Differential Effects of Microtubule Inhibitors on Axonal Branching and Elongation of Cultured Rat Hippocampal Neurons

Atsushi Aoyagi, Hiroshi Saito and Kazuho Abe*

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113, Japan

Received February 13, 1995 Accepted April 6, 1995

ABSTRACT—We investigated the effects of microtubule inhibitors, taxol and colchicine, on the axonal branching and elongation in cultured rat hippocampal neurons. Taxol (50 nM) did not affect the morphology of neurons cultured under the control conditions, but significantly reduced the axonal branching stimulated by basic fibroblast growth factor. The axonal elongation stimulated by astrocyte-conditioned medium was not affected by the same concentration of taxol. Colchicine (10 nM) showed similar effects as taxol. These results suggest that microtubules play more important roles in axonal branching than in axonal elongation.

Keywords: Fibroblast growth factor, Neurite branching, Microtubule inhibitor

Neuronal cells form their complex shape of neurites by two processes, branching and elongation. Investigations of cellular mechanisms underlying neurite branching and elongation of brain neurons are very important not only for understanding the brain development but also for the therapy of neurodegenerative disorders such as Alzheimer's disease. We have recently found that basic fibroblast growth factor (bFGF), which has been proposed to function as a neurotrophic factor in the brain (1, 2), selectively promotes the bifurcation and growth of axonal branches without affecting the elongation rate of primary axons in cultured rat hippocampal neurons (3). Many polypeptide growth factors or neuromodulators have been reported to promote the axonal elongation (4-6), but, to our knowledge, bFGF is the first substance that selectively affects the axonal branching of brain neurons. Furthermore, in our previous study (3), bFGF increased the complexity of axonal branches, whereas astrocyte-conditioned medium (ACM) stimulated the axonal elongation but not branching. The contrastive effects of bFGF and ACM indicate that axonal branching and elongation are independently regulated by different factors or mechanisms.

Microtubules are generally thought to play important roles in neuronal architecture (7). The effects of microtubule inhibitors on the neurite outgrowth were reported in cultured peripheral neurons (8, 9), but there has been no report in cultured brain neurons. In the present study, we compared the effects of microtubule inhibitors, taxol and colchicine, on bFGF-stimulated axonal branching and ACM-stimulated axonal elongation in primary cultured rat hippocampal neurons.

bFGF used in the present study is an acid-resistant mutein of human bFGF, CS23 (a generous gift from Takeda Chemical Industries, Ltd., Osaka). The activity of CS23 on brain neurons is virtually the same as those of the wild type of human bFGF and bovine bFGF (10, 11). Astrocyte-conditioned medium (ACM) was prepared from confluent rat hippocampal astrocyte cultures (3). Dissociated hippocampal neurons were prepared from 18-day-old embryos of Wistar rats as described in our previous paper (3, 10). Briefly, the cells were dissociated by incubation with 0.25% trypsin and 0.01076 DNase I at 37°C for 30 min, followed by pipetting, and then they were suspended in modified Eagle's medium containing 10% fetal bovine serum (3). The dissociated cells were plated on polylysine-coated 35-mm culture dishes (10 cm²/dish) at a density of 2500 cells/cm². After the plated cells were cultured in the serum-containing medium for 24 hr, the medium was changed to serum-free modified Eagle's medium containing 10% fetal bovine serum (3). The dissociated cells were plated on polylysine-coated 35-mm culture dishes (10 cm²/dish) at a density of 2500 cells/cm². After the plated cells were cultured in the serum-containing medium for 24 hr, the medium was changed to serum-free modified Eagle's medium containing N2 supplements (3). The cells were cultured in the serum-free medium for a further 24 hr. We selected pyramidal-like neurons that had established axons and dendrites during this preliminary period (for 48 hr in total) and that were free from contact with other cells, and took photographs of them. Immediately after the recording,
Fig. 1. Representative photomicrographs showing rat hippocampal neurons cultured for 48 hr under the following conditions: A, In the control medium; B, In the presence of 1 ng/ml bFGF; C, In the presence of 50% (v/v) ACM; D, In the presence of 50 nM taxol; E, In the presence of 1 ng/ml bFGF and 50 nM taxol; F, In the presence of 50% (v/v) ACM and 50 nM taxol. Bars = 100 μm.

Fig. 2.
bFGF or other drugs were added to cultures. The same cells were photographed 24 or 48 hr after addition of the drugs. Under these experimental conditions, more than 90% of the selected neurons survived for the next 48 hr. However, in the case where the selected neurons died within 48 hr, the data of these cells were omitted. Primary cultured hippocampal pyramidal neurons extend one long process and several short processes as shown in Fig. 1. By morphological and immunocytological criteria, the long process and several short processes have been identified as axon and dendrites, respectively (12, 13). The number of branch points per axon and the axon length were measured as illustrated in Fig. 2.

Figure 1 shows representative photomicrographs of hippocampal neurons cultured in the absence or presence of 1 ng/ml bFGF or 50% (v/v) ACM. Addition of bFGF promoted the axonal branching without affecting the elongation of primary axons (Fig. 1B), while 50% (v/v) ACM remarkably promoted the axonal elongation but not branching (Fig. 1C), consistent with our previous observation (3). The microtubule inhibitor taxol (50 nM) (Sigma Chemical Co., St. Louis, MO, USA) alone did not affect the morphology of neurons cultured under the control conditions (Figs. 1D, 2A and B). However, the axonal branching-promoting effect of bFGF was significantly attenuated by the presence of 50 nM taxol (Figs. 1E and 2A). The axonal elongation-promoting effect of ACM was not affected by the same concentration of taxol (Figs. 1F and 2B). Colchicine (Sigma Chemical Co.), another microtubule inhibitor, showed similar effects as taxol. Colchicine (10 nM) alone did not affect the morphology of control or ACM-treated neurons, but significantly attenuated the effect of bFGF (Fig. 2, C and D).

Since taxol and colchicine showed no effect on neurite elongation in the presence of ACM, we checked if these agents were active in this condition. As shown in Fig. 3, higher concentrations (50 and 100 nM) of colchicine decreased the number of surviving neurons in the medium containing 50% (v/v) ACM as well as in the control medium. Higher concentrations (100–1000 nM) of taxol caused shrinkage of neurites in the presence of ACM as well as in the control medium (data not shown). Therefore, the possibility that ACM absorbs taxol or colchicine can be ruled out.

The main finding of this study was that taxol and colchicine selectively blocked the bFGF-stimulated axonal branching of cultured hippocampal neurons. The microtubule inhibitors, at concentrations that blocked the action of bFGF, did not affect the axonal elongation in the control or ACM-treated neurons. This means that bFGF-stimulated axonal branching is more susceptible to the inhibitors. Microtubules may play more important roles in axonal branching than in axonal elongation. Considering that taxol stabilizes microtubules (14) and colchicine inhibits microtubule polymerization (15), both processes of microtubule disassembly and reorganization may be required for elongating axons to divide into two or more daughter neurites.

In conclusion, we found that in cultured rat hippocampal neurons, the bFGF-stimulated axonal branching is more susceptible to blocking by microtubule inhibitors.
The action of bFGF will definitely be a useful model for studying the role of microtubules in the axonal branch formation of brain neurons.

REFERENCES


