Baikal Skullcap Extract Stimulates Neurite Growth in Cultures of Rat Dorsal Root Ganglia

A. Ya. Shurygin, N. V. Bezzubov, E. A. Ignatova, S. M. Nikolaev*, and I. V. Viktorov**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 7, pp. 56-58, June, 2002
Original article submitted February 18, 2002

The effect of baikal skullcap extract on the development of dorsal root ganglia from 1-2-day-old rats in organotypic cultures was studied. Baikal skullcap extract produced a dose-dependent stimulating effect on neurite growth in neurons of dorsal root ganglia.

Key Words: *baikal skullcap extract; dorsal root ganglia; organotypic culture; neurites; regeneration*

Preparations of baikal skullcap (*Scutellaria baicalensis Georgii*) [1] produce various therapeutic effects and are used for prophylaxis and treatment of many cardiovascular, gastroenterological, and immune diseases. Clinical observations show that baikal skullcap is effective in the treatment of enhanced excitability of the nervous system, neuroses, and insomnia [1-3]. These data prompted *in vitro* study of the possible neurotrophic properties of baikal skullcap extract (BSE).

MATERIALS AND METHODS

Dorsal root ganglia (DRG) from 1-2-day-old Wistar rats were used in the experiments. DRG were cultured for 7 days in a medium containing 50 ml minimum Eagle's medium, 33 ml equine serum, 12 ml Hanks solution, 1 ml glutamine (20 mM), and 4 ml glucose (20%). BSE was extracted from roots of baikal skullcap [3] and added to the culture medium in concentrations of 0.1 (n=40) and 0.01% (n=30). The ganglia (n=50) cultured without BSE served as the control.

The cultures were fixed with 10% neutral paraformaldehyde and stained with 0.3% trypan blue. The effect of preparation on DRG development was evaluated by routine quantitative parameters: maximum neurite length in the outgrowth zone at the end of culturing and the number of neurites and neurite bundles crossing a 200-μ radial line at a distance of 250 μ from the explant [4,6,8].

The results were processed statistically using Student t test.

RESULTS

Addition of BSE to the culture medium modulated DRG growth: the number and length of neurites in the outgrowth zone increased compared to the control cultures. Vital phase-contrast microscopy and histological studies showed that the outgrowth zone contained regenerating neurites and neurite bundles consisting of several processes (Fig. 1). Neurite growth was accompanied by migration of Schwann cells, however, myelin was not found at these terms of culturing.

BSE increased the number of regenerating neurites and neurite bundles in the outgrowth zone (Fig. 1), which suggests better survival of cultured neurons and more intensive branching of regenerating neurites. Addition of 0.1% BSE to the culture medium significantly increased the number of neurites in DRG outgrowth zone (Fig. 2, *a*). Histological studies revealed

Kuban' Research-and-Production Laboratory of Physiologically Active Substances, Krasnodar; *Institute of Common and Experimental Biology, Siberian Department of the Russian Academy of Sciences, Ulan-Ude; **Brain Research Institute, Russian Academy of Medical Sciences, Moscow. ***Address for correspondence:*** ivic@4unet.ru. Viktorov I. V.

0007-4888/02/1341-047$27.00

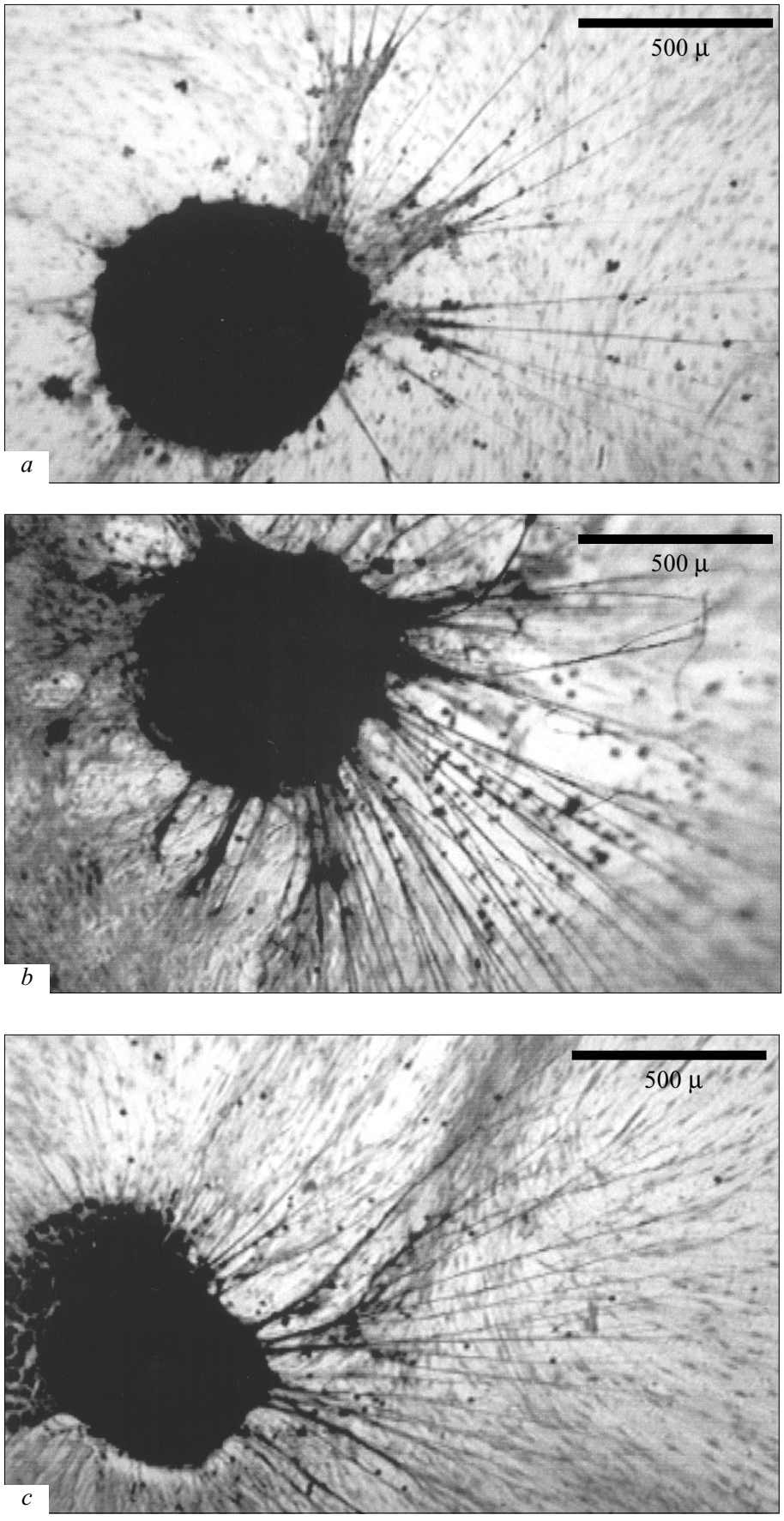

Fig. 1. Effect of baikal skullcap extract on neurite outgrowth in cultured rat dorsal root ganglia. Trypan blue staining. *a*) control, *b, c*) 0.1 and 0.01% extract in the nutrient medium, respectively.

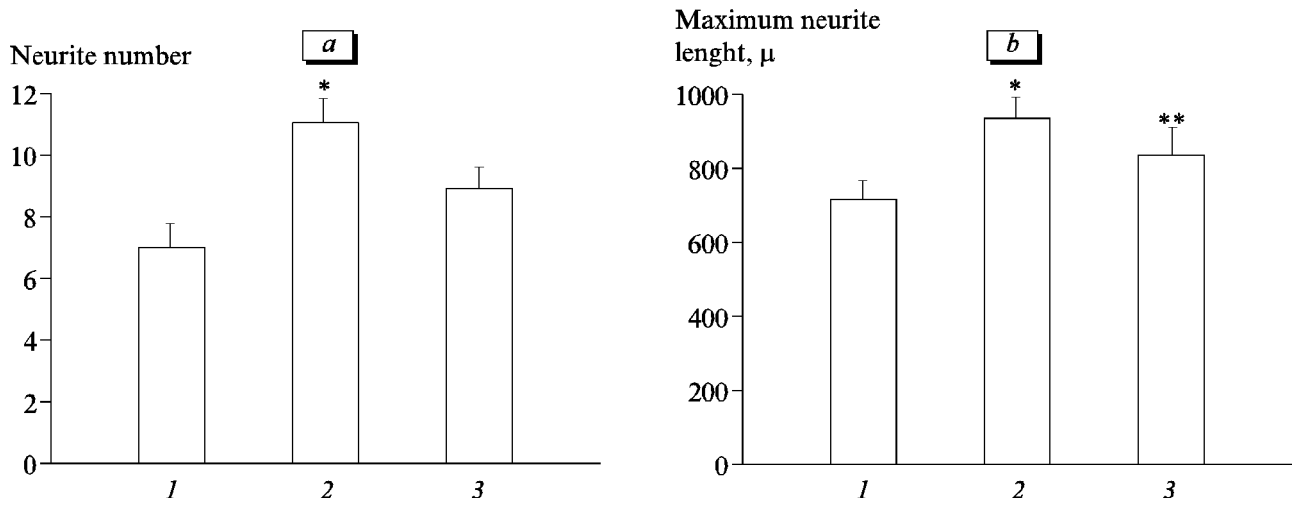

Fig. 2. Effect of baikal skullcap extract on the number of neurites in the outgrowth zone (*a*) and their maximum length (*b*). *1*) control, *2, 3*) 0.1 and 0.01% baikal skullcap extract, respectively. *$p<0.01$, **$p<0.05$ compared to the control.

significant differences between the maximum neurite lengths in the control and experimental cultures (Fig. 2, *b*).

Thus, our experiments demonstrated a dose-dependent neurotrophic effect of BSE-containing culture medium on regeneration of DGR neurons and intensity of neurite growth. The most pronounced effect was observed after addition of 0.1% BSE.

The stimulating effect of BSE probably depends on the presence of antioxidants, in particular, flavonoids (25.7% of BSE extract) [3,5] possessing pronounced neuroprotective properties [7] and some other unknown bioactive neurotropins [3].

Hence, BSE possessing neurotrophic properties can be used for the development of new pharmacological preparations for stimulation of regenerative processes after damage to the peripheral nervous system.

REFERENCES

1. T. G. Bykhasheeva, *Ecological and Biological Peculiarities of Scutellaria Baicalensis Georgi in Baikal Region*, Abstract of Cand. Med. Sci. Dissertation, Ulan-Ude (2000).
2. E. D. Gol'dberg, A. M. Dygai, V. I. Litvinenko, *et al., Baikal Skullcap. Phytochemistry and Pharmacological Properties* [in Russian], Tomsk (1994).
3. G. Kh. Damdinova, *Nootropic Action of Baikal Skullcap*, Abstract of Cand. Med. Sci. Dissertation, Ulan-Ude (2001).
4. M. V. Kozlova, A. Ya. Shurygin, I. P. Sidorenko, and V. U. Kalenchuk, *Neirofiziologiya*, **20**, No. 4, 239-246 (1988).
5. S. B. Seredenin and A. D. Durnev, *Pharmacological Genome Protection* [in Russian], Moscow (1992).
6. L. G. Khaspekov, I. V. Viktorov, and N. A. Shashkova, *Guidebook on the Cultivation of the Nervous Tissue. Methods. Technique. Problems.* [in Russian], Moscow (1988), pp. 167-180.
7. H. De Groot and U. Rauen, *Fundam. Clin. Pharmacol.*, **12**, 249-255 (1998).
8. T. Lahtinen and A. Eranko, *Med. Biol.*, **62**, 189-197 (1984).