Stimulatory Effect of Green Tea Extract on the Growth of Neurites in the Rat Spinal Ganglion Culture

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Addition of green tea extract in concentrations of 0.004-0.006% to the nutrient medium markedly stimulated the growth of spinal ganglion neurites of 1-2-day-old rats.

Key Words: *green tea extract; polyphenols; tissue culture; spinal ganglia; neurite growth*

The search for substances with neurotrophic effects among natural compounds of plant origin is an important trend in the creation of new drugs for the prevention and treatment of nervous diseases. Extracts of many medicinal plants, *e.g.* green tea extract (GTE), include compounds with a wide spectrum of biological activity. We previously showed that Scutellaria baikalensis extracts potentiated the growth of neurites in cultured rat spinal ganglion (SG) [1].

Here we studied the effects of ethanol GTE on neurite growth in SG cultures.

MATERIALS AND METHODS

The study was carried out on 540 SG of 1-2-day-old Wistar rats. SG were cultured as described previously [1]. The nutrient medium contained 50 ml minimum Eagle's medium, 33 ml equine serum, 12 ml Hanks' solution, 1 ml glutamine (200 mM), and 4 ml glucose (20%). Dry GTE obtained by ethanol extraction with subsequent ethanol evaporation and lyophilization was added to the nutrient medium in final concentrations of 0.04, 0.008, 0.006, 0.004, and 0.002%. SG cultures grown in nutrient medium without GTE served as the control. The effect of the test preparation on SG was evaluated by the maximum length and number of neurites [1].

The results were statistically processed using Student's *t* test.

RESULTS

GTE in concentrations of 0.004-0.006% had the most pronounced stimulatory effect on the growth of SG neurites.

Stimulation of neurite growth in the presence of 0.006% GTE was most demonstrative compared to the control (Table 1). On day 2 the number of neurites in SG treated with GTE increased, while the length of neurites did not differ from the control. On day 3 of culturing neurite growth in the presence of GTE was significantly higher ($p \leq 0.01$). On day 4 the differences between the control and experiment were maximum ($p \leq 0.001$) for both parameters. On day 5 the effect of GTE was lower and the growth parameters of experimental ganglia corresponded to the control.

GTE in a concentration of 0.004% exhibited the same regularities during culturing (Table 2), but the differences between the experiment and control on day 2 were negligible. On day 3 of culturing the experimental SG neurite growth was markedly stimulated: the differences in the maximum length and number of neurites were significant ($p \leq 0.001$). On days 4 and 5 these differences persisted: the number and maximum length of neurites in experimental cultures were significantly higher than in the control.

GTE in a concentration of 0.002% less intensively modified the growth of SG. A negligible increase of the

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maximum length and number of neurites in experimental SG in comparison with the control was observed on days 2 and 4 of culturing. The maximum concentration of GTE (0.04%) caused high mortality (85%) of SG. Survived SG had just solitary neurites in the growth zone. GTE concentration of 0.008% caused no destructive changes in SG, but their growth parameters virtually did not differ from the control.

The results of our study demonstrate for the first time the stimulatory effect of GTE on regenerative growth of neurites in cultures of rat SG and supplement previous data on the neurotrophic and neuroprotective effects of green tea, which can be explained by high content of antioxidants (flavonoids, polyphenol (-)-epigallocatechine-3-gallate (EGCG)), theanine (γ-glutamylethylamide), and vitamins [3,6,8,9,11,12].

Theanine is affine for AMPA and kainate subtypes of glutamate receptors. When injected into cerebral ventricles of Mongolian gerbils, it prevented neuronal death in the hippocampal CA1 field during global ischemia and in CA3 field under conditions of toxic effect of kainic acid [3]. EGCG injected intraperitoneally (25-50 mg/kg) prevented neuronal death in the hippocampal CA1 field in Mongolian gerbils with global ischemia [5]. GTE (0.5-1.0 mg/kg) and EGCG (2 and 10 mg/kg) prevented the death of substantia nigra dopaminergic neurons (experimental Parkinson's disease) in mice treated with 6-hydroxydopamine and N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine damaging these neurons [6]. Similar neuroprotective effect of EGCG was observed after treatment of cultured pheochromocytoma (PC-12) and human neuroblastoma cells (SH-SY5Y) with dopaminergic neurocytotoxins [7,9,10]. Green tea belongs to a group of foodstuffs preventing excessive production of nitrogen oxide, which, as a free radical and precursor of many toxins, can be the cause of neurodegenerative diseases [14]. Recent studies demonstrating the pronounced anticarcinogenic effect of EGCG *in vitro* and *in vivo* [4,13-15] are particularly interesting from this viewpoint. However, clinical data confirming this effect are scanty, which prompts further investigation of the anticarcinogenic effects of green tea polyphenols.

TABLE 1. Effect of GTE in a Concentration of 0.006% on SG ($M \pm m$)

Duration of culturing, group	Maximum length of neurites, μ	Number of neurites
2 days		
control (n=10)	568.00±105.99	10.22±1.24
experiment (n=9)	765.00±94.45	14.38±1.48*
3 days		
control (n=33)	802.00±69.22	10.78±1.23
experiment (n=38)	1096.56±62.88**	16.64±1.25**
4 days		
control (n=23)	808.52±110.78	8.74±1.30
experiment (n=2)	1401.22±81.31***	16.25±1.52***
5 days		
control (n=9)	832.44±109.19	7.89±2.14
experiment (n=12)	991.67±104.87	7.75±0.90

Note. Here and in Table 2: *p<0.05, **p<0.01, ***p<0.001 compared to the control.

TABLE 2. Effect of GTE in a Concentration of 0.004% on SG ($M \pm m$)

Duration of culturing, group	Maximum length of neurites, μ	Number of neurites
2 days		
control (n=17)	565.41±38.96	16.29±2.84
experiment (n=14)	709.71±67.46	16.36±2.46
3 days		
control (n=49)	717.94±64.17	9.04±1.01
experiment (n=53)	1253.25±83.23 ***	18.85±1.38***
4 days		
control (n=37)	626.38±50.92	7.92±0.89
experiment (n=37)	1004.84±114.87**	13.08±1.58**
5 days		
control (n=6)	664.50±56.70	3.17±1.22
experiment (n=9)	1145.00±174.79*	10.78±1.88**

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