the values obtained from five DRG cells. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$ compared to the value before the application of H-89.

retrograde axonal transport (Hirokawa 1998; Hirokawa *et al.* 1998), NPY is not likely to act on individual motor proteins themselves. Probably, NPY acts on mechanisms interacting with every motor protein, such as energy metabolism or microtubule assembly and disassembly. Further studies are necessary to clarify these mechanisms.

Physiologically, DRG neurones express NPY receptors (Mantyh *et al.* 1994; Zhang *et al.* 1994*a*, 1994*b*, 1995; Hökfelt *et al.* 1998; Marchand *et al.* 1999; Zhang *et al.*1999). Under physiological conditions, NPY is released from sympathetic and parasympathetic neurones (Lundberg *et al.* 1990; Schalling *et al.* 1991), the adrenal medulla (Schalling *et al.* 1991), and intrinsic spinal

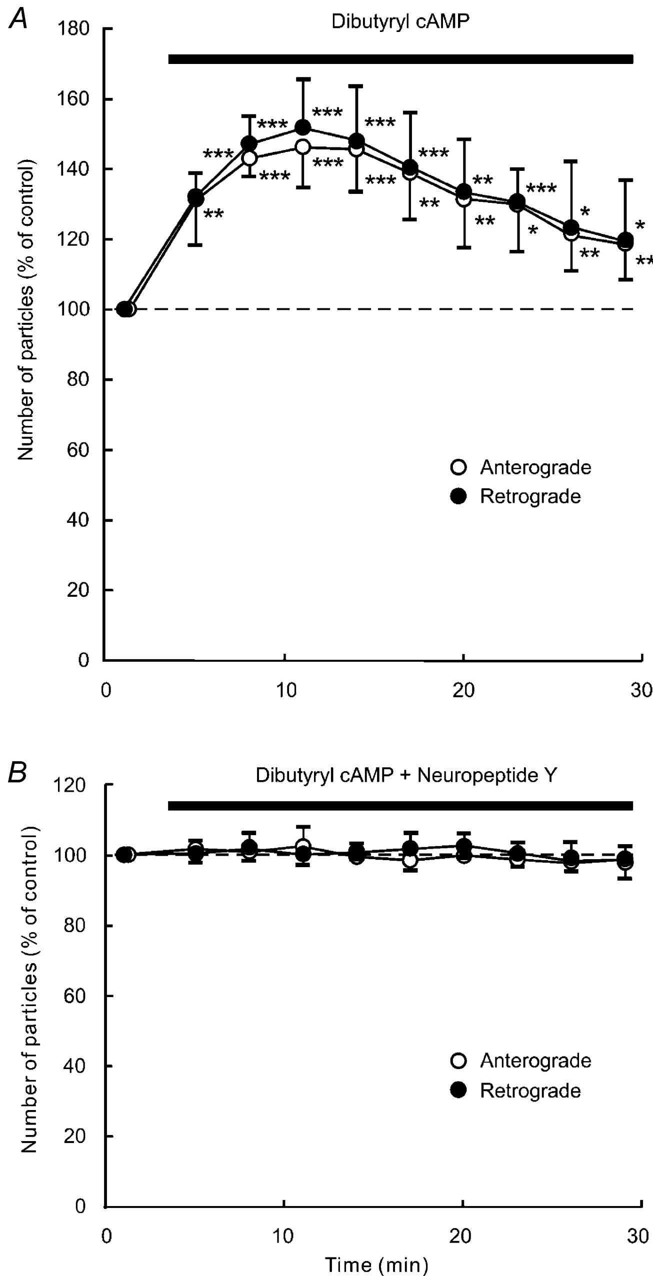

Figure 11. Effects of dbcAMP (*A*) and a combination of dbcAMP and NPY (*B*)

Data represent percentage changes in the number of transported particles. Cells were treated with membrane-permeable cAMP analogue dbcAMP (1 mM) alone (*A*), or a combination of dbcAMP (1 mM) and NPY (1 μM; *B*). Note that NPY counteracted the effect of dbcAMP. Each data point indicates the mean (± S.D.) of the values obtained from five DRG cells. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$ compared to the value before the application.

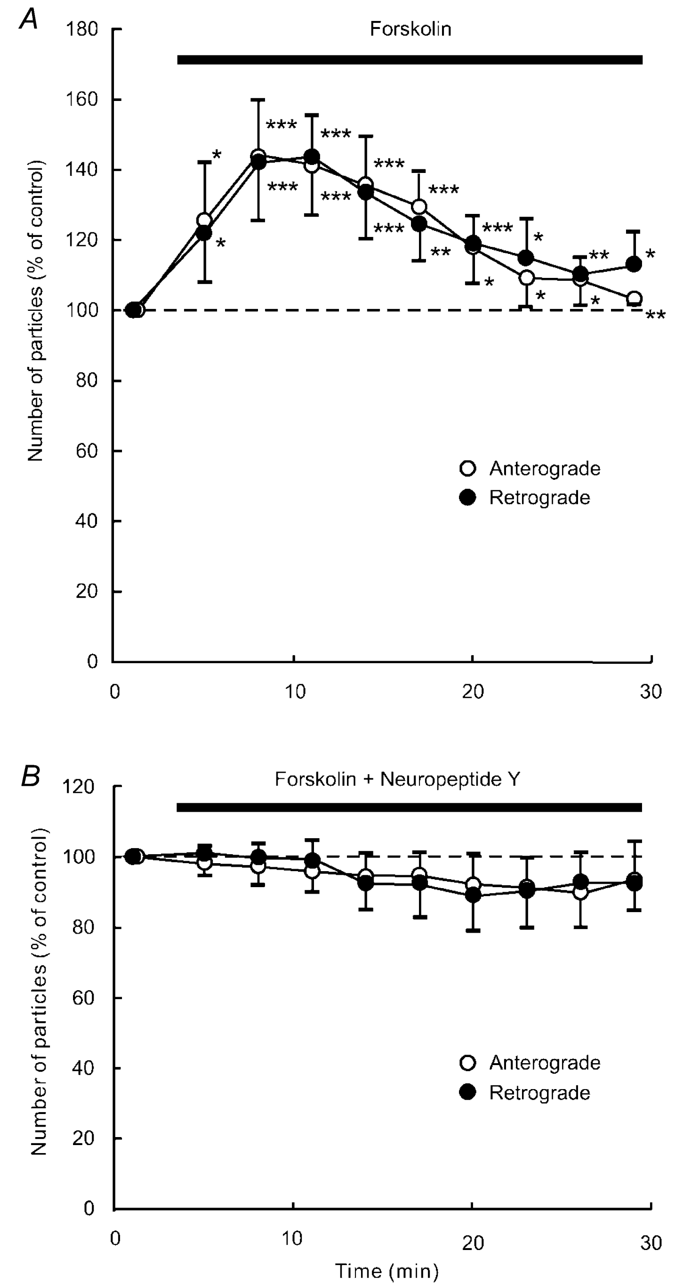

Figure 12. Effects of forskolin (*A*) and a combination of forskolin and NPY (*B*)

Data represent percentage changes in the number of transported particles. Cells were treated with the adenylate cyclase activator forskolin (1 μM) alone (*A*), or a combination of forskolin (1 μM) and NPY (1 μM; *B*). Note that NPY counteracted the effect of forskolin. Each data point indicates the mean (± S.D.) of the values obtained from five DRG cells. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$ compared to the value before the application.

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neurones (Mark *et al.* 1997). It is suggested that NPY released from these tissues can reach the DRG neurones through the circulation (Zhang *et al.* 1994*a*; Hökfelt *et al.* 1998). Thus, the inhibitory action of NPY on axonal transport may be operational under physiological conditions. Alternatively, once peripheral sensory nerves have been damaged by injury or inflammation, the synthesis and release of NPY is markedly changed. Immunoreactivity for NPY is observed in injured-side DRG neurones but not in normal DRG neurones (Wakisaka *et al.* 1991, 1992; Landry *et al.* 2000). In the spinal cord where primary afferent fibres terminate, the expression of NPY is also increased after peripheral nerve injury (Wakisaka *et al.* 1991, 1992). Neuropeptide Y is released from central terminals of DRG neurones after nerve injury (Mark *et al*, 1998), while such release does not occur in a normal state (Mark *et al.* 1997). Thus, NPY may exert its inhibitory effect on axonal transport not only in a normal state but also in an injury state.

It has been suggested that NPY released in response to nerve injury possesses antinociceptive effects (Hua *et al.* 1991; Broqua *et al.* 1996; Naveilhan *et al.* 2001). Application of NPY into the dorsal horn of the spinal cord inhibits the release of substance P from sensory neurones (Duggan *et al.* 1991). Substance P is one of the major transmitters conveying nociceptive signals to the spinal cord (De Koninck & Henry, 1991; Salter & Henry, 1991). Therefore, NPY is implicated to exert an antinociceptive effect by blocking directly the release of transmitters from DRG neurones (Duggan *et al.* 1991). It has been suggested that axonal transport is closely related to transmitter release (Wooten *et al.* 1975; Giachetti & Said, 1979; Lundberg *et al.* 1989; Keast & Stephensen, 2000). In particular, neuropeptides, synthesised within the cell body, are axonally transported toward axon terminals (Giachetti & Said, 1979; Steiner *et al.* 1984; Lundberg *et al.* 1989; Sossin & Scheller, 1991). In fact, blockade of axonal transport by colchicine reduces the release of sensory neuropeptides (Lecci *et al.* 1996). Thus, the present results are in agreement with the inhibitory action of NPY on peptide release described by others (Duggan *et al.* 1991).

Cellular mechanisms of the NPY effect

The present study has also clarified the cellular mechanisms for the inhibitory effect of axonal transport induced by NPY. The present immunocytochemical study revealed that a large portion (85.9 %) of DRG cells cultured for 48 h were immunoreactive for the NPY Y_1 receptor. The NPY Y_1 agonist [Leu^{31},Pro^{34}]-NPY and the NPY Y_2 agonist NPY_{13-36} both inhibited anterograde and retrograde axonal transport. Thus, both Y_1 and Y_2 receptors are likely to participate in mediating the inhibitory effect of NPY on axonal transport.

We further investigated the signal transduction mechanisms involved in the NPY-induced inhibition of axonal transport. Pre-treatment with pertussis toxin, a G protein inhibitor, blocked the inhibitory effect of NPY on axonal transport. Each application of the adenylate cyclase inhibitor SQ-22536 and the protein kinase A inhibitor H-89 mimicked and occluded the effect of NPY. The membrane-permeable cAMP, dbcAMP, and the adenylate cyclase activator, forskolin, each produced a transient increase in axonal transport. The application of dbcAMP or forskolin in combination with NPY negated the decreasing effect of NPY alone. Thus, it is assumed that NPY may reduce adenylate cyclase activity, causing a decrease in endogenous cAMP. The decrease in endogenous cAMP can be counteracted by the action of exogenous cAMP, dbcAMP. These results suggest that the NYP receptor-mediated inhibition of axonal transport results from the activation of Gi proteins and subsequent inhibition of adenylate cyclase and protein kinase A activity. This is consistent with previous studies showing that all NPY receptors including Y_1 and Y_2 receptors are coupled to G proteins and their activation leads to inhibition of cAMP synthesis (Kassis *et al.* 1987; Westlind-Danielsson *et al.* 1987; Aakerlund *et al.* 1990; Michel *et al.* 1990; Wahlestedt *et al.* 1990; Michel, 1991; Larhammar *et al.* 1992; Herzog *et al.* 1992; Shigeri & Fujimoto, 1992; Wan & Lau, 1995; Larhammar, 1996; Larhammar *et al.* 1998). The present results are also supported by previous studies on axonal transport. It has been shown that membrane-permeable cAMP increases both anterograde and retrograde axonal transport in certain types of cultured neurones (Takenaka *et al.* 1994; Hashimoto *et al.* 1997) including mouse DRG neurones (Hiruma *et al.* 2000*a*). Furthermore, the adenylate cyclase/protein kinase A cascade has been proposed as an important pathway that regulates both anterograde and retrograde axonal transport (Takenaka *et al.* 1994; Takenaka & Kawakami, 1996; Hiruma *et al.* 2000*a*). The present study as well as previous studies noted that the increasing effect of dbcAMP and forskolin was transient, while the inhibitory effect of application of protein kinase A inhibitors such as H-89 and KT5720 was sustained during the application. The reason for this is not yet clear. Perhaps, because the intracellular energy supply should be limited, the excessive increase in axonal transport may be suppressed by mechanisms for effective use of energy.

We have previously shown that some neuropeptides and mediators regulate axonal transport of DRG neurones. Substance P (Hiruma *et al.* 2000*b*) and histamine (Amano *et al.* 2001) inhibit axonal transport, whereas calcitonin gene-related peptide (Hiruma *et al.* 2000*b*) and prostaglandin E_2 (Hiruma *et al.* 2000*a*) enhance axonal transport. Alternatively, we have clarified that various intracellular mechanisms are responsible for the regulation of axonal transport. For example, axonal transport is inhibited by increases in concentrations of intracellular Ca^{2+} (Kanai *et al.* 2001) or Cl^- (Hiruma *et al.*

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1999*b*), by suppression of protein kinase C (Hiruma *et al.* 1999*a*), or by activation of Ca^{2+}/calmodulin protein kinase II (Kanai *et al.* 2001). As described above, the activation of cAMP-dependent protein kinase A, which is related to prostaglandin E_2 (Hiruma *et al.* 2000*a*), enhances axonal transport. Although it has not yet been studied, various neuropeptides, neurotransmitters, and chemical mediators are each possible links to such intracellular signallings for the regulation of axonal transport. The present study firstly shows the role of NPY on axonal transport and its signal transduction mechanisms, i.e. the inhibition of the cAMP/adenylate cyclase/protein kinase A pathway.

As described above, the present immunocytochemical study using an antiserum against NPY Y_1 receptor proteins revealed that the majority (85.9 %) of cultured mouse DRG cells were positive for NPY Y_1 receptors. In the present experiment on axonal transport, NPY and its agonists were effective on each neurone tested. In agreement with the present data, electrophysiological studies reported by Walker *et al.* (1988) indicated that NPY inhibited Ca^{2+} current in every cultured DRG cell tested. However, previous ligand binding studies have shown that NPY receptors are expressed in only 5–20 % of DRG neurones *in situ* in normal rats, rabbits, and monkeys (Mantyh *et al.* 1994). *In situ* hybridisation study has also shown that NPY Y_1 receptor mRNA is expressed in 20 % of normal DRG neurones of the rat (Zhang *et al.* 1994*a*). Similarly, immunohistochemical studies using antibody to the C-terminal portion of the Y_1 receptor have shown that approximately 20–25 % of the total neurones of rat dorsal root ganglia *in situ* are Y_1 receptor immunoreactive in rats (Zhang *et al.* 1994*a*). One possible explanation for such differences in the ratio of NPY receptor expression is that it is due to the preparations where the cell size is differently distributed. It has been described that Y_1 receptors are present mainly in small-sized (Zhang *et al.* 1994*a*, 1994*b*, 1995, 1999) and small- and medium-sized (Marchand *et al.* 1999) DRG neurones in a normal state. In isolated and cultured DRG cells of the adult rat, large DRG cells with diameter $> 50\ \mu m$ or with cell area $> 1000\ \mu m^2$ are rare (Gold *et al.* 1996; Hu *et al.* 1997; Segond von Banchet *et al.* 1999), whereas more than 20 % of the adult rat DRG neurones are such large-sized cells *in situ* (Battaglia & Rustioni, 1988; McCarthy & Lawson, 1990; Mantyh *et al.* 1994; Zhang *et al.* 1997). In our preparations, approximately 3 % of cultured DRG cells were large cells with diameter $> 50\ \mu m$. Since large cells are lost in the process of cell isolation as described by Gold *et al.* (1996), the ratio of Y_1-positive cells detected in this study may be higher than that obtained by *in situ* study. Our results indicated that the proportion of Y_1-positive cells was not affected by the period of culture. Therefore, culture conditions do not seem to influence the expression of the Y_1 receptor.

In conclusion, we have demonstrated that NPY, acting at NPY Y_1 and Y_2 receptors, inhibits anterograde and retrograde axonal transport. The effect seems to be mediated by pertussis toxin-sensitive G proteins and the adenylate cyclase/protein kinase A pathway. These results suggest that NPY is one of the modulatory factors for axonal transport under physiological and pathological conditions.

REFERENCES

Aakerlund, L., Gether, U., Fuhlendorff, J., Schwartz, T. W. & Thastrup, O. (1990). Y_1 receptors for neuropeptide Y are coupled to mobilization of intracellular calcium and inhibition of adenylate cyclase. *FEBS Letters* **260**, 73–7 8.

Amano, R., Hiruma, H., Nishida, S., Kawakami, T. & Shimizu, K. (2001). Inhibitory effect of histamine on axonal transport in cultured mouse dorsal root ganglion neurons. *Neuroscience Research* **41**, 201–206.

Battaglia, G. & Rustioni, A. (1988). Coexistence of glutamate and substance P in dorsal root ganglion neurons of the rat and monkey. *Journal of Comparative Neurology* **277**, 302–312.

Brimijoin, S., Lundberg, J. M., Brodin, E., Hökfelt, T. & Nilsson, G. (1980). Axonal transport of substance P in the vagus and sciatic nerves of the guinea pig. *Brain Research* **191**, 443–457.

Broqua, P., Wettstein, J. G., Rocher, M. N., Gauthier-martin, B., Riviere, P. J. . M., Junien, J.-L. & Dahl, S. G. (1996). Antinociceptive effects of neuropeptide Y and related peptides in mice. *Brain Research* **724**, 25–32.

De Koninck, Y. & Henry, J. L. (1991). Substance P-mediated slow excitatory postsynaptic potential elicited in dorsal horn neurons *in vivo* by noxious stimulation. *Proceedings of the National Academy of Sciences of the USA* **88**, 11344–1134 8.

Duggan, A. W., Hope, P. J. & Lang, C. W. (1991). Microinjection of neuropeptide Y into the superficial dorsal horn reduces stimulus-evoked release of immunoreactive substance P in the anaesthetized cat. *Neuroscience* **44**, 733–740.

Fernandez, H. L. & Hodges-Savola, C. A. (1994). Axoplasmic transport of calcitonin gene-related peptide in rat peripheral nerve as a function of age. *Neurochemical Research* **19**, 1369–1377.

Forman, D. S., Lynch, K. J. & Smith, R. S. (1987). Organelle dynamics in lobster axons: anterograde, retrograde and stationary mitochondria. *Brain Research* **412**, 96–106.

Giachetti, A. & Said, S. I. (1979). Axonal transport of vasoactive intestinal peptide in sciatic nerve. *Nature* **281**, 574–57 5.

Gold, M. S., Dastmalchi, S. & Levine, J. D. (1996). Co-expression of nociceptor properties in dorsal root ganglion neurons from the adult rat *in vitro*. *Neuroscience* **71**, 265–275.

Harmar, A. & Keen, P. (1982). Synthesis, and central and peripheral axonal transport of substance P in a dorsal root ganglion-nerve preparation *in vitro*. *Brain Research* **231**, 379–385.

Hashimoto, Y., Hori, H., Kawakami, T., Kusakabe, T. & Takenaka, T. (1997). Effect of dibutyryl cyclic AMP on axoplasmic transport in the hippocampus. *Brain Research* **755**, 343–346.

Herzog, H., Hort, Y. J., Ball, H. J., Hayes, G., Shine, J. & Selbie, L. A. (1992). Cloned human neuropeptide Y receptor couples to two different second messenger systems. *Proceedings of the National Academy of Sciences of the USA* **89**, 5794–579 8.

Hirokawa, N. (1998). Kinesin and dynein superfamily proteins and the mechanism of organelle transport. *Science* **279**, 519–5 26.

Journal of Physiology

Hirokawa, N., Noda, Y. & Okada, Y. (1998). Kinesin and dynein superfamily proteins in organelle transport and cell division. *Current Opinion in Cell Biology* **10**, 60–73.

Hiruma, H., Ichikawa, T., Kobayashi, H., Hoka, S., Takenaka, T. & Kawakami, T. (2000*a*). Prostaglandin E2 enhances axonal transport and neuritogenesis in cultured mouse dorsal root ganglion neurons. *Neuroscience* **100**, 885–891.

Hiruma, H., Maruyama, H., Katakura, T., Simada, Z. B., Nishida, S., Hoka, S., Takenaka, T. & Kawakami, T. (1999*a*). Axonal transport is inhibited by a protein kinase C inhibitor in cultured isolated mouse dorsal root ganglion cells. *Brain Research* **826**, 135–138.

Hiruma, H., Nishida, S., Katakura, T., Kusakabe, T., Takenaka, T. & Kawakami, T. (1999*b*). Extracellular potassium rapidly inhibits axonal transport of particles in cultured mouse dorsal root ganglion neurites. *Journal of Neurobiology* **38**, 225–233.

Hiruma, H., Saito, A., Ichikawa, T., Kiriyama, Y., Hoka, S., Kusakabe, T., Kobayashi, H. & Kawakami, T. (2000*b*). Effects of substance P and calcitonin gene-related peptide on axonal transport in isolated and cultured adult mouse dorsal root ganglion neurons. *Brain Research* **883**, 184–191.

Hökfelt, T., Broberger, C., Zhang, X., Diez, M., Kopp, J., Xu, Z.-Q., Landry, M., Bao, L., Schalling, M., Koistinaho, J., Dearmond, S. J., Prusiner, S., Gong, J. & Walsh, J. H. (1998). Neuropeptide Y: some viewpoints on a multifaceted peptide in the normal and diseased nervous system. *Brain Research Reviews* **26**, 154–1 66.

Hu, H.-Z., Li, Z.-W. & Si, J.-Q. (1997). Evidence for the existence of substance P autoreceptor in the membrane of rat dorsal root ganglion neurons. *Neuroscience* **77**, 535–541.

Hua, X.-Y., Boublik, J. H., Spicer, M. A., Rivier, J. E., Brown, M. R. & Yaksh, T. L. (1991). The antinociceptive effects of spinally administered neuropeptide Y in the rat: systematic studies on structure-activity relationship. *Journal of Pharmacology and Experimental Therapeutics* **258**, 243–248.

Kanai, A., Hiruma, H., Katakura, T., Sase, S., Kawakami, T. & Hoka, S. (2001). Low-concentration lidocaine rapidly inhibits axonal transport in cultured mouse dorsal root ganglion neurons. *Anesthesiology* **95**, 675–680.

Kassis, S., Olasmaa, M., Terenius, L. & Fishman, P. H. (1987). Neuropeptide Y inhibits cardiac adenylate cyclase through a pertussis toxin-sensitive G protein. *Journal of Biological Chemistry* **262**, 3429–3431.

Kashihara, Y., Sakaguchi, M. & Kuno, M. (1989). Axonal transport and distribution of endogenous calcitonin gene-related peptide in rat peripheral nerve. *Journal of Neuroscience* **9**, 3796–3802.

Keast, J. R. & Stephensen, T. M. (2000). Glutamate and aspartate immunoreactivity in dorsal root ganglion cells supplying visceral and somatic targets and evidence for peripheral axonal transport. *Journal of Comparative Neurology* **424**, 577–587.

Keen, P., Harmar, A. J., Spears, F. & Winter, E. (1982). Biosynthesis, axonal transport and turnover of neuronal substance P. *Ciba Foundation Symposium.* **91**, 145–164.

Landry, M., Holmberg, K., Zhang, X. & Hökfelt, T. (2000). Effect of axotomy on expression of NPY, galanin, and NPY Y_1 and Y_2 receptors in dorsal root ganglia and the superior cervical ganglion studied with double-labeling in situ hybridization and immunohistochemistry. *Experimental Neurology* **162**, 361–384.

Larhammar, D. (1996). Structural diversity of receptors for neuropeptide Y, peptide YY and pancreatic polypeptide. *Regulatory Peptides* **65**, 165–174.

Larhammar, D., Blomqvist, A. G., Yee, F., Jazin, E., Yoo, H. & Wahlested, C. (1992). Cloning and functional expression of a human neuropeptide Y/peptide YY receptor of the Y1 type. *Journal of Biological Chemistry* **267**, 10935–10938.

Larhammar, D., Söderberg, C. & Lundell, I. (1998). Evolution of the neuropeptide Y family and its receptors. *Annals of the New York Academy of Sciences* **839**, 35–40.

Lecci, A., Patacchini, R., De Giorgio, R., Corinaldesi, R., Theodorsson, E., Giuliani, S., Santicioli, P. & Maggi, C. A. (1996). Functional, biochemical and anatomical changes in the rat urinary bladder induced by perigangliar injection of colchicine. *Neuroscience* **71**, 285–296.

Lundberg, J. M., Franco-Cereceda, A., Lacroix, J.-S. & Pernow, J. (1990). Neuropeptide Y and sympathetic neurotransmission. *Annals of the New York Academy of Sciences* **611**, 166–174.

Lundberg, J. M., Rudehill, A., Sollevi, A., Fried, G. & Wallin, G. (1989). Co-release of neuropeptide Y and noradrenaline from pig spleen *in vivo* : importance of subcellular storage, nerve impulse frequency and pattern, feedback regulation and resupply by axonal transport. *Neuroscience* **28**, 475–486.

McCarthy, P. W. & Lawson, S. N. (1990). Cell type and conduction velocity of rat primary sensory neurons with calcitonin gene-related peptide-like immunoreactivity. *Neuroscience* **34**, 623–632.

Mantyh, P. W., Allen, C. J., Rogers, S., Demaster, E., Ghilardi, J. R., Mosconi, T., Kruger, L., Mannon, P. J., Taylor, I. L. & Vigna, S. R. (1994). Some sensory neurons express neuropeptide Y receptors: potential paracrine inhibition of primary afferent nociceptors following peripheral nerve injury. *Journal of Neuroscience* **14**, 3958–39 68.

Marchand, J. E., Cepeda, M. S., Carr, D. B., Wurm, W. H. & Kream, R. M. (1999). Alterations in neuropeptide Y, tyrosine hydroxylase, and Y-receptor subtype distribution following spinal nerve injury to rats. *Pain* **79**, 187–200.

Mark, M. A., Colvin, L. A. & Duggan, A. W. (1998). Spontaneous release of immunoreactive neuropeptide Y from the central terminals of large diameter primary afferents of rats with peripheral nerve injury. *Neuroscience* **83**, 581–589.

Mark, M. A., Jarrott, B., Colvin, L. A., MacMillan, S. J. A. & Duggan, A. W. (1997). The release of immunoreactive neuropeptide Y in the spinal cord of the anaesthetized rat and cat. *Brain Research* **754**, 195–203.

Michel, M. C. (1991). Receptors for neuropeptide Y: multiple subtypes and multiple second messengers. *Trends in Pharmacological Sciences* **12**, 389–394.

Michel, M. C., Schlicker, E., Fink, K., Boublik, J. H., Göthert, M., Willette, R. N., Daly, R. N., Hieble, J. P., Rivier, J. E. & Motulsky, H. J. (1990). Distinction of NPY receptors *in vitro* and *in vivo*. I. NPY-(18–36). discriminates NPY receptor subtypes *in vitro*. *American Journal of Physiology* **259**, E131–139.

Naveilhan, P., Hassani, H., Lucas, G., Blakeman, K. H., Hao, J.-X., Xu, X.-J., Wiesenfeld-Hallin, Z., Thorén, P. & Ernfors, P. (2001). Reduced antinociception and plasma extravasation in mice lacking a neuropeptide Y receptor. *Nature* **409**, 513–517.

Naveilhan, P., Neveu, I., Arenas, E. & Ernfors, P. (1998). Complementary and overlapping expression of Y1, Y2 and Y5 receptors in the developing and adult mouse nervous system. *Neuroscience* **87**, 289–302.

Okada, Y., Sato-Yoshitake, R. & Hirokawa, N. (1995). The activation of protein kinase A pathway selectively inhibits anterograde axonal transport of vesicles but not mitochondria transport or retrograde transport *in vivo*. *Journal of Neuroscience* **15**, 3053– 3064.

Journal of Physiology

SALTER, M. W. & HENRY, J. L. (1991). Responses of functionally identified neurones in the dorsal horn of the cat spinal cord to substance P, neurokinin A and physalaemin. *Neuroscience* **43**, 601–610.

SCHALLING, M., FRANCO-CERECEDA, A., HEMSÉN, A., DAGERLIND, Å., SEROOGY, K., PERSSON, H., HÖKFELT, T. & LUNDBERG, J. M. (1991). Neuropeptide Y and catecholamine synthesizing enzymes and their mRNAs in rat sympathetic neurons and adrenal glands: studies on expression, synthesis and axonal transport after pharmacological and experimental manipulations using hybridization techniques and radioimmunoassay. *Neuroscience* **41**, 753–766.

SEGOND VON BANCHET, G., PETERSEN, M. & SCHAIBLE, H.-G. (1999). Expression of neurokinin-1 receptors on cultured dorsal root ganglion neurons from the adult rat. *Neuroscience* **90**, 677–684.

SHIGERI, Y. & FUJIMOTO, M. (1992). Two different signal transductions of neuropeptide Y1 receptor in SK-N-MC cells. *Biochemical and Biophysical Research Communications* **187**, 1565–1571.

SOSSIN, W. S. & SCHELLER, R. H. (1991). Biosynthesis and sorting of neuropeptides. *Current Opinion in Neurobiology* **1**, 79–83.

STEINER, D. F., DOCHERTY, K. & CARROLL, R. (1984). Golgi/granule processing of peptide hormone and neuropeptide precursors: a minireview. *Journal of Cellular Biochemistry* **24**, 121–130.

TAKENAKA, T. & KAWAKAMI, T. (1996). Signal transduction mechanism responsible for changes in axoplasmic transport caused by neurotransmitters. *Neurochemical Research* **21**, 553–556.

TAKENAKA, T., KAWAKAMI, T., HIKAWA, N. & GOTOH, H. (1990). Axoplasmic transport of mitochondria in cultured dorsal root ganglion cells. *Brain Research* **528**, 285–290.

TAKENAKA, T., KAWAKAMI, T., HORI, H. & BANDOU, Y. (1994). Effect of neurotransmitters on axoplasmic transport: how adrenaline affects superior cervical ganglion cells. *Brain Research* **643**, 81–85.

WAHLESTEDT, C., GRUNDEMAR, L., HÅKANSON, R., HEILIG, M., SHEN, G. H., ZUKOWSKA-GROJEC, Z. & REIS, D. J. (1990). Neuropeptide Y receptor subtypes, Y1 and Y2. *Annals of the New York Academy of Sciences* **611**, 7–26.

WAKISAKA, S., KAJANDER, K. C. & BENNETT, G. J. (1991). Increased neuropeptide Y (NPY)-like immunoreactivity in rat sensory neurons following peripheral axotomy. *Neuroscience Letters* **124**, 200–203.

WAKISAKA, S., KAJANDER, K. C. & BENNETT, G. J. (1992). Effects of peripheral nerve injuries and tissue inflammation on the levels of neuropeptide Y-like immunoreactivity in rat primary afferent neurons. *Brain Research* **598**, 349–352.

WALKER, M. W., EWALD, D. A., PERNEY, T. M. & MILLER, R. J. (1988). Neuropeptide Y modulates neurotransmitter release and Ca^{2+} currents in rat sensory neurons. *Journal of Neuroscience* **8**, 2438–2446.

WAN, C. P. & LAU, B. H. S. (1995). Neuropeptide Y receptor subtypes. *Life Sciences* **56**, 1055–1064.

WESTLIND-DANIELSSON, A., UNDÉN, A., ABENS, J., ANDELL, S. & BARTFAI, T. (1987). Neuropeptide Y receptors and the inhibition of adenylate cyclase in the human frontal and temporal cortex. *Neuroscience Letters* **74**, 237–242.

WOOTEN, G. F., KOPIN, I. J. & AXELROD, J. (1975). Effects of colchicine and vinblastine on axonal transport and transmitter release in sympathetic nerves. *Annals of the New York Academy of Sciences* **253**, 528–534.

ZARBIN, M. A., WAMSLEY, J. K. & KUHAR, M. J. (1990). Anterograde transport of opioid receptors in rat vagus nerves and dorsal roots of spinal nerves: pharmacology and sensitivity to sodium and guanine nucleotides. *Experimental Brain Research* **81**, 267–278.

ZHANG, X., BAO, L., XU, Z.-Q., KOPP, J., ARVIDSSON, U., ELDE, R. & HÖKFELT, T. (1994*a*). Localization of neuropeptide Y Y1 receptors in the rat nervous system with special reference to somatic receptors on small dorsal root ganglion neurons. *Proceedings of the National Academy of Sciences of the USA* **91**, 11738–11742.

ZHANG, X., JI, R.-R., NILSSON, S., VILLAR, M., UBINK, R., JU, G., WIESENFELD-HALLIN, Z. & HÖKFELT, T. (1995). Neuropeptide Y and galanin binding sites in rat and monkey lumbar dorsal root ganglia and spinal cord and effect of peripheral axotomy. *European Journal of Neuroscience* **7**, 367–380.

ZHANG, X., SHI, T., HOLMBERG, K., LANDRY, M., HUANG, W., XIAO, H., JU, G. & H&OACUTE;KFELT, T. (1997). Expression and regulation of the neuropeptide Y Y2 receptor in sensory and autonomic ganglia. *Proceedings of the National Academy of Sciences of the USA* **94**, 729–734.

ZHANG, X., TONG, Y.-G., BAO, L. & HÖKFELT, T. (1999). The neuropeptide Y Y1 receptor is a somatic receptor on dorsal root ganglion neurons and a postsynaptic receptor on somatostatin dorsal horn neurons. *European Journal of Neuroscience* **11**, 2211–2225.

ZHANG, X., WIESENFELD-HALLIN, Z. & HÖKFELT, T. (1994*b*). Effect of peripheral axotomy on expression of neuropeptide Y receptor mRNA in rat lumbar dorsal root ganglia. *European Journal of Neuroscience* **6**, 43–57.

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