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PC12 Pheochromocytoma and Sympathetic Nervous System Derived Trophic Factors Augment Growth of Neuroblastoma

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Abstract—*A trophic factor secreted by PC12 rat pheochromocytoma augments growth of C1300 neuroblastoma clonal lines S20, N18 and C46, but does not affect growth of the NIE 115 line. A trophic factor present in newborn sympathetic ganglia has the same biological effect on neuroblastoma cell lines. PC12 cells and sympathetic ganglia are both of neural crest origin; possibly both secrete the same trophic factor.*

INTRODUCTION

WE HAVE SHOWN previously that the sympathetic nervous system (SNS) modulates the growth of mouse neuroblastoma (C1300 NB) both *in vitro* and *in vivo*. We have shown that ablation of the SNS, in mice, prior to injection of C1300 NB tumor cells significantly suppresses tumor growth [1, 2]. We have also shown that the SNS contains and secretes a trophic factor which augments the growth of C1300 and some of its clonal lines *in vitro*. Co-culturing of C1300 NB explants with superior cervical ganglia (SCG) augmented the growth of C1300 NB significantly [3]. Furthermore, media conditioned with SCG, as well as supernatants from freshly excised, homogenized SCG significantly augmented the growth of C1300 NB and some clonal lines derived from it [4]. We now present data on a trophic factor secreted by PC12 rat pheochromocytoma cells in culture which has the same biological effect on clonal lines of C1300 NB *in vitro* as the SNS trophic factor.

MATERIALS AND METHODS

PC12 cells were seeded on collagen-coated Petri dishes (20 cm^2) at 10^6 cells/dish and cultured in RPMI medium containing 10% heat-inactivated fetal bovine serum (FBS), 5% horse serum, 2% L-glutamine and 1% penicillin/streptomycin. After 3 days in culture, the starting medium was replaced by DME medium containing 1% FBS and was incubated for 24 h prior to removal of conditioned medium. Control medium contained 1% FBS in DME and was incubated for 24 h on collagen-coated Petri dishes.

Sympathetic cervical ganglia were obtained from 1-day-old Wistar–Furth rats. For each experiment, four ganglia were suspended in 1 ml of DME, sonicated on ice at a setting of 3 for 5 s using a Bronson model 18 S Sonifier cell disrupter and then filtered through a 0.22 μm microfilter. The protein content of the filtrate obtained from four SCG averages 22 μg/ml.

The following clonal lines of C1300 NB were used in these experiments: S20—cholinergic line, N18 and C46—neither line produced neurotransmitters—and NIE 115 a cell line that was originally adrenergic but at the time of these experiments was not producing neurotransmitters.

S20, N18, C46 or NIE 115 cells were seeded onto 9 cm^2 Petri dishes at 5×10^4 cells/dish and cultured either in DME medium containing 1% FBS and 10 μg of SCG filtrate or in PC12-conditioned medium. Cells cultured in DMEM with 1% FBS served as a control. Each experiment was done in triplicate. After 4 days in culture, cells in experimental and control dishes were removed and counted. Significance was calculated using Student's *t* test.

RESULTS

Both SCG filtrates and PC12-conditioned medium significantly augmented the growth of S20, N18, and C46 cells but did not influence the growth of NIE 115 cells.

A. The number of NB cells grown in SCG filtrate (E) or control medium (C) was: *S20 cells*

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$3.7 \pm 0.7 \times 10^5$ cells/dish (E), $1.4 \pm 0.3 \times 10^5$ cells/dish (C) $n = 4$ $P < 0.05$. *N18 cells* $3.6 \pm 0.8 \times 10^5$ cells/dish (E), $1.3 \pm 0.2 \times 10^5$ cells/dish (C) $n = 4$ $P < 0.05$. *C46 cells* $2.5 \pm 0.3 \times 10^5$ cells/dish (E); $1.3 \pm 0.1 \times 10^5$ cells/dish (C) $n = 4$ $P < 0.01$. *NIE 115 cells* $1.5 \pm 0.2 \times 10^5$ cells/dish (E), $1.4 \pm 0.1 \times 10^5$ cells/dish (C) $n = 3$, difference not significant.

B. The number of NB cells grown in PC12-conditioned medium (E) and control medium (C) was as follows: *S20* $3.8 \pm 0.5 \times 10^5$ cells/dish (E), $1.6 \pm 0.3 \times 10^5$ cells/dish (C), $n = 5$ $P < 0.01$. *N18* $2.3 \pm 0.4 \times 10^5$ cells/dish (E), $1.0 \pm 0.1 \times 10^5$ cells/dish (C) $n = 6$, $P < 0.01$. *C46* $2.7 \pm 0.4 \times 10^5$ cells/dish (E), $1.5 \pm 0.3 \times 10^5$ (C) $n = 6$ $P < 0.05$. *NIE 115* $2.8 \pm 0.3 \times 10^5$ cells/dish (E), $2.3 \pm 0.4 \times 10^5$ cells/dish (C) $n = 4$, difference not significant. The data are summarized in Fig. 1. The trophic/mitogenic factor present in SCG and in PC12-conditioned medium are inactivated by heat and trypsin, suggesting that they are peptides.

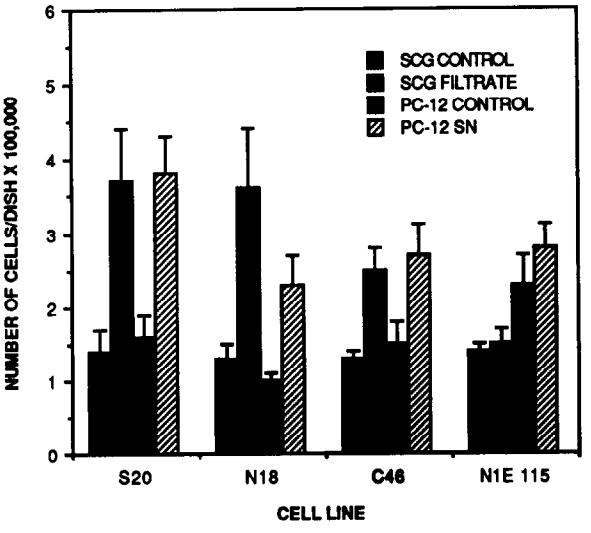

Fig. 1. Effect of PC12-conditioned medium and SCG filtrate on the growth of C1300 NB clonal lines. Vertical bars show standard error of the mean.

DISCUSSION

We have shown that the PC12 rat pheochromocytoma tumor line secretes a trophic factor which augments the growth of NB clonal lines S20, N18, and C46, but does not influence the growth of NIE 115. A trophic factor present in filtrates of newborn SCG has the same effect, i.e. it augments the growth of S20, N18, and C46 clonal lines of C1300 NB but does not influence the growth of the NIE 115 clonal line.

We reported previously that the SNS modulates growth of mouse neuroblastoma. We have shown that, in mice with a chemically destroyed SNS, growth of C1300 NB and S20 tumors was significantly suppressed. Those NB clonal lines that were sensitive to sympathectomy *in vivo* had augmented growth in the presence of the SCG trophic factor *in vitro* [5]. The SGC trophic factor is relatively specific for neuroblastoma since it did not influence growth of rat C6 glioma or mouse A10 breast adenocarcinoma *in vitro*. In addition, heart and skeletal muscle homogenates, lymph node homogenates and fibroblast-conditioned medium did not influence growth of S20 NB cells. We have also shown that the proliferative effect of the SCG factor on neuroblastoma cells diminishes with dilution and is still significant at dilution containing 4.4 μg of SCG filtrate protein/ml of medium but not at a dilution containing 0.9 μg of protein/ml of medium [4].

The PC12 line was established from a rat adrenal pheochromocytoma [6]. In several aspects PC12 resembles noradrenergic chromaffin cells and sympathetic neurons. Pheochromocytomas (which arise from the adrenal medulla) and sympathetic neurons are both of neural crest origin. PC12 cells synthesize and store dopamine and norepinephrine but not epinephrine. The adrenal medulla can be viewed as a modified sympathetic ganglion which lacks nerve terminals and which secretes neurotransmitters directly to the circulation [7–9]. Therefore, it is not surprising that sympathetic ganglia and a tumor derived from the adrenal medulla should contain and/or secrete trophic factors with similar activities and possibly the same trophic factor.

We have demonstrated the presence of a trophic factor in sympathetic ganglia and in an adrenal medulla-derived tumor, which augments growth of neuroblastoma. Sympathetic chains and adrenals are preferential sites at which neuroblastomas originate. Perhaps the two are linked.

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