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REGULAR ARTICLE

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Axonal and dendritic transport in Purkinje cells of cerebellar slice cultures studied by microinjection of horseradish peroxidase

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Abstract Axonal and dendritic transport in single Purkinje neurons of cerebellar slice cultures was quantified as single transport distances. Examination of the cells within a vital tissue was regarded as being an approach to the in situ condition. The Purkinje cells were organotypically integrated in the in vitro tissues and extended long axonal projections connecting synapses to the target neurons. The tracer horseradish peroxidase (HRP) was applied via microinjection to the somata of the Purkinje cells and the injected neurons were incubated thereafter for defined time-intervals. The tracer was transported anterogradely into the neuron processes. The measurements on both the axonal and the dendritic transport of microinjected HRP revealed continuous transportation with increasing times of postincubation. This transport was reduced by the use of microtubule-depolymerizing drugs. The axonal transport of the tracer was either retarded in colchicine-treated cells or continuously reduced for up to 50% in vinblastine-treated neurons. Thus, a correlation of axonal transport to the microtubules was demonstrated. The dendrites were filled with the tracer after 60 min of postincubation. Dendritic transport was reduced by the use of vinblastine, and not significantly by colchicine. The results strongly support the dependence of neuronal transport on microtubules as a component of the cytoskeleton.

Key words Axonal transport · Purkinje cell · Organotypic culture · Microinjection · Antimitotic drugs · Cytoskeleton · Dendritic transport · Rat

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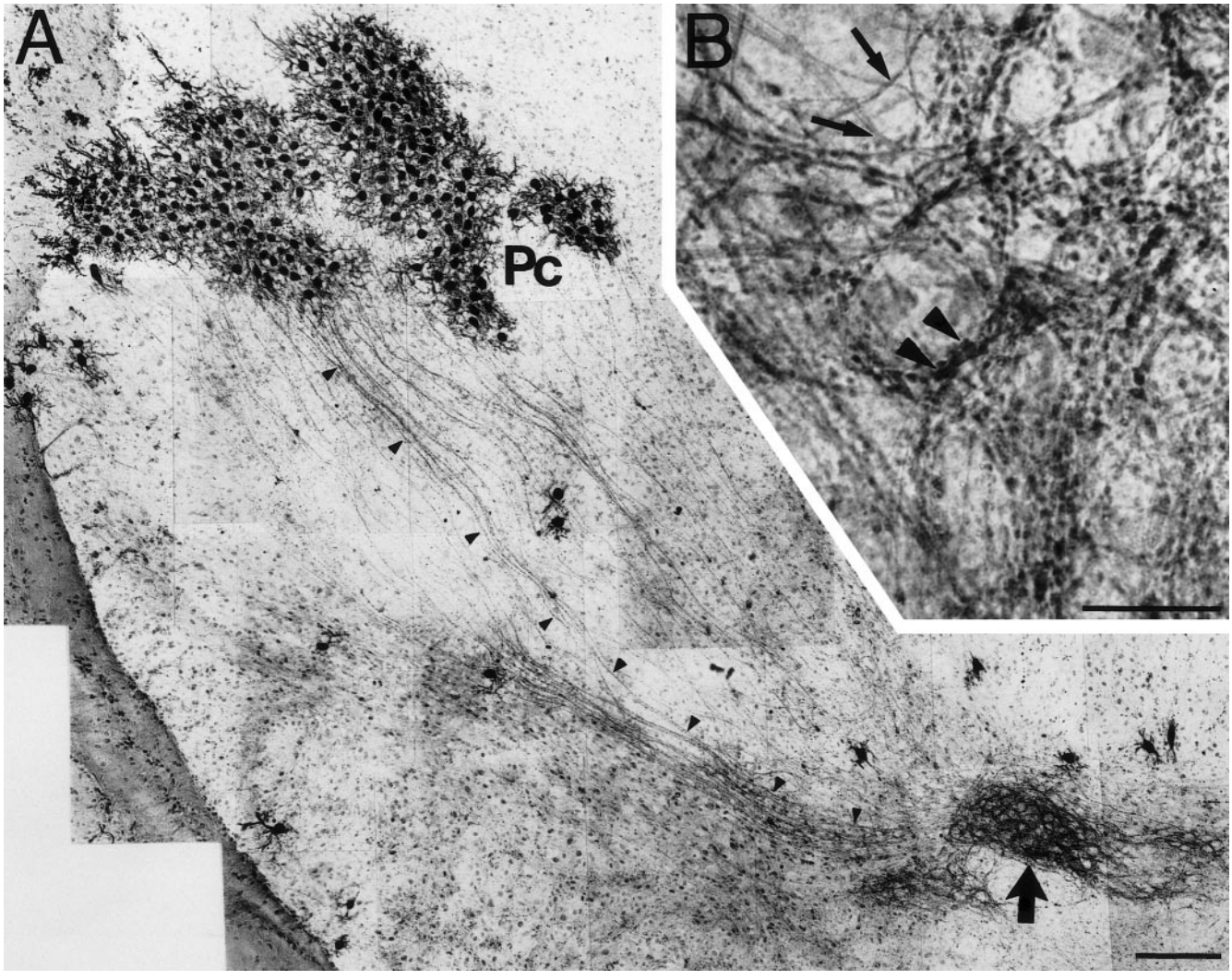
Introduction

Axonal transport is characterized by movements that, independent of electrical activity, carry macromolecules, vesicles or organelles from their sites of synthesis in the neuron somata to sites of utilization at synaptic contacts at the axon ending. This transport is an active process involving elements of the neuronal cytoskeleton. The role of microtubules has been extensively investigated (for a review, see Hammerschlag et al. 1994); they might be used as tracks for the transported material (Price et al. 1991). Transported vesicles are connected to the microtubules and movement is mediated by the motorproteins kinesin and dynein (Brady 1985, 1991; Shpetner et al. 1988; Schnapp and Reese 1989; Cyr and Brady 1992; Hirokawa 1996; Larcher et al. 1996; Lin et al. 1996). Kinesins are thought to be microtubule sidearms (Hirokawa et al. 1985).

Similarly, dendritic transport has been correlated with microtubules. Since the organization of the dendritic microtubules is different from their axonal counterparts, e.g. there is no uniform polarity of the plus-ends, a specific differentiation between active transport in axons and dendrites has been postulated (Overly et al. 1996).

Early investigations on axonal transport were based on the whole nerve comprising many thousands of axons in which movements were visualized by the application of radioactively labelled amino acids (Ochs and Burger 1958; Lasek et al. 1968). Various types of axonal transport, e.g. retrograde transport (Kristensson et al. 1971; Lasek 1968), slow anterograde transport (Lasek 1968, 1970) and fast anterograde transport (Lasek 1970), were classified. The structural hypothesis of axonal transport was postulated in which all proteins were regarded as transported subcellular membranous vesicles (Tytell et al. 1981; Lasek and Brady 1982).

A novel approach to axonal transport was the study of single axons. One method involved the extraction of the axoplasm from the squid giant axon combined with video-enhanced differential interference contrast light microscopy, which displayed the movement of particles along micro-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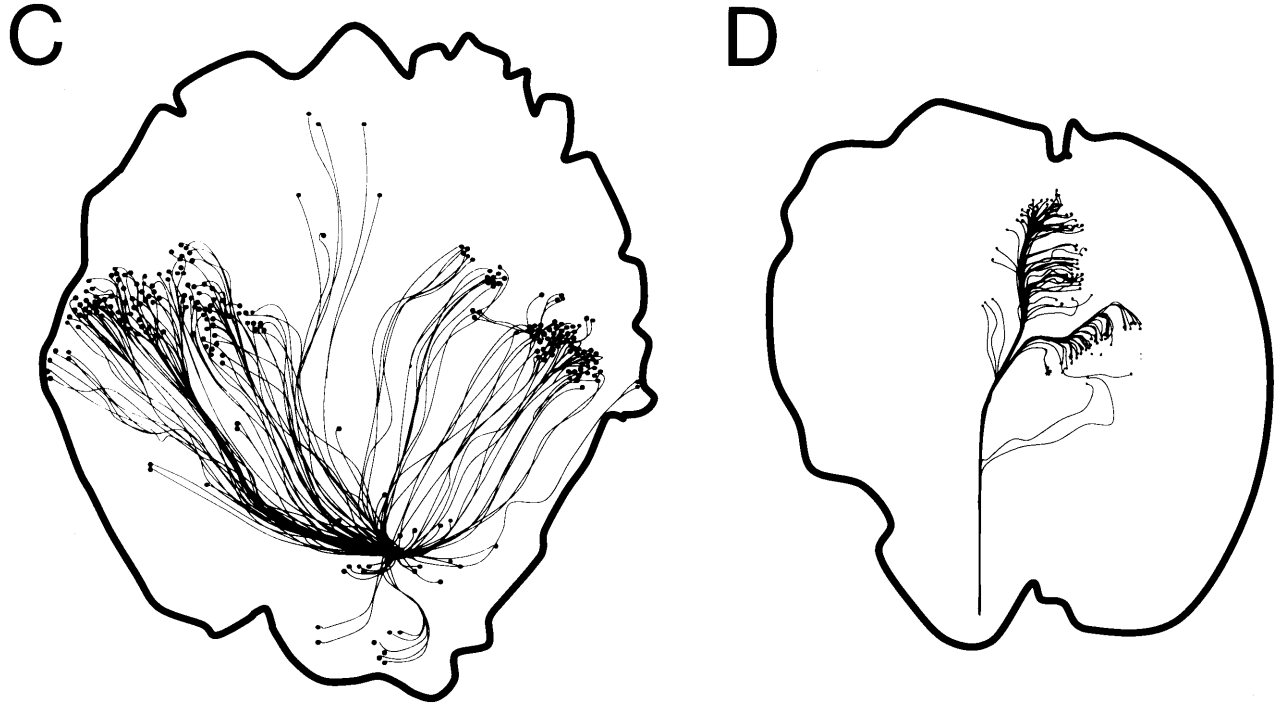
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tubules (Allen et al. 1985; Brady et al. 1985; Schnapp et al. 1985; Vale et al. 1985).

The microinjection of macromolecules directly into the neuron has revealed two new aspects. First, the marker moves throughout the neuron and offers the possibility of investigating the transport of macromolecules or exogenous materials that are nonpermeable to cellular membranes (Schwartz and Goldberg 1982). Second, the cellular environment can be determined during the experimental procedure. Primary objects of investigation have been giant cells, e.g. the squid axon or the abdominal ganglion of *Aplysia* (Goldberg 1982; Nemhauser and Goldberg 1985; Koike 1987).

The microinjection technique according to Graessmann and Graessmann (1986) allows the injection of minimal quantities of tracer (10^{-10} to 10^{-11} ml) into small cells. In the in vitro model of dissociated dorsal route ganglion cells of embryonic chicken, the microtubule-correlated transport of a microinjected tracer is inhibited by the microtubule-depolymerizing drug colchicine supplemented to the medium, whereas the possible microfilament-correlated transport is unaffected by the actin-depolymerizing drug cytochalasin B (Meller 1992, 1994).

The aim of this study has been to quantify correlations of fast anterograde transport to the microtubules of the neuronal cytoskeleton in single Purkinje cells (PC) within a vital tissue. For this, microinjection in identified PC of cerebellar rollertube cultures according to Gähwiler (1980, 1981) has been adopted as the model system. As a basis for subsequent studies of transport, we first sought to demonstrate the organotypic development of PC in the in vitro tissues and, especially, axonal proliferation, by immunocytochemical investigations. For the studies of axonal and dendritic transport, the mean distances of transported tracer were measured during defined intervals of postincubation after microinjection. The possible dependence of the transport processes on neuronal microtubules has been demonstrated by the use of microtubule-depolymerizing drugs.

Materials and methods

Culture methods

Slice cultures of rat cerebellum were obtained from newborn rat pups according to the rollertube technique (Gähwiler 1980, 1981). Briefly, the cerebella of neonatal rats were cut sagittally into approximately 400-µm-thick slices. Slices were attached to a cover glass with a plasma clot (15 µl; Cocalico, Reamstown, Pa.) coagulated with thrombin (60 U/15 µl; Calbiochem, La Jolla) and incubated in closed tubes at 37°C in a roller drum incubator. The nutrient medium Basal Medium Eagle (BME) was supplemented with 25% Gey's-salts, 25% horse serum, 1% glutamin, 6.5 mg/ml glucose and 25 ng/ml nerve growth factor. It was renewed twice weekly. The tissues were incubated for at least 14 days.

Microinjection

Capillaries (Hilgenberg, Germany; borosilicate glass: 1.5 mm/0.2 mm) were pulled (0.6±0.2 µm) according to Graessmann and Graessmann (1986).The capillary was filled with 2 µl tracer, viz. horseradish peroxidase (HRP) in a 2% dilution in phosphate-buffered saline (PBS) pH 7.2, immediately before use.

The microinjection of the PC was carried out under visual control on an inverted microscope (Axiovert 35, Zeiss, Germany) with the aid of phase-contrast optics. For microinjection, we used the high pressure microinjector 5242 (Eppendorf, Germany) and the micromanipulator 5170 (Eppendorf). The microinjector set a constant pressure of 80–100 hPa at the tip. For injection, the pressure was increased to 100–120 hPa for 0.7–0.8 s. The success of injection was visible as a brief swelling of the neuron soma. As many PC as possible were injected within 30 min.

During the injections, the tissues were kept under heat control, the medium never being warmer than 28°C. Neuron functions were thus reduced to a minimum and the transport of the tracer after microinjection was negligible until postincubation in the roller drum incubator. The postincubations were set for defined intervals of 30 min between 1–3 h after microinjection. These intervals were sufficient to demonstrate the movement of HRP into the neuron processes. Transport was stopped by fixation in 1% glutaraldehyde and 1.5% paraformaldehyde in phosphate buffer (PB) pH 7.0 and the distances of transported HRP were demonstrated with diaminobenzidine according to Graham and Karnovsky (1966). About 8–12 cells in each slice were measurable after histochemical processing. The distances were measured by a computer-aided image analysing system ("analysis", SIS, Münster, Germany) connected to an Olympus microscope BH-2 via video camera.

Cytoskeleton-depolymerizing drugs

Drugs affecting microtubules were added to the nutrient medium 120 min before microinjection of HRP and were not removed from the medium until fixation. Colchicine (Sigma, Germany) was maintained as a stock solution of 10 mg/ml in distilled water and diluted to a final concentration of 10 µg/ml in the nutrient medium. Vinblastine (Sigma) was stored as a stock solution of 1 mg/ml in BME and added to the medium at a concentration of 10 µg/ml.

Immunocytochemistry

Cultures were fixed at 14 days in vitro in 4% paraformaldehyde in PBS for 30 min. The tissues were thoroughly rinsed in PBS and exposed to 1% Triton X-100 in PBS for another 45 min before being treated with 3% hydrogen peroxide in 10% methanol. The primary antibodies anti-calbindin D28 k (CaBP; 1:200 dilution, Sigma) or anti-parvalbumin (PV; 1:500 dilution, Sigma), both directed against calcium-binding proteins, were incubated overnight at 4°C diluted in PBS containing 0.5% Triton X-100 and 0.25% bovine serum albumin. This buffer was also used for the subsequent wash steps and dilutions of antibodies.

For histochemical staining, the tissues were processed by using the peroxidase-antiperoxidase method of Sternberger (1986). For documentation, the labelled cells were either photographed (AGFA-Pan 25ASA, b/w film) or drawn directly from specimens with a com-

◀ **Fig. 1** Purkinje cells of cerebellar rollertube slices of newborn rats after 14 days in vitro and immunhistochemical labelling with anti-calbindin (**A–C**) or anti-parvalbumin (**D**). **A** The PC were arranged in a wide cluster (*Pc*) and their axons (*arrowheads*) extended directly to the interneurons of the deep cerebellar nuclei (the region of the interneurons is marked with an *arrow*), where they branched and formed bulbous endings. **B** Section of the region of the cerebellar nuclei neurons at higher magnification; incoming axons are marked with *arrows*, bulbi with *arrowheads*. Compare the targeted axonal projections in the drawings of whole slices (**C** and **D**): each spot represents the PC soma and the *associated lines* represent the PC axons. x17. The PC axons either extended directly to their target neurons (**C**) or formed tight bundles before they reached their target (**D**). *Bar:* 25 µm in **A**, 10 µm in **B**

aided image analysing system ("analysis", SIS) connected to an Olympus microscope BH-2 via video camera.

Transmission electron microscopy

For ultrastructural analysis, the cultures were fixed in 2% glutaraldehyde for 30 min and embedded in epoxy resin via propylene oxide. Ultrathin sections of the PC were cut parallel to the tissue surface to "hit" the neuron sidearms at their longitudinal axis.

Results

Morphology

As a basis for our studies of the transport of macromolecules, the development of the PC axons and dendrites was investigated by immunostainings with anti-CaBP and anti-PV. Within 14 days in vitro, the PC in cerebellar rollertube cultures from newborn rats were integrated into the in vitro tissues as the typical PC layer found in the in vivo cerebellum (Fig. 1D) or as clusters (Fig. 1A). The PC axons extended long projections with a defined target: the cells of the deep cerebellar nuclei (either the nucleus dentatus, n. interpositus or n. fastigus, not identified; Fig. 1A). The PC axons occurred either in tight bundles (Fig. 1D) or in a loose configuration (Fig. 1A,C). They ended up in multiple ramifications that formed small bulbi around the cerebellar nuclei neurons as described for in vivo PC (Fig. 1B).

Axonal transport

General considerations

For the following investigations, the cerebellar cultures of newborn rats were incubated for at least 14 days and no longer than 18 days. Microinjection studies were then carried out within several hours. Analysis of HRP transport was based on qualitative and quantitative data.

The structure of the HRP-labelled axons was judged according to the following criteria. HRP transport was considered to be "normal" when the tracer was detectable in the axon for at least several hundred micrometers. It was considered to be disturbed when the tracer was only demonstrable in isolated spots.

The distances of transported HRP for each interval of postincubation between 30 and 180 min (approximately 80–100 values per postincubation interval) were measured directly on the specimen. The mean values are summarized in Fig. 4.

Transport of injected HRP under normal culture conditions

The morphology of the injected PC was comparable to the immunostained PC (Fig. 2A–C). HRP transport had occurred from the cell soma into the dendrites and axons. Computer-aided drawings of specimens that contained PC axons at 180 min postincubations demonstrated axonal projections comparable to those found in immunolabelled specimens (Fig. 3A, B). However, the PC target cells could not be ascertained in the microinjection experiments. The microinjection of HRP into several PC of the same cluster revealed a synchronous transport of the tracer into the axons.

The mean distances of transported HRP continuously increased from an initial 880 μm to a saturation of 1170 μm after 180 min (Fig. 4) and thus demonstrated the temporal movement of HRP into the axons. The mean value for a PC axon after 14 days in vitro, as measured in an immunostained specimen (anti-CaBP and anti-PV), was 1500 μm.

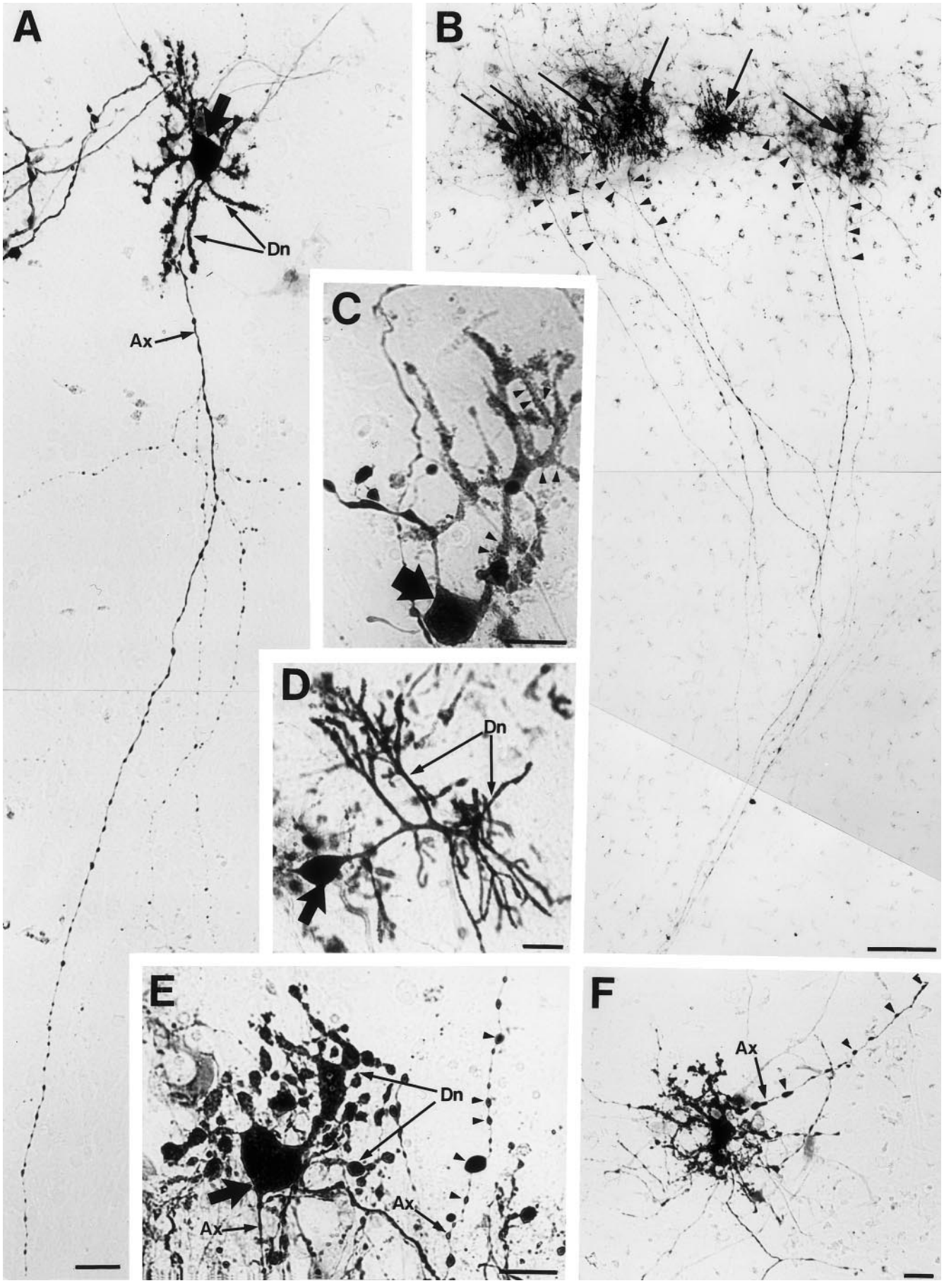
Axonal transport of injected HRP in PC treated with colchicine and vinblastine

To study the effects of microtubule-depolymerizing drugs on axonal transport, the rollertube cultures were preincubated for 2 h in medium supplemented with 10 μg/ml colchicine or vinblastine and then injected, postincubated and processed as described. The mean values for each interval are summarized in Fig. 4, which demonstrates the retardation of HRP transport in colchicine medium and the continuous reduction caused by vinblastine, both of which are statistically significant ($P<0.001$, $df=4$, $n=135$, two-way analyis of variance).

The mean distance of transported HRP in colchicine-conditioned medium was initially 550 μm, i.e. 45% compared with the controls. It increased to approximately 770 μm (90–150 min) and finally to 1170 μm, so that the initial reduction fell to 25% during further postincubations until the transport distance was the same as in controls after 180 min. The mean reduction was statistically significant ($P<0.001$, $df=2$, $n=84$; Scheffé Post Hoc test, colchicine-treated cells compared with controls). Higher concentrations of colchicine (20 μg/ml) were lethal to the PC even during the preincubation of 2 h.

The avarage distances of transported HRP in vinblastine-treated PC ranged from 440 to 590 μm (Fig. 4). This is

Fig. 2A–F Axonal and dendritic transport in the Purkinje cells of cerebellar rollertube cultures of newborn rats after 14 days in vitro. HRP was microinjected into the cell somata followed by a postincubation of 180 min. **A** The injected tracer was transported from the somata (*thick arrows*) into the axons (*Ax*) and into the dendrites (*Dn*). **B** When several neighbouring PC were injected with HRP, the tracer was transported axonally over comparable distances revealing bundles of axons (*arrows* PC, *arrowheads* axons showing transported HRP). **C** The PC exhibited no differences in morphology after microinjection and transportation of the tracer, the dendrites were narrow and covered with spines (*arrowheads*) and the soma was smooth. **D** The morphology did not change in those PC that were microinjected while being treated with colchicine. In the microinjected PC treated with vinblastine, HRP was axonally transported over short distances (**F**) and in irregular spots (**E,F**) and the dendrites appeared to be deformed, as HRP was found only in spots (**E** *arrowheads*). *Bars* 10 μm in **A,C–F**, 50 μm in **B** ►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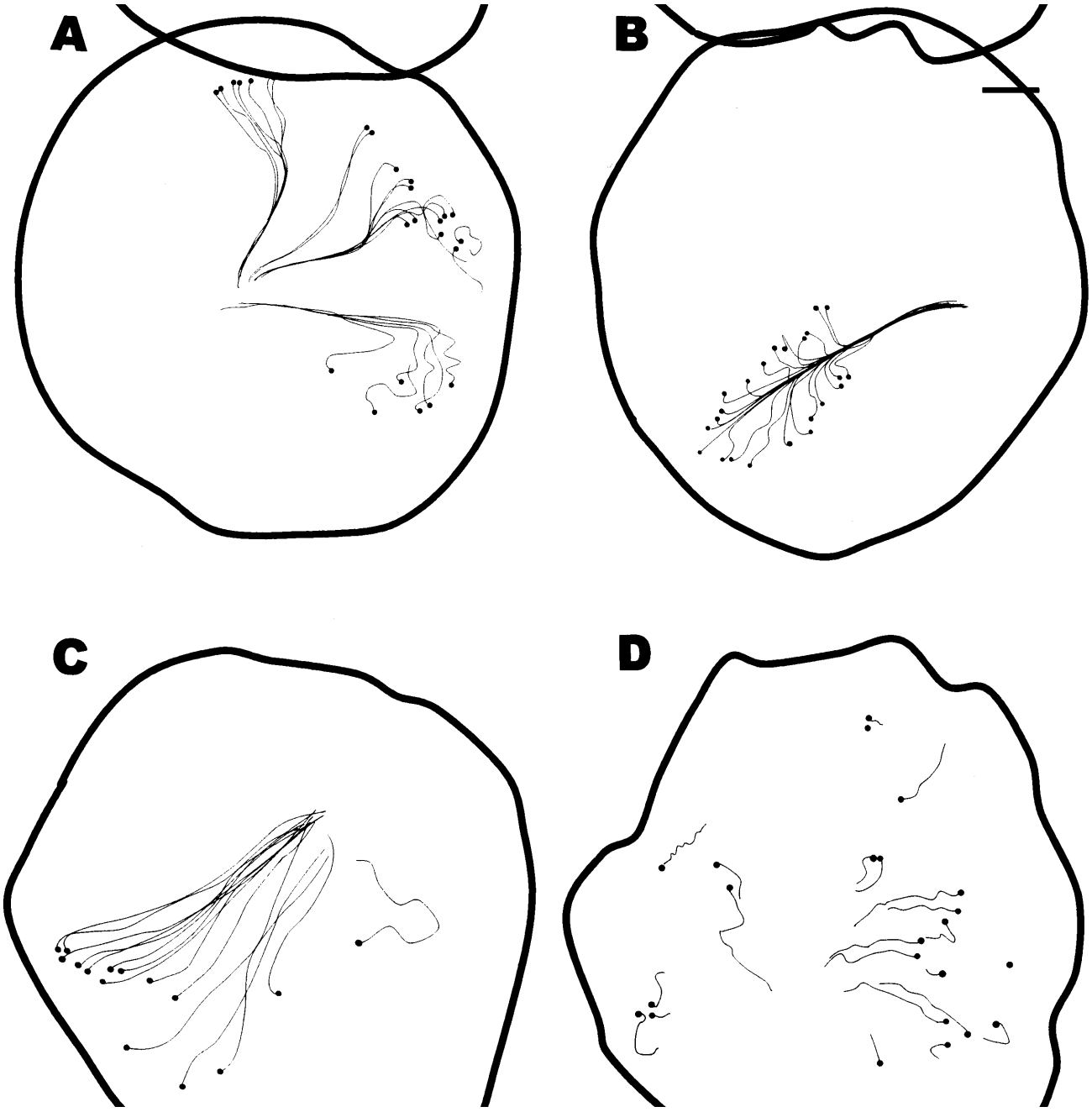

Fig. 3A–D Axonal transport in Purkinje cells of cerebellar rollertube slices after 14 to 18 days in vitro, microinjection with HRP and postincubation for 180 min. The PC including the axons stained with the transported HRP were drawn directly from the specimen: each figure represents a slice, each *spot* represents a PC and the *associated lines* represent the axons. **A,B** PC injected under normal culture conditions. Axonal transport occurred as expected and the axons demonstrated an orientation as found by immunhistochemical staining (compare with drawings in Fig. 1). Axonal transport in PC that were microinjected with HRP while being treated with colchicine occurred over comparable distances to the controls (**C**). Axonal transport in those PC treated with vinblastine occurred only over short distances (**D**). *Bar* 0.5 mm

a reduction of the axonal transport by 40%–50%, independent of the duration of postincubation ($P<0.001$, $df=2$, $n=94$, Scheffé Post Hoc test, vinblastine-treated cells compared with controls).

Morphology of PC axons in colchicine and vinblastine

The axons of microinjected PC that were postincubated for 180 min were drawn and compared with immunolabelled

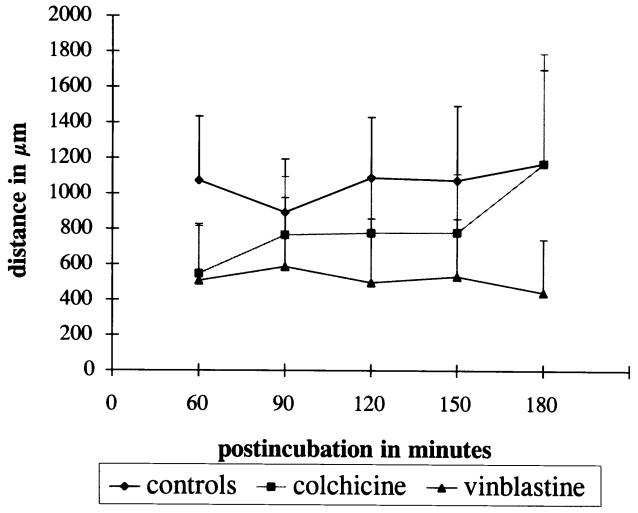

Fig. 4 Axonal HRP transport. Purkinje cell somata were microinjected with 2% HRP in normal culture medium and postincubated for increasing intervals from 60 to 180 min; the distances of axonally transported HRP were measured after histological processing. The mean values calculated from 80–100 single distances are summarized in the plot. For drug treatment, the PC were preincubated in conditioned medium (10 µg/ml colchicine or vinblastine) for 120 min and then microinjected and processed as described without removal of the drug

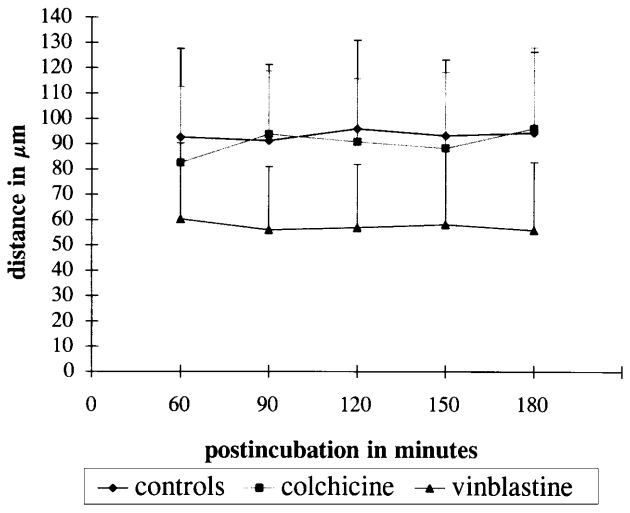

Fig. 5 Dendritic HRP transport. Purkinje cell somata were microinjected with 2% HRP in normal culture medium and postincubated for increasing intervals from 60 to 180 min; the distances of dendritically transported HRP were measured after histological processing. The mean values calculated from 60–80 single distances are summarized in the plot. For drug treatment, the PC were preincubated in conditioned medium (10 µg/ml colchicine or vinblastine) for 120 min and then microinjected and processed as described without removal of the drug

PC axons (Fig. 3, compare also with drawings in Fig. 1). The colchicine-treated specimens were not apparently different from the controls. However, in those slices treated with vinblastine, the distances of transported HRP were strikingly shortened (Figs. 3D, 2F). Ultrastructurally, we found typical vinblastine paracrystals of tubulin monomers. In the colchicine-treated PC, the microtubules were not visibly reduced (data not shown).

Dendritic transport

The cultured PC transported injected HRP into axons and dendrites in a similar manner. To quantify the dendritic transport, we proceeded in the same way as described for axonal transport. Results are given quantitatively and qualitatively.

Dendritic transport was regarded to have occurred "normally" when the HRP was detectable in the whole dendrite (Fig. 2A, C). It was regarded as interrupted when the dendrites were labelled merely with spots of HRP. The distances of transported HRP into the dendrites for each interval of postincubation between 30 and 180 min (60–80 values per interval) were measured directly on the specimens. The mean values are summarized in Fig. 5.

Dendritic transport of injected HRP under culture conditions

There were no differences between the morphology of dendrites of the injected and immunolabelled PC. The dendritic transport in the PC extended to a distance of about 90 µm even within a postincubation of 60 min (Fig. 5) and agreed with the average length of a PC dendrite in culture as calculated from immunolabelled neurons.

Dendritic transport of injected HRP under colchicine and vinblastine treatment

There was a significant difference in morphology between the colchicine- and vinblastine-treated neurons. Whereas the colchicine-treated PC seemed to have unchanged dendrites (Fig. 2D), the vinblastine-treated PC appeared to be deformed (Fig. 2E, F). HRP transport occurred discontinuously. Often these dendrites seemed to be shortened and thickened.

HRP transport in colchicine-treated cells measured approximately 80 µm after 60 min postincubation, i.e. there was a reduction by 10%; this corresponded to the 90-min values of the controls (Fig. 5). The retardation was not significant when statistically calculated in a two-way analysis of variance ($P>0.05$; $df=4$; $n=110$).

Dendritic transport under vinblastine treatment attained distances of 55–60 µm (Fig. 5). This was a reduction by 35%–40% and was independent of the duration of postincubation ($P<0.001$, $df=4$, $n=77$, Scheffé Post Hoc test, vinblastine-treated cells compared with controls).

Discussion

Morphology of the cerebellar cultures

A basic precondition for our model was the ability to investigate typical neurons within a functional tissue. Thus, we intended to find organotypic features in the in vitro PC. The cerebellar rollertube slices formed cytoarchitectural features that were comparable to those described in other slice culture models (Seil 1979; Jäger et al. 1988; Stoppini et al. 1991; Tanaka et al. 1994; Tauer et al. 1996). The adult in vitro PC expressed the morphology described earlier (Hendelman and Aggerwal 1980; Blank and Seil 1982; Calvet et al. 1983; Tauer et al. 1996). Axons proliferated long targeted projections to the cells of the deep cerebellar nuclei. The axonal branches ended as bulbi of different sizes similar to those in vivo (Meller 1987). We concluded that, in the rollertube cultures, the PC axons were organotypically connected to their target cells. This idea was supported by previous findings. In static slice cultures, it was established that the PC axons extended functional active connections to their target cells (Audinat et al. 1990; Mouginot and Gähwiler 1995; Seil 1996). Recently, axonal regenerations in vitro were shown to be correctly targeted as long as tissues were explanted at stages younger than P5 (Li et al. 1994; Linke et al. 1995; Dusart et al. 1997).

Axonal transport of injected HRP

The tracer HRP is a macromolecule that is actively transported in the axon after microinjection. Broadwell and coworkers (1980) have provided evidence for the active transport of endogenous peroxidase that clearly demarcates it from mere diffusion. They have found peroxidase packed in lysosomes that resemble precipitations from the agranular endoplasmic reticulum. In the axons of crabs injected polystyrene beads are transported anterogradely, like endogenous material, depending on ATP (Adams and Bray 1983).

Our results confirm the anterograde transportation of microinjected HRP for the period of postincubations. Likewise, the distances of the transported tracer increase depending on the duration of postincubation. However, the plot shows a maximum at a level 400 μm shorter than the average length of a PC axon found in an immunolabelled specimen. A plausible explanation for this effect might be an insufficient quantity of injected HRP. This has still to be determined, e.g. with HRP at higher concentrations 4% and more.

Drugs affecting microtubules

Both colchicine and vinblastine are known to be microtubule-depolymerizing drugs (Paulson and McClure 1975). Colchicine interferes with the enddynamics of microtubules, resulting in the depolymerization of the filament (Vandecandelaere et al. 1997). Vinblastine causes the formation of tubulin paracrystals (Haskins et al. 1981; Na and Timasheff 1992). The minus-ends of the microtubules are depolymerized (Panda et al. 1996).

Both drugs are also described to cause a blockade of axonal transport. When HRP is injected into dissociated neurons of the dorsal root ganglia of embryonic chicks, its transport is disturbed following treatment with colchicine (Meller 1992). Recently, colchicine has been used to inhibit the retrograde transport of trophic factors in the retinae of embryonic chicks (Primi and Clarke 1997). Vinblastine interrupts the anterograde transport of mitochondria in neurons of the sympathetic ganglia (Morris and Hollenbeck 1995) and its analogue vincristine causes a reversible blockade of retrograde transport (MacFarlane et al. 1997).

In our model of measuring the single distances of transported HRP stopped at different times of postincubation from 60 to 180 min after drug treatment and microinjection, we demonstrate a reduction of the HRP-transport distances, apart from a complete blockade. Moreover, in the examinations of certain PC within an organotypic tissue, the drugs colchicine and vinblastine have different effects. The treatment of the PC with vinblastine results in a reduction of up to 50% of the transport. Colchicine has a weaker efficacy and causes a retardation of the transport. This effect is abolished after 180 min and axonal transport completely recovers from sustained treatment with colchicine.

The relaxation of the axonal transport of injected HRP in the PC during treatment with colchicine deviates from results gained on the microinjection of HRP in dissociated neurons of the dorsal root ganglion (Meller 1992, 1994). This might be the result of the organotypic culturing of the examined neurons. Probably, the PC start assembling new microtubules, thus counteracting the blockade. This idea is supported by the finding that colchicine does not completely inactivate the microtubule ends, so that new free tubulin retains the ability to assemble (Farrell and Wilson 1984). As demonstrated by ultrastructural examination, the colchicine-treated cells contain similar numbers of microtubules compared with controls. On the contrary, vinblastine blocks microtubule elongation entirely as long as it is associated with the ends (Toso et al. 1993). Additionally, the affinity of vinblastine for self-assembled tubulin is considered to be much higher than for free tubulin dimers (Toso et al. 1993). This might explain the stronger impact of vinblastine on the observed transport.

Apart from this, the in vitro tissues may have created a barrier around the PC. For instance, the effect of colchicine is diminished in myelinated axons compared with naked ones (Fink et al. 1973). It remains to be determined when and to what extent myelination in the cerebellar rollertube cultures takes place. In static slice cultures, myelination of the projecting axons occurs within 3 weeks in vitro (Hendelman et al. 1985; Notterpek et al. 1993).

Dendritic transport of injected HRP

Similar to axonal transport, a close correlation of dendritic transport to cellular cytoskeleton, especially with respect to

the microtubules, has been postulated. However, the different organizations of the microtubules in dendrites and axons have led to the assumption of a specific differentiation between axonal and dendritic transport (Overly et al. 1996). The microtubules in the axon are oriented with their plus-ends towards the distal tip, thus expressing a uniform polarity, whereas in the dendrites, the microtubules are oriented non-uniformly with their plus-ends both towards the dendrite tip and to the cell body (Baas et al. 1988, 1989; Burton 1988). The loss of uniform polarity is regarded to occur via the transport of the tubules into the dendrite branches (Sharp et al. 1995). In contrast to axons, dendrites contain free ribosomes that provide a limited independence for the dendrites concerning their demand for proteins (Grafstein and Forman 1980).

Here, dendritic transport in the PC is reduced, especially by vinblastine. The involvement of the microtubules in dendritic transport becomes obvious. Moreover, the reason for the interrupted transport, even at short distances, might be based on microtubules in dendrites being shorter but more abundant than in axons (Yu and Baas 1994).

Microtubule-associated proteins (MAPs) are thought to mediate transport along microtubules (Mercken et al. 1995; Larcher et al. 1996; Sato-Harada et al. 1996). MAPs are attendant proteins of all microtubules and are responsible for the stabilization and the polymerization of the filaments (for a review, see Maccioni and Cambiazo 1995). They might be cross-linkers between microtubules and neurofilaments (Price et al. 1991) or between microtubules and membranous organelles (Sato-Harada et al. 1996). Their role in the fast axonal transport of macromolecules can be determined in further investigations with the microinjecton technique, together with the specific blockade of MAPs.

Conclusions

In the present study of the model system for axonal and dendritic transport in the Purkinje cells of organotypically cultured rollertube tissues by microinjection of a tracer, we describe a further link in the chain of investigations of fast anterograde transport in relation to the neuronal cytoskeleton. By measuring transportation distances, we have demonstrated the dependence of axonal and dendritic transport on microtubules in projecting neurons of the central nervous system. The decisive advantage of our model has been its resemblance to the in situ situation and the observation of targeted connections of the axonal projections to cerebellar nuclei cells. This model can thus be used for further investigations of the axonal transport of neurotransmitters at synapses on target cells.

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References

Adams RJ, Bray D (1983) Rapid transport of foreign particles microinjected into crab axons. Nature 303:718–720

Allen RD, Weiss DG, Hayden JH, Brown DT, Fujiwake H, Simpson M (1985) Gliding movement of and bidirectional transport along single native microtubules from squid axoplasm: evidence for an active role of microtubules in cytoplasmic transport. J Cell Biol 100:1736–1752

Audinat E, Knöpfel T, Gähwiler BH (1990) Responses to excitatory amino acids of Purkinje cells and neurones of the deep nuclei in cerebellar slice cultures. J Physiol (Lond) 430:297–313

Baas PW, Deitch JS, Black MM, Banker GA (1988) Polarity orientation of microtubules in hippocampal neurons: uniformity in the axon and nonuniformity in the dendrite. Proc Natl Acad Sci USA 85:8335–8339

Baas PW, Black MM, Banker GA (1989) Changes in microtubule polarity orientation during the development of hippocampal neurons in culture. J Cell Biol 109:3085–3094

Blank NK, Seil FJ (1982) Mature Purkinje cells in cerebellar tissue cultures: an ultrastructural study. J Comp Neurol 208:169–176

Brady ST (1985) A novel brain ATPase with properties expected for the fast axonal transport motor. Nature 317:73–75

Brady ST (1991) Molecular motors in the nervous system. Neuron 7:521–533

Brady ST, Lasek RJ, Allen RD (1985) Video microscopy of fast axonal transport in extruded axoplasm. Cell Motil 5:81–101

Broadwell RD, Olivier C, Brightman MW (1980) Neuronal transport of acid hydrolases and peroxidase within the lysosomal system of organelles. Involvement of agranular reticulum-like cisterns. J Comp Neurol 190:519–532

Burton PR (1988) Dendrites of mitral cell neurons contain microtubules of opposite polarity. Brain Res 473:107–115

Calvet M-C, Calvet J, Camacho R, Eude D (1983) The dendritic trees of Purkinje cells: a computer assisted analysis of HRP labeled neurons in organotypic cultures of kitten cerebellum. Brain Res 280:199–215

Cyr JL, Brady ST (1992) Molecular motors in axonal transport. Mol Neurobiol 6:137–155

Dusart I, Airaksinen MS, Sotelo C (1997) Purkinje cell survival and axonal regeneration are age dependent: an in vitro study. J Neurosci 17:3710–3726

Farrell KW,Wilson L (1984) Tubulin-colchicine complexes differentially poison opposite microtubule ends. Biochemistry 23:3741–3748

Fink BR, Byers MR, Middaugh ME (1973) Dynamics of colchicine effects on rapid axonal tansport and axonal morphology. Brain Res 56:299–311

Gähwiler BH (1980) Morphological differentiation of nerve cells in thin organotypic cultures derived from rat hippocampus and cerebellum. Proc R Soc Lond Biol 211:287–290

Gähwiler BH (1981) Organotypic monolayer cultures of nervous tissue. J Neurosci Methods 4:329–342

Goldberg DJ (1982) Microinjection into an identified axon to study the mechanism of fast axonal transport. Proc Natl Acad Sci USA 79:4818–4822

Graessmann M, Graessmann A (1986) Microinjection of tissue culture cells using glass capillaries: methods. In: Celis JE (ed) Microinjection and organelle transplantation techniques. Methods and applications. Academic Press, London, pp 3–13

Grafstein B, Forman DS (1980) Intracellular transport in neurons. Physiol Rev 60:1167–1283

Graham RC, Karnovsky MJ (1966) The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney. Ultrastructural cytochemistry by a new technique. J Histochem Cytochem 14:291–302

Hammerschlag R, Cyr JL, Brady ST (1994) Axonal transport and the neuronal cytoskeleton. In: Siegel GJ (ed) Basic neurochemistry: molecular, cellular, and medical aspects. Raven, New York, pp 545–571

Haskins KM, Donoso JA, Himes RH (1981) Spirals and paracrystals induced by *Vinca* alkaloids: evidence that microtubule-associated proteins act as polycations. J Cell Sci 47:237–247

Hendelman WJ, Aggerwal AS (1980) The Purkinje neuron. I. A Golgi study of its development in the mouse and in culture. J Comp Neurol 193:1063–1079

Hendelman WJ, DeSavigny N, Marshall KC (1985) Growth and myelination of explant cultures in defined medium. In Vitro Cell Dev Biol 21:129–134

Hirokawa N (1996) The molecular mechanism of organelle transport along microtubules: the identification and characterization of Kifs (kinesin superfamily proteins). Cell Struct Funct 21:357–367

Hirokawa N, Bloom GS, Vallee RB (1985) Cytoskeletal architecture and immunocytochemical localization of microtubule-associated proteins in regions of axons associated with rapid axonal transport: the β,β'-iminodipropionitrile-intoxicated axon as a model system. J Cell Biol 101:227–239

Jäger CB, Kapoor R, Llinas R (1988) Cytology and organization of rat cerebellar organ cultures. Neuroscience 26:509–538

Koike H (1987) The disturbance of the fast axonal transport of protein by passive stretching of an axon in *Aplysia*. J Physiol (Lond) 390:489–500

Kristensson K, Olsson Y, Sjöstrand J (1971) Axonal uptake and retrograde transport of exogenous proteins in the hypoglossal nerve. Brain Res 32:399–406

Larcher J-C, Boucher D, Lazereg S, Gros F, Denoulet P (1996) Interaction of kinesin motor domains with a- and b-tubulin subunits at a tau-independent binding site. Am Soc Biochem Mol Biol 22117–22124

Lasek RJ (1968) Axoplasmic transport in cat dorsal root ganglion cells: as studied with ^{3}H-L-leucine. Brain Res 7:360–377

Lasek RJ (1970) Axonal transport of proteins in dorsal root ganglion cells of the growing cat: a comparison of growing and mature neurons. Brain Res 20:121–126

Lasek RJ, Brady ST (1982) The structural hypothesis of axonal transport: two classes of moving elements. In: Weiss DG (ed) Axoplasmic transport. Springer, Berlin Heidelberg New York, pp 397–405

Lasek R, Joseph BS, Whitlock DG (1968) Evaluation of a radioautographic tracing method. Brain Res 8:319–336

Li D, Field PM, Yoshioka N, Raisman G (1994) Axons regenerate with correct specificity in horizontal slice culture of the postnatal rat entorhino-hippocampal system. Eur J Neurosci 6:1026–1037

Lin SXH, Pfister KK, Collins CA (1996) Comparison of the intracellular distribution of cytoplasmic dynein and kinesin in cultured cells: motor protein location does not reliably predict function. Cell Motil Cytoskeleton 34:299–312

Linke R, Heimrich B, Frotscher M (1995) Axonal regeneration of identified septohippocampal projection neurons in vitro. Neuroscience 68:1–4

Maccioni RB, Cambiazo V (1995) Role of microtubule-associated proteins in the control of microtubule assembly. Physiol Rev 75:835–864

MacFarlane BV, Wright A, Benson HAE (1997) Reversible blockade of retrograde axonal transport in the rat sciatic nerve by vincristine. J Pharm Pharmacol 49:97–101

Meller K (1987) The cytoskeleton of cryofixed Purkinje cells of the chicken cerebellum. Cell Tissue Res 247:155–165

Meller K (1992) Axoplasmic transport of horseradish peroxidase in single neurons of the dorsal root ganglion studied in vitro by microinjection. Cell Tissue Res 270:139–148

Meller K (1994) Intracellular injection of transport markers and antibodies into in vitro cultured nerve cells. Neurosci Prot 50:1–10

Mercken M, Fischer I, Kosik KS, Nixon RA (1995) Three distinct axonal transport rates for tau, tubulin, and other microtubule-associated proteins: evidence for dynamic interactions of tau with microtubules in vivo. J Neurosci 15:8259–8267

Morris RL, Hollenbeck PJ (1995) Axonal transport of mitochondria along microtubules and f-actin in living vertebrate neurons. J Cell Biol 131:1315–1326

Mouginot D, Gähwiler BH (1995) Characterization of synaptic connections between cortex and deep nuclei of the rat cerebellum in vitro. Neuroscience 64:699–712

Na GC, Timasheff SN (1982) In vitro vinblastine-induced tubulin paracrystals. J Biol Chem 257:10387–10391

Nemhauser I, Goldberg DJ (1985) Structural effects in axoplasm of DNase I, an actin depolymerizer that blocks fast axonal transport. Brain Res 334:47–58

Notterpek LM, Bullock PN, Malek-Hedayat S, Fisher R, Rome LH (1993) Myelination in cerebellar slice cultures: development of a system amenable to biochemical analysis. J Neurosci Res 36:621–634

Ochs S, Burger E (1958) Movement of substances proximo-distally in the nerve axons as studied with spinal cord injections of radioactive phosphorus. Am J Physiol 194:499–506

Overly CC, Rieff HI, Hollenbeck PJ (1996) Organelle motility and metabolism in axons vs dendrites of cultured hippocampal neurons. J Cell Sci 109:971–980

Panda D, Jordan MA, Chin Chu K, Wilson L (1996) Differential effects of vinblastine on polymerization and dynamics at opposite microtubule ends. J Biol Chem 271:29807–29812

Paulson JC, McClure WO (1975) Microtubules and axoplasmic transport. Inhibition of transport by podophyllotoxin: an interaction with microtubule protein. J Cell Biol 67:461–467

Price RL, Lasek RJ, Katz MJ (1991) Microtubules have special physical associations with smooth endoplasmic reticula and mitochondria in axons. Brain Res 540:209–216

Primi M-P, Clarke PGH (1997) Early retrograde effects of blocking axoplasmic transport in the axons of developing neurons. Dev Brain Res 99:259–262

Sato-Harada R, Okabe S, Umeyama T, Kanai Y, Hirokawa N (1996) Microtubule-associated proteins regulate microtubule function as the track for intracellular membrane organelle transports. Cell Struct Funct 21:283–295

Schnapp BJ, Reese TS (1989) Dynein is the motor for retrograde axonal transport of organelles. Proc Natl Acad Sci USA 86:1548–1552

Schnapp BJ, Vale RD, Sheetz MP, Reese TS (1985) Single microtubules from squid axoplasm support bidirectional movement of organelles. Cell 40:455–462

Schwartz JH, Goldberg DJ (1982) Studies on the mechanism of fast axoplasmic transport in single identified neurons. In: Weiss DG (ed) Axoplasmic transport. Springer, Berlin Heidelberg New York, pp 351–361

Seil FJ (1979) Cerebellum in tissue culture. Rev Neurosci 4:105–177

Seil FJ (1996) Neural plasticity in cerebellar cultures. Prog Neurobiol 50:533–556

Sharp DJ, Yu W, Baas PW (1995) Transport of dendritic microtubules establishes their nonuniform polarity orientation. J Cell Biol 130:93–103

Shpetner HS, Paschal BM, Vallee RB (1988) Characterization of the microtubule-activated ATPase of brain cytoplasmic dynein (MAP1 C). J Cell Biol 107:1001–1009

Sternberger LA (1986) The unlabeled antibody peroxidase-antiperoxidase (PAP) method. In: Sternberger LA (ed) Immunocytochemistry, 3rd edn. Wiley, New York, pp 90–209

Stoppini L, Buchs P-A, Muller D (1991) A simple method for organotypic cultures of nervous tissue. J Neurosci Methods 37:173–182

Tanaka M, Tomita A, Yoshida S, Yano M, Shimizu H (1994) Observation of the highly organized development of granule cells in rat cerebellar organotypic cultures. Brain Res 641:319–327

Tauer U, Volk B, Heimrich B (1996) Differentiation of Purkinje cells in cerebellar slice cultures: an immunocytochemical and Golgi EM study. Neuropathol Appl Neurobiol 22:361–369

Toso RJ, Jordan MA, Farrell KW, Matsumoto B, Wilson L (1993) Kinetic stabilization of microtubule dynamic instability in vitro by vinblastine. Biochemistry 32:1285–1293

Tytell M, Black MM, Garner JA, Lasek RJ (1981) Axonal transport: each major rate component reflects the movement of distinct macromolecular complexes. Science 214:179–181

Vale RD, Schnapp BJ, Reese TS, Sheetz MP (1985) Movement of organelles along filaments dissociated from the axoplasm of the squid giant axon. Cell 40:449–454

Vandecandelaere A, Martin SR, Engelborghs Y (1997) Response of microtubules to the addition of colchicine and tubulin-colchicine: evaluation of models for the interaction of drugs with microtubules. Biochem J 323:189–196

Yu W, Baas PW (1994) Changes in microtubule number and length during axon differentiation. J Neurosci 14:2818–2829