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Specific responses of axons and dendrites to cytoskeleton perturbations: an in vitro study

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SUMMARY

Several factors can influence the development of axons and dendrites in vitro. Some of these factors modify the adhesion of neurons to their substratum. We have previously shown that the threshold of neuron-substratum adhesion necessary for initiation and elongation of dendrites is higher than that required for axonal growth. To explain this difference we propose that, in order to antagonize actin-driven surface tension, axons primarily rely on the compression forces of microtubules whereas dendrites rely on adhesion. This model was tested by seeding the cells in conditions allowing the development either of axons or of axons and dendrites, then adding cytochalasin B or nocodazole 1 hour or 24 hours after plating. The addition of cytochalasin B, which depolymerizes actin filaments and thus reduces actin-tensile forces, increases the length of both axons and dendrites, indicating that both axons and dendrites have to antagonize surface tension in order to elongate. The addition of nocodazole, which acts primarily on microtubules, slightly reduces dendrite elongation and totally abolishes axonal growth. Similar results are obtained when the drugs are added 1 or 24 hours after plating, suggesting that the same mechanisms are at work both in initiation and in elongation. Finally, we find that in the presence of cytochalasin B axons adopt a curly morphology, a fact that could be explained by the importance of tensile forces in antagonizing the asymmetry created by polarized microtubules presenting a uniform minus/plus orientation.

Key words: cytoskeleton, neuronal polarity, axons, dendrites

INTRODUCTION

We have previously demonstrated that the development of neuronal polarity can be modulated in culture through region-specific neuro-astroglial interactions (Denis-Donini et al., 1984; Chamak et al., 1987; Autillo-Touati et al., 1988; Rousselet et al., 1988, 1990). These studies employed chiefly cells from the mesencephalon and striatum, and were extended to sympathetic neurons (Buckenstein and Higgins, 1988a,b; Lein and Higgins, 1989) and more recently to neurons from spinal cord and hippocampus (Qian et al., 1992), thus generalizing the idea of differential regulation for the development of each neuronal compartment.

Neuronal adhesion and spreading can be modified by specific concentrations of soluble or substratum-attached matrix molecules (Chamak and Prochiantz, 1989; Lochter et al., 1991; Lafont et al., 1992). Thus, systematic morphological comparisons between neurons cultured in the presence of soluble or substratum-attached matrix molecules revealed to us a strict correlation between the definition of neuronal polarity and the level of cell adhesion (Chamak and Prochiantz, 1989; Rousselet et al., 1990; Lafont et al., 1992).

In fact, we consistently observed that the adhesion threshold necessary for dendrite growth is higher than that for initiation and elongation of the axon. Neurons with axons only or with axons and dendrites can therefore be produced by adjusting neuron-substratum adhesion conditions (Chamak and Prochiantz, 1989; Rousselet et al., 1990). This can be easily achieved by adding low concentrations of heparan sulfate (HS) or dermatan sulfate (DS) to the culture medium. Neurons cultured in the presence of HS are unipolar with a highly developed axon and almost no dendrites. In contrast, DS promotes the elongation of both axons and dendrites (Lafont et al., 1992).

The importance of neuron-substratum adhesion in the elongation of dendrites can be explained by the hypothetical scheme of Fig.1. In this model, axons and dendrites use different strategies to antagonize actin-tensile forces. Axons, as already suggested by several authors (Dennerll et al., 1988, 1989; Zheng et al., 1991), use the compression

forces of microtubules with a minus/plus polarized orientation, whereas dendrites rely more on adhesion forces. This model is supported by the fact that axonal microtubules are all uniformly oriented with their plus end distal to the cell body, allowing for the vectorization of compression forces (Baas et al., 1988). This situation differs from that observed in the dendrites, which contain microtubules with the two orientations. In addition, compared with proximal dendrites, developing axons contain a very high concentration of polymerized microtubules (Autillo-Touati et al., 1988; Rousselet et al., 1990).

As stated above, the molecular basis for surface tensile forces probably resides in the presence of polymerized cortical actin within the neurites and underneath neuronal membranes (Bray et al., 1987; Lamoureux et al., 1990). In fact, microtubule bundles and actin filaments represent two antagonistic systems of tension and compression and it seems that the regulated interplay between these two systems is the basis for directional elongation of neurites (Dennerll et al., 1988, 1989; Madraperla and Adler, 1989; Zheng et al., 1991).

In the present paper, we report the effect of cytochalasin B and nocodazole on total neurite growth and on the growth of axons and dendrites. If the model of Fig. 1 is correct, it is predicted that cytochalasin B (an inhibitor of actin polymerization) will favor the growth of all neurites by reducing actin-driven tensile forces, but that axons should be more sensitive than dendrites to the presence of nocodazole, a drug that primarily affects microtubule polymerization. We have therefore quantified the sensitivity of axons and dendrites to the addition of nocodazole or cytochalasin. This was done either in the presence of HS (allowing the growth of axons) or in the presence of DS (allowing both axons and dendrites to grow) (Lafont et al., 1992). These experiments confirmed several points of the model and gave rise to new information on the role of actin-tubulin interplay in axonal shape.

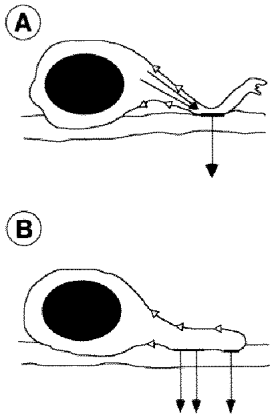

Fig. 1. The jumping axon and spreading dendrites model. In this model, axons (A) and dendrites (B) use two different strategies to antagonize actin-driven tensile forces (open arrowheads). Axons increase the intracellular viscosity by accumulating bundles of microtubules with similar orientations (black arrowhead in A) between adhesion points (black horizontal bars). Dendrites, at least in their initial segment, relate more on continuous adhesion to the substratum (B). Adhesion forces are represented by downwardly oriented arrows.

MATERIALS AND METHODS

Cell culture

Pure neuronal cultures were obtained as described previously (Rousselet et al., 1988, 1990). In brief, dissociated mesencephalic cells prepared from 14-day-old rat embryos (IFFA CREDO, France) were seeded on polyornithine (1.5 µg/ml, M_r 40,000, Sigma)-coated culture dishes (16 mm diameter wells) at densities of 25×10^3 cells/cm^2 (for morphological and immunocytochemical analysis) or 10^5 cells/cm^2 (for adhesion assays). Chemically defined medium (CDM) consisted of DMEM/F12 (1:1, Gibco) supplemented with 33 mM glucose, 2 mM L-glutamine, 3 mM $NaHCO_3$, 5 mM Hepes pH 7.4, 5 i.u./ml penicillin, 5 µg/ml streptomycin, 25 µg/ml insulin, 100 µg/ml transferrin, 2×10^{-8} M progesterone, 6×10^{-5} M putrescin, 3×10^{-8} M selenium and 0.1% ovalbumin. Bovine kidney heparan sulfate (HS, Sigma) or bovine mucosa dermatan sulfate (DS, Sigma) were added to the medium 30 min after seeding at a concentration of 10 µg/ml. Nocodazole (NZ, Sigma) or cytochalasin B (CytB, Sigma) were added 60 min or 24 h after plating at concentrations of 0.05 µg/ml (0.16 µM) or 1.0 µg/ml (3.32 µM) (NZ), and 1 µg/ml (2 µM) (CytB), respectively. Although most experiments were done with mesencephalic neurons, similar results were obtained with brainstem, cortex or striatal neurons prepared from 14- or 16-day-old rat embryos.

Quantification of cell survival and adhesion

Cells were dissociated and the number of living cells was determined by trypan blue exclusion just before plating. Survival rates after different times in culture were also calculated by the trypan blue exclusion procedure. Cells from 3 independent fields were counted directly under the microscope. Adhesion was quantified at various times following plating on polyornithine-coated plastic culture dishes. The plates were agitated at 250 revs/min (Giratory Shaker, model G2, New Brunswick) for 10 min at room temperature. Attached cells were fixed with 2.5% glutaraldehyde in PBS, washed, stained with toluidine blue and counted in 3 independent fields under the microscope.

Table 1. Percentages of surviving neurons after 2, 18, 30 or 48 hours with or without cytochalasin B or nocodazole

	Time in culture (hours)			
Conditions	3	18	30	48
Control				
CDM	85 ± 1.4	81 ± 4.8	79 ± 8.3	73 ± 8.0
HS	84 ± 2.3	83 ± 4.6	81 ± 3.9	75 ± 4.6
DS	85 ± 1.7	79 ± 6.9	81 ± 5.2	77 ± 7.5
Cytochalasin B				
CDM	86 ± 2.1	71 ± 9.3	70 ± 6.5	59 ± 9.5
HS	86 ± 3.2	80 ± 4.1	81 ± 2.4	70 ± 6.4
DS	86 ± 0.1	75 ± 8.6	77 ± 4.9	70 ± 8.9
Nocodazole				
CDM	83 ± 3.3	77 ± 7.3	72 ± 9.3	61 ± 8.9
HS	85 ± 2.5	81 ± 5.0	79 ± 5.3	70 ± 7.7
DS	85 ± 2.5	81 ± 4.5	79 ± 3.6	72 ± 5.8

Values are the results of 3 independent experiments. Neuronal survival was not modified significantly in any of the tested conditions (P>0,05, Student's t-test). CDM, chemically defined medium; HS, addition of heparan sulfate; DS, addition of dermatan sulfate.

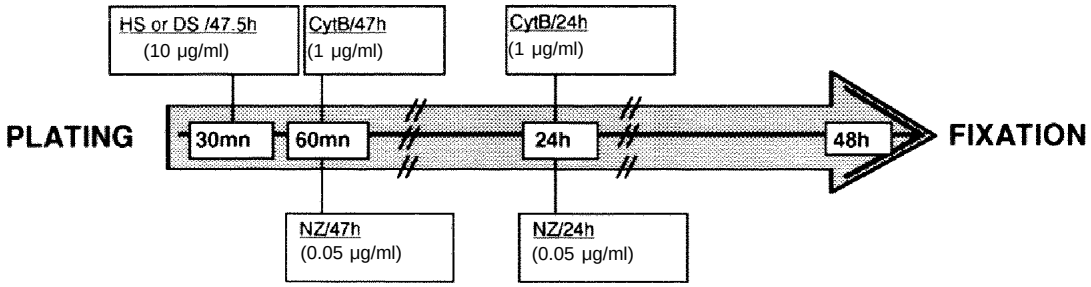

Fig. 2. Experimental conditions. Neurons were plated on polyornithine-coated glass coverslips and heparan sulfate (HS) or dermatan sulfate (DS) was added 30 min (mn) later at a concentration of 10 µg/ml. Cytochalasin B (1 µg/ml) or nocodazole (0.05 µg/ml) was added 1 hour or 24 hours after plating and left until fixation at 48 hours.

Immunocytochemistry

Cells seeded on polyornithine-coated (15 µg/ml) 16 mm diameter glass coverslips were cultured for 2 days, fixed with methanol:acetic acid (95:5, v/v) for 6 min at −20°C, incubated with monoclonal (1:50) or polyclonal (1:200) antibodies, washed 5 times and further incubated with biotinylated anti-mouse (1:200, Amersham) or Texas red-conjugated anti-rabbit (1:100, Byosis) Igs. Cells were washed 5 times, incubated with fluorescein-conjugated streptavidin (1:200, Amersham), washed 5 more times and mounted in Mowiol. All incubations were carried out at 37°C for 45 min. Washes and dilutions were in PBS plus 5% FCS except for the wash preceding mounting, which was in distilled water. The monoclonal anti-NF-H antibody was provided by P. Levitt and the anti-MAP2 and anti-NCAM polyclonal antibodies were given by A. Fellous an C. Goridis, respectively.

Morphological analysis

Cells were fixed with methanol:acetic acid (95:5, v/v) for 5 min at −20°C and stained with the appropriate antibodies (see Results). For each experiment 50 neurons per condition were digitalized and analysed with a morphological analysis software (IMSTAR, France). All statistics were calculated with the help of the Statgraphics software (STSC, Inc.).

Table 2. Neuron-substratum adhesion after 3, 20, 30 or 48 hours with or without cytochalasin B or nocodazole

Culture times in the different conditions	HS	DS
3 hours:		
Control	56 ± 6.7	98 ± 10.2
Cytochalasin B	65 ± 3.5	94 ± 9.8
Nocodazole	71 ± 8.5	105 ± 3.6
20 hours:		
Control	52 ± 2.3	98 ± 12.6
Cytochalasin B	65 ± 1.9	96 ± 21.8
Nocodazole	53 ± 1.9	104 ± 14.5
30 hours:		
Control	62 ± 18.2	94 ± 20.5
Cytochalasin B	63 ± 20.6	115 ± 36.2
Nocodazole	66 ± 18.4	106 ± 1.2
48 hours:		
Control	62 ± 12.5	89 ± 10.5
Cytochalasin B	60 ± 22.5	88 ± 2.3
Nocodazole	66 ± 9.3	113 ± 12.5

Values are the result of 3 independent experiments. The addition of cytochalasin B or nocodazole resulted in no significant modification of neuron-substratum adhesion (P>0,05, Student's t test).

Electron microscopy

Cells were fixed with glutaraldehyde (4% in PBS) for one hour at room temperature and surfixed with osmium tetroxide (1% in PBS), before inclusion in Epon and dehydratation (Lafont et al., 1992). Contrast was enhanced with uranyl acetate (4%). Examination was with a Jeol 1200 electron microscope.

RESULTS

Definition of the experimental conditions

In a previous study of the glycosaminoglycans (GAGs) (Lafont et al., 1992) we determined that 10 µg/ml is the optimal concentration of heparan sulfate (HS) or dermatan sulfate (DS) for survival of neurons and the growth of dendrites and axons (DS) or of axons only (HS). Glycosaminoglycans were added 30 min after plating (Fig. 2) to allow for the initial attachment of neurons to the culture substratum. Procedures leading to 98% pure neuronal culture as assessed by neurofilament staining have been previously described (Rousselet et al., 1988).

Nocodazole (NZ) (0.05 µg/ml or 1 µg/ml) and cytochalasin B (CytB) (1 µg/ml) were added 1 or 24 hours after plating and left until the end of the 48 hour culture period (Fig. 2). The two concentrations of NZ gave identical results. Depolymerization was verified biochemically (western blotting, not shown) and by electron microscopy (Fig. 3). Examination of cell bodies and neurites clearly shows the presence of actin fascicles and of microtubules in a non-treated cell (Fig. 3A). The addition of NZ (Fig. 3B,C,D) eliminates the polymerized microtubules and spares the actin bundles (Fig. 3D). Addition of CytB decreases the

Table 3. Average number of primary neurites in neurons grown in the presence of HS or DS and with or without cytochalasin B or nocodazole

	HS	DS
Control	1.3±0.1	2.4±0.6
CytB/47h	1.1±0.1	2.3±0.8
NZ/47h	0.4±0.2	1.7±0.5
CytB/24h	1.2±0.2	2.5±0.5
NZ/24h	0.8±0.2	1.4±0.5

Values are the results from 4 independent experiments, 300 neurons were analysed for each condition.

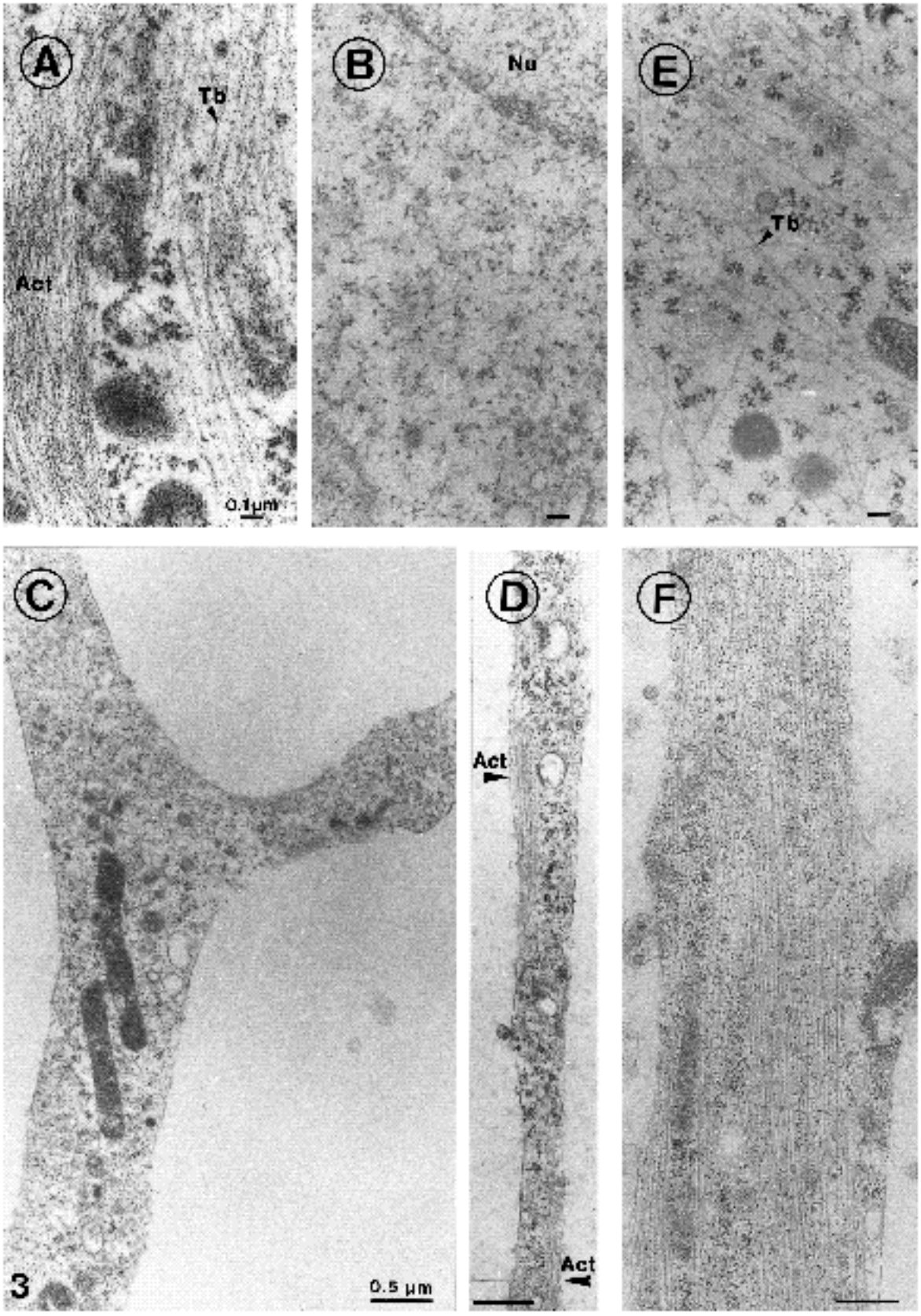

Fig. 3. Ultrastructural aspects of cell bodies and neurites of neurons cultured for 2 days in control conditions (A), in the presence of 1 µg/ml nocodazole (B-D) or in the presence of 1 µg/ml cytochalasin B (E,F). Act, actin; Nu, nucleus; Tb, microtubule.

content in polymerized actin without affecting the microtubules (Fig. 3E,F).

As shown in Table 1, CytB and NZ added 1 hour after plating did not significantly modify neuronal survival, during the first 48 hours, irrespective of wether GAGs were present. The results presented below are thus not due to the selective survival of sub-populations with different morphologies.

Since adhesion seems to be a key factor in the development of neuronal polarity, we verified that the addition of NZ or CytB did not modify the respective effects of DS and HS on neuron-substratum adhesion. Table 2 demonstrates that the differences in adhesion created by adding either GAG 30 min after plating were maintained during the entire culture period, independently of the presence of cytoskeleton drugs.

Fig. 4 illustrates the typical morphologies obtained in control conditions (CDM, Fig. 4A,B) or in the presence of the two complex sugars, HS (Fig. 4C,D) and DS (Fig. 4E,F). The same figure also demonstrates the specificity of the markers (anti-NF-H and anti-MAP2 antibodies) used to characterize axons and dendrites. The percentage of neu-

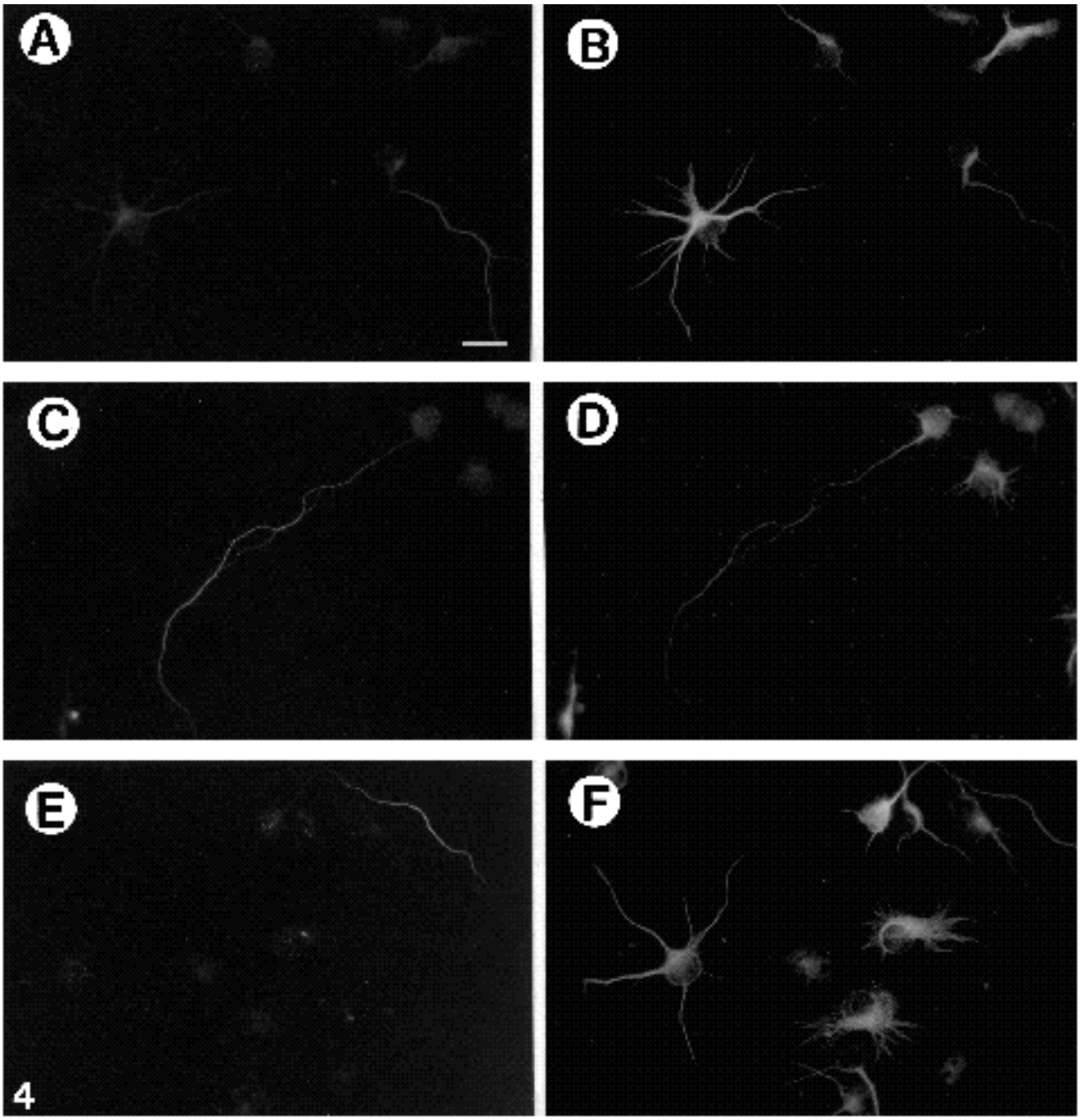

Fig. 4. Double labelling of mesencephalic neurons with anti-NF-H (A,C,E) and anti-MAP2 (B,D,F) antibodies in control conditions (A,B), in HS (C,D) or in DS (E,F). Dendrites are strongly labelled by the anti-MAP2 antibody and some weak MAP2 staining is also visible in the axons. In contrast, the anti-NF-H staining is axon-specific. Bar, 20 μm.

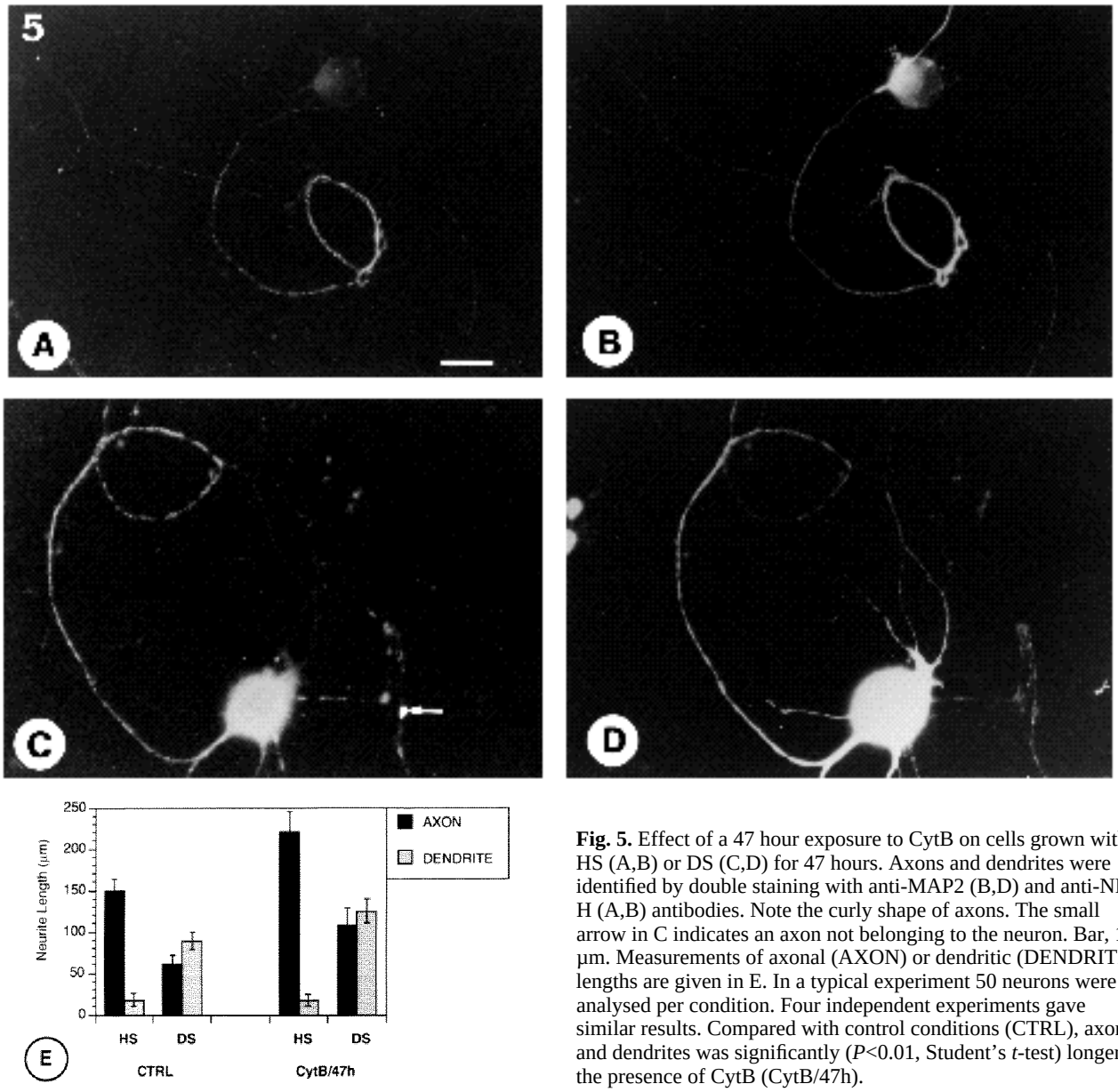

Fig. 5. Effect of a 47 hour exposure to CytB on cells grown with HS (A,B) or DS (C,D) for 47 hours. Axons and dendrites were identified by double staining with anti-MAP2 (B,D) and anti-NF-H (A,B) antibodies. Note the curly shape of axons. The small arrow in C indicates an axon not belonging to the neuron. Bar, 10 µm. Measurements of axonal (AXON) or dendritic (DENDRITE) lengths are given in E. In a typical experiment 50 neurons were analysed per condition. Four independent experiments gave similar results. Compared with control conditions (CTRL), axons and dendrites was significantly (P<0.01, Student's t-test) longer in the presence of CytB (CytB/47h).

rons strongly NF-H positive increased from 34 ± 8% (n=600, 2 experiments) in the absence of GAGs to 66 ± 7% (n=600, 2 experiments) and 56 ± 8% (n=600, 2 experiments) in the presence of HS and DS, respectively. The specific effects of HS and DS on the growth of axons and dendrites were thoroughly described by Lafont et al. (1992) and can be evaluated in the histogram of Fig. 5 where lengths of axons and dendrites after 48 hours in the presence of HS or DS are quantified.

Cytochalasin B increased the outgrowth of axons and dendrites

CytB added 1 hour after plating and left for 47 hours before fixation and analysis did not modify the effects of HS and DS on the number of primary neurites and on the initiation of axons and dendrites, but significantly (P<0,01; Student's t-test) increased the length of all neurites (Table 3 and Fig. 5). Indeed, in the presence of HS (Fig. 5A,B,E), CytB increased the length of axons by more that 50% (221 ± 24 µm versus 150 ± 18 µm) whereas, in the presence of DS (Fig. 5C,D,E), CytB increased the length of axons (108 ± 20 µm versus 61 ± 11 µm) and dendrites (125 ± 15 µm versus 89 ± 10 µm).

We consistently observed that, in the presence of CytB, some neurites developed a "curly" appearance. These curly neurites were always double stained with the anti-MAP2 and anti-NFH antibodies and therefore identified as axons (Fig. 5A-D). In contrast, dendrites, only stained with the anti-MAP2 antibody (Fig. 5C,D), did not exhibit this striking curly morphology.

Similar effects on growth were observed when CytB was added to neurons already developed (24 hours after plating) and left for the final 24 hours of culture (Fig. 6E). However, in this latter situation, increases in growth were

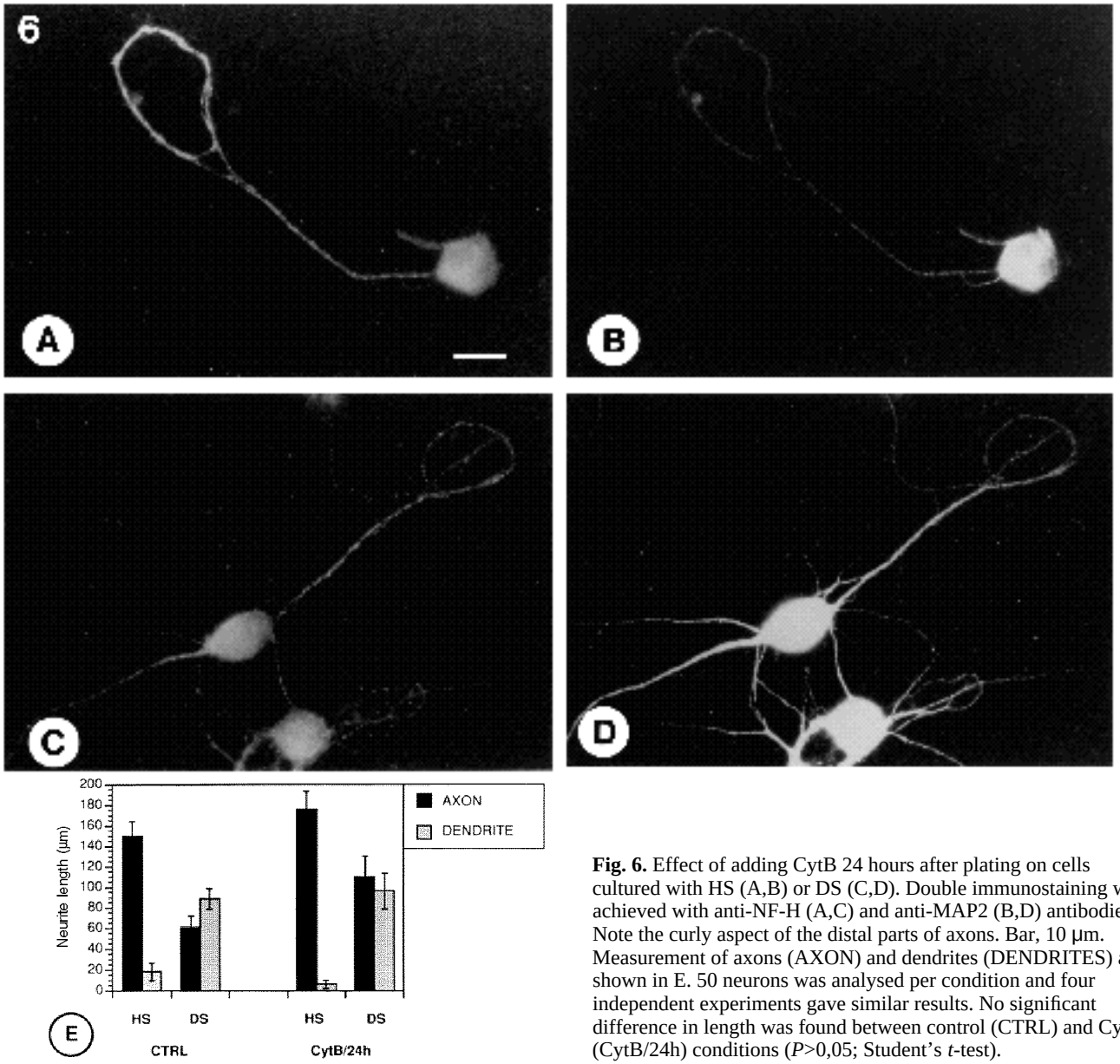

Fig. 6. Effect of adding CytB 24 hours after plating on cells cultured with HS (A,B) or DS (C,D). Double immunostaining was achieved with anti-NF-H (A,C) and anti-MAP2 (B,D) antibodies. Note the curly aspect of the distal parts of axons. Bar, 10 µm. Measurement of axons (AXON) and dendrites (DENDRITES) are shown in E. 50 neurons was analysed per condition and four independent experiments gave similar results. No significant difference in length was found between control (CTRL) and CytB (CytB/24h) conditions (P>0,05; Student's t-test).

less marked, as is to be expected from the twofold reduction in the time of drug action. The addition of CytB 24 hours after plating modified the morphology of only the distal segments of axons, which, as illustrated in Fig. 6 (A-D), were induced to curl. In some cases, the axon's distal end fasciculated upon itself, as indicated by the intense fluorescence of some of these axonal extremities (Fig. 6A).

It is likely that the point at which the axons start to adopt the curly morphology coincides with the position of the growth cone at the time of addition of CytB. This in turn suggests that depolymerization of actin filaments affects events occurring at the distal tip of elongating neurites and primarily disrupts the steering of the axonal growth cone. This latter point is clearly illustrated in the experiment of Fig. 7, in which the use of an anti-NCAM antibody allowed for a clear visualization of a long and curly axon and of several dendrites only partially affected by the addition of CytB for the last 24 hours.

Nocodazole preferentially reduced axonal growth

In the experiments with NZ, cytoskeletal markers for polarity could not be used, since MAP2 is associated with the microtubules and NZ can modify the distribution of intermediate filaments (Carmo-Fonseca and David-Ferreira, 1990; Gyoeva and Gelfand, 1991). Therefore neurons were labelled with an anti-NCAM antibody (Fig. 8). This staining revealed several thin filopodial extensions which were not visible with previous cytoskeletal markers. Only the main neuritic shafts were counted in the statistical analysis.

Consequently, in experiments involving NZ, the longest neurite was considered as the "axon" (histograms of Figs 8 and 9). As previously demonstrated (Lafont et al., 1992), this criterion of identification is always valid in the presence of HS, but is valid in only 70% of cases in the presence of DS, which permits the elongation of some very long dendrites. Dendrite length was estimated by subtracting the

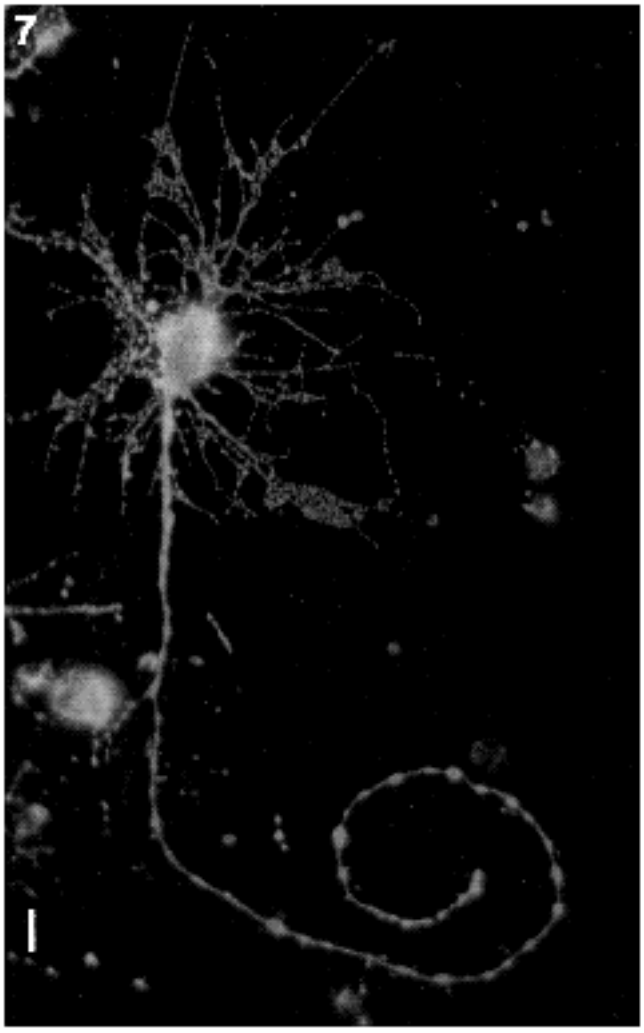

Fig. 7. Morphological effect of CytB on axonal shape. Neurons were plated with DS and CytB was added for the last 24 h before fixation and staining with the anti-NCAM antibody. Note the length, the curly aspect and the "varicosities" of the axon. Bar, 10 μm.

length of the axon from total neurite length. In the presence of DS and NZ the length of the axon was thus slightly (at most 30%) over-estimated at the expense of that of the dendrites.

NZ added 1 hour after plating and left for 47 hours dramatically decreased neurite elongation in the presence of HS (Fig. 8A,B,E). In comparison, as seen in Fig. 8 (C,D,E), growth inhibition was less marked with DS, demonstrating that NZ acts primarily on the initiation and elongation of the axon and is less deleterious to dendrite growth. The morphology of dendrites growing in the absence of microtubules can also be seen at the ultrastructural level in Fig. 3 (C,D).

This primary effect of NZ on the axon is further indicated by experiments in which NZ was added to the culture 24 hours after plating (Fig. 9A,B). In Fig. 9C, the lengths of axons and dendrites are shown for cultures arrested 48 hours after plating without (CTRL) or with NZ addition at 24 hours (NZ/24h). In the presence of HS, addition of NZ at 24 hours clearly inhibited further axonal elongation and could even provoke some retraction. This clearcut growth inhibition of the axon was also observed in the presence of DS.

The effects on dendrites are more difficult to interpret because of the systematic under-estimation of dendritic length (see above). Therefore, in spite of the fact that, in the presence of DS, the differences in total neurite lengths between control and NZ/24h did not exceed 33%, we cannot totally exclude a slight effect of NZ on the elongation of 24-hour-old dendrites.

DISCUSSION

The present study demonstrates that actin depolymerization increases the growth of all neurites and has no effect per se on neuronal polarity. In addition, the curly aspect of axons in the presence of CytB suggests that actin has an important function in the navigation of axonal growth cones. It is also clear that elongation of dendrites is less sensitive than that of axons to NZ. These observations fit well with the model in Fig. 1 proposing that axon growth, in contrast to that of dendrites, depends more on tubulin polymerization than on a continuous adhesion to the substratum.

To evaluate this different behaviour, axons and dendrites were identified by immunocytochemistry. The best markers presently available are MAP2 (a protein enriched in dendrites) and a neurofilament isoform present in young axons (Matus et al., 1986; Higgins et al., 1988; Pennypacker et al. 1991; Lafont et al., 1992). Using antibodies against these two proteins, we could easily identify axons and dendrites. Some neurites were stained with both antibodies, these were considered as axons because, whereas NF staining is very specific, MAP2 can be present in immature axons (Higgins et al., 1988).

In experiments with NZ, antibodies were not used because this drug is known to modify the distribution of MAP2 and intermediate filaments (Carmo-Fonseca and David-Ferreira, 1990; Gyoeva and Gelfand, 1991; Lafont, unpublished results). The axon was therefore defined as the longest neurite. This choice, based on previous experiments (Lafont et al., 1992), is always valid in the presence of HS. However, in the presence of DS, where 30% of the longest neurites are in fact dendrites, it leads to a slight under-estimation of the dendritic length.

The axon growth-promoting effect of CytB is in accord with the observations of Dennerll et al. (1988, 1989), who demonstrated the importance of the interactive forces between actin and microtubules in a mechanically imposed growth model. Their results and those of Bray (1984), Albrecht-Buehler (1987), Lamoureux et al. (1990), Zheng et al. (1991) and Winckler and Salomon (1991) suggest that the decrease of actin tension created by mechanically applied forces or by cytochalasin allows rapid microtubule assembly and, consequently, stimulates neurite elongation.

Our results, demonstrating that axonal growth is accelerated by CytB but specifically antagonized by NZ, fully

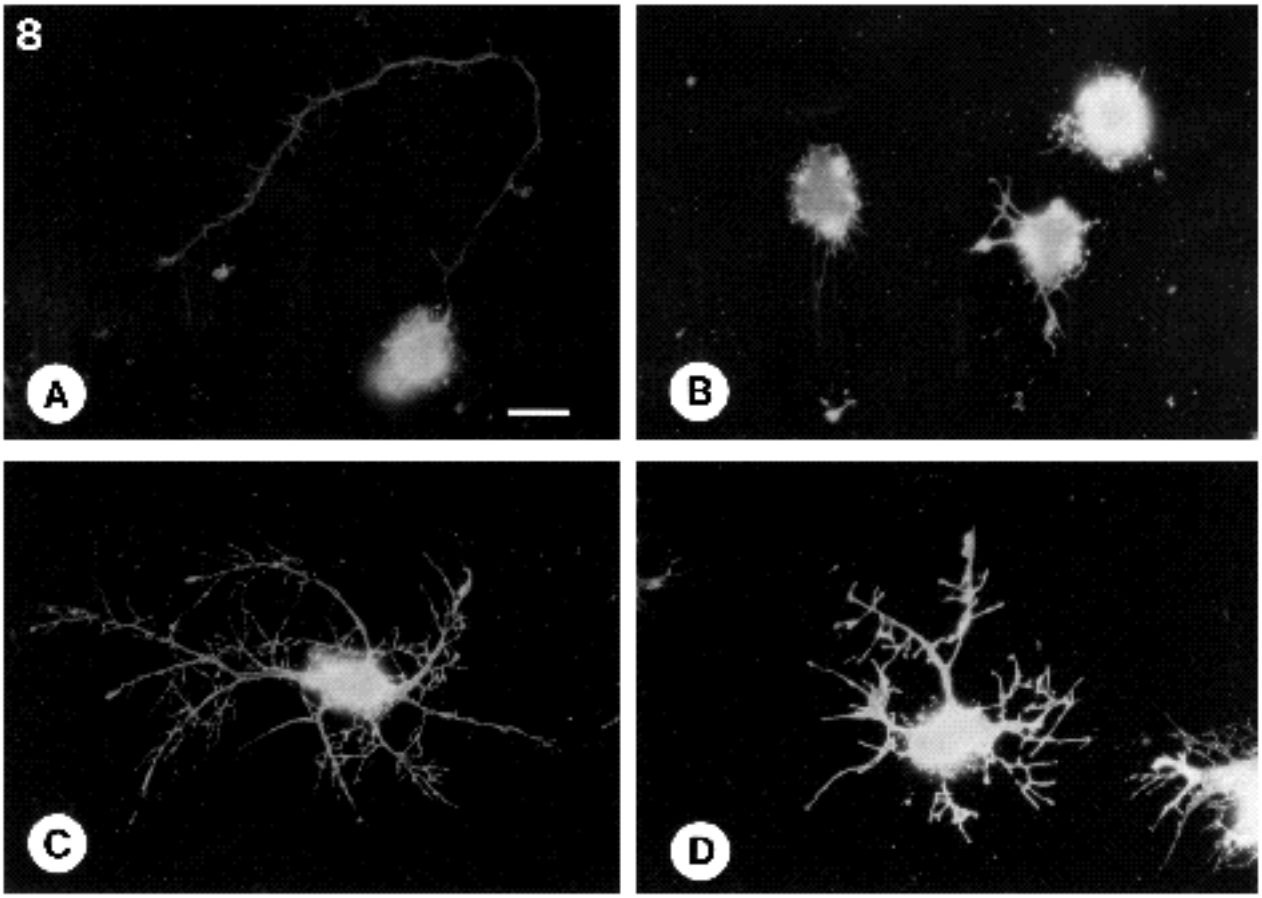

Fig. 8. Effect of the continuous presence of NZ on neuritic outgrowth. Cells were cultured with HS (A,B) or DS (C,D) and NZ was added 1 hour after plating (B,D). Cells were immunostained with a polyclonal anti-NCAM antibody. Note that growth inhibition by NZ is more dramatic in HS (B) than in DS (D). Bar, 10 μm. Quantification of the length of the longest neurite (AXON) as well as the calculation of dendrite length (DENDRITE) are shown in E (see in the text for explanations). 50 neurons were analysed per condition and four independent experiments gave similar results. Axonal length was significantly reduced by NZ (P<0.01 Student's t-test). However, dendrite growth (total neurite length minus axon length) was not significantly reduced by NZ.

confirm this model. However, the relative resistance of dendrite growth to the addition of NZ suggests that the strategy developed by the dendrites to antagonize actin tensile forces is not primarily based on microtubule assembly, but, as proposed in the model in Fig. 1, on adhesion. This conclusion is in good agreement with that of several of our previous studies (Chamak and Prochiantz, 1989; Rousselet et al., 1988, 1990; Lafont et al., 1992) and fits with the report by Lamoureux et al. (1990) showing that neurite outgrowth in the presence of NZ can be rescued only if cellular adhesion is enhanced by the presence of extracellular matrix molecules.

The observation that dendrites, as opposed to axons, can only grow in high-adhesion conditions suggests that their concentration of polymerized microtubules is not sufficient to antagonize actin-driven tensile forces. In a previous study we showed that the concentration of polymerized microtubules in proximal dendrites is lower than that in young axons (Rousselet et al., 1990). In addition to this concentration difference, it is noteworthy that, within axons, all microtubules have the same orientation with their plus ends distal to the cell bodies, whereas within dendrites microtubules with the two orientations co-exist. This difference might have distinct consequences on the direction and intensity of compression forces within the two types of neurites and explain why dendrite growth depends so much on adhesion (Baas et al., 1988).

The interplay between adhesion, viscosity and elasticity in the definition of cell shape is not unique to neurons and has been observed in other experimental models (Albrecht-Buehler, 1987; Breitfield et al., 1990; Matter et al., 1990; Winckler and Salomon, 1991). It is clear, however, that a

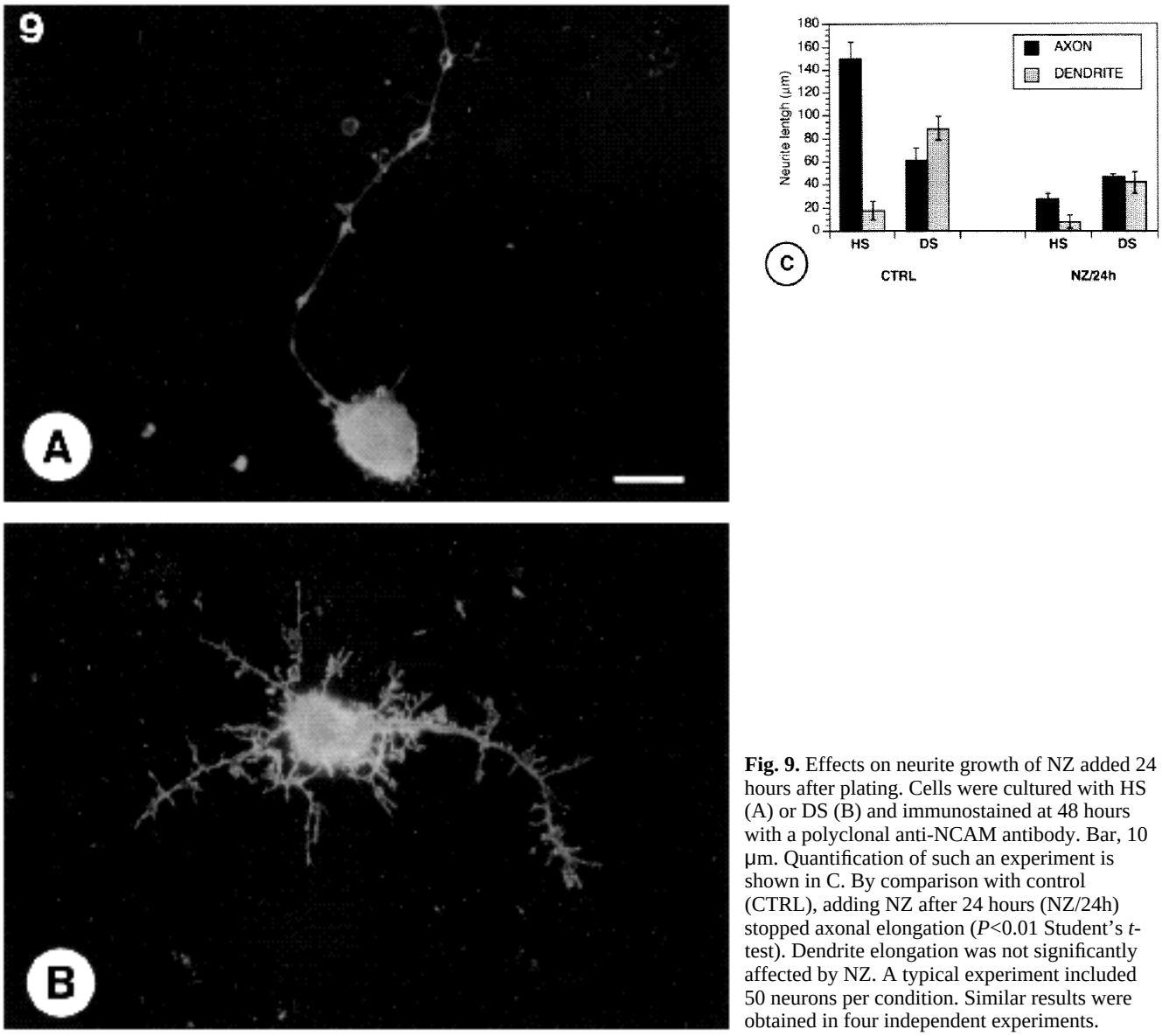

Fig. 9. Effects on neurite growth of NZ added 24 hours after plating. Cells were cultured with HS (A) or DS (B) and immunostained at 48 hours with a polyclonal anti-NCAM antibody. Bar, 10 µm. Quantification of such an experiment is shown in C. By comparison with control (CTRL), adding NZ after 24 hours (NZ/24h) stopped axonal elongation (P<0.01 Student's t-test). Dendrite elongation was not significantly affected by NZ. A typical experiment included 50 neurons per condition. Similar results were obtained in four independent experiments.

purely mechanical model such as the one presented in Fig. 1 is limited and that, in order to understand the establishment and maintenance of cell polarity, one has to take many other parameters into account.

For example, considering the similarity between the axon and the apical compartment of some non-neuronal polarized cells, we might predict that NZ would affect the intracellular traffic in the direction of the axon (Breitfield et al., 1990; Dotti and Simons, 1990; Matter et al., 1990; Gilbert et al., 1991). Another point not to be under-estimated is the role of extrinsic factors (including growth factors, neuromediators and cell or surface adhesion molecules) in regulating the levels of second messengers and the degrees of phosphorylation of molecules interacting with the membrane and the cytoskeleton (Schuch et al., 1989; Doherty et al., 1991).

The present study favours the hypothesis that axons and dendrites differ in their requirements for initiation and elongation, dendrites being more dependent on adhesive properties of the substratum. These findings imply that axons differ from dendrites very early in the course of their in vitro development, but give no indication as to when and how the "polarity decision" is actually made. We cannot, in particular, exclude the possibility that polarity decisions are made before neurite growth.

In addition to its effect on neurite growth, CytB induces a striking curly morphology in axons, an observation also made by Madreperla and Adler (1989) on retinal cells. We propose that this phenomenon is due to the centripetal orientation of axonal microtubules (Heacock and Agranoff, 1977; Heidemann et al., 1981; Baas et al., 1987, 1988, 1989). This curly morphology is maintained for the length of the entire axon if CytB is added at the onset of the culture, and is restricted to axonal extremities if the drug is only added later in the culture.

This latter point indicates that actin acts at the level of the elongating axonal shaft and that tensile forces participate in the steering of the growth cone. This is dramatically

illustrated in Fig.7. Our results thus support the idea that, in contrast to the situation in the frog (Reinsch et al., 1991), in mammals microtubule elongation occurs mainly at the distal end of the axon (Baas and Ahmad, 1992; Okabe and Hirokawa, 1992).

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