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EXPERIMENTAL ARTICLES

An Inhibitor of Serine Proteases, Serpinb1a, Modulates Differentiation of PC12 Cells but Not Cultured Neurons

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Abstract—It was shown that serpinb1a is expressed during differentiation of PC12 cells induced by nerve growth factor (NGF). Here, we found that overexpression of serpinb1a in PC12 cells weakly but significantly increases PC12 survival during staurosporine-induced apoptosis. Immunoprecipitation of serpinb1a after its overexpression in PC12 showed that this protein interacts with active caspase-3 in both resting cells and cells that were stimulated by staurosporine. NGF-induced PC12 differentiation resulted in the formation of outgrowths and a considerable increase in caspase-3 activity in 24 h after the beginning of NGF treatment. This increase in the activity of caspase-3 lasted for at least 48 h. Overexpression of serpinb1a in PC12 cells suppressed the growth of neurites during NGF-induced differentiation; this effect was observed only at 48 h. Experiments performed with primary cultures of neocortical and hippocampal neurons showed that serpinb1a overexpression results in relatively weak changes in morphology: serpinb1a decreased the number of secondary dendrites in the cortical and average length of secondary dendrites in hippocampal neurons. The results of the experiments suggests that serpinb1a may interact with caspase-3 and influence the differentiation of PC12 cells but not neuronal cells.

Keywords: pheochromocytoma PC12, neuronal culture, caspase-3, serpinb1a, nerve growth factor

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INTRODUCTION

The hypothesis that processes that occur during apoptosis and differentiation are interrelated is widely accepted. It has been shown that the same molecular mechanisms function at some stages of apoptosis and cell differentiation and these processes have strong morphological similarity [1–3]. Both in apoptosis and in differentiation, an important role is played by proteases called caspases, in particular caspase-3, which is one of the principal proteases in apoptosis. During apoptosis, the activity of caspases is uncontrolled because the cell must die; however, during differentiation, the control over the activity of caspases is crucial. The absence of strict control over caspase activity during differentiation may result in cell destruction. Hence, the mechanisms that control the functioning of caspase-3 during the execution of non-apoptotic functions are of special interest. First, activation of caspase-3 may occur in certain cell compartments where the active caspase has a number of vital functions. Second, the cells may express certain proteins that can suppress activity of caspase-3 in some cell compartments.

It has been shown that induction of differentiation of neuron-like PC12 cells leads to changes in the expression of a large number of genes and that the pattern of these changes depends on the stimulus that induced differentiation [4]. Among proteins whose expression is strongly changed during differentiation of PC12 cells, serpinb1a has a special role because it is an inhibitor of serine and cysteine proteases. The serpinb1a expression strongly increases during differentiation of PC12 cells induced by nerve growth factor (NGF) [4–6], remains at a high level for 12 h [5], and returns to the basal level in 24 h [6]. It was shown that this increase in the serpinb1a expression may mediate the protective effect of NGF during apoptosis due to a decrease in the activity of caspase-3 [6]. Suppression of serpinb1a expression during NGF-induced differentiation of PC12 cells led to the slowing of neurite growth [5]; however, in another study, this treatment did not significantly affect the morphology of differ-

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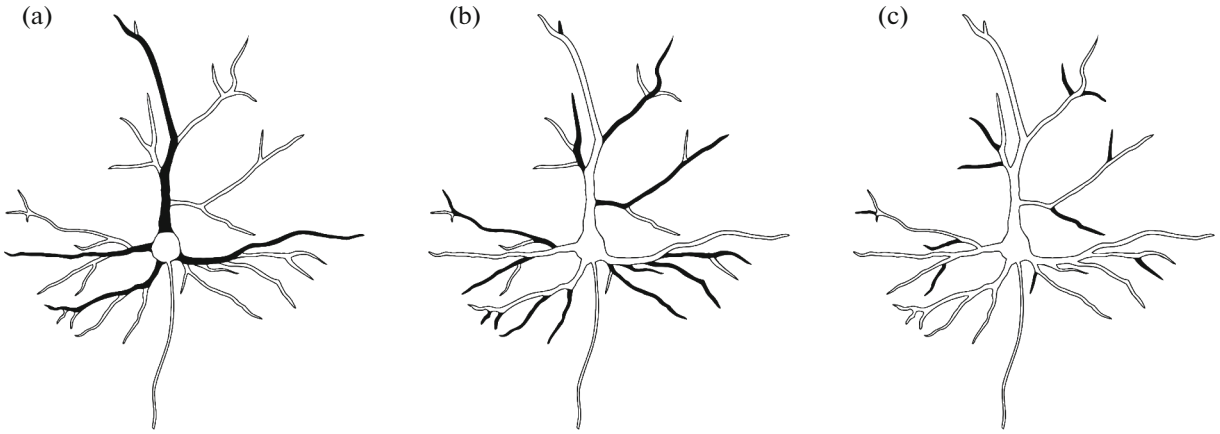

Fig. 1. Measurements of primary (a), secondary (b), and tertiary (c) dendrites in neurons. Neurites whose length was measured are shown in black.

entiating cells [6]. Thus, serpinb1a is involved in processes that occur during differentiation of PC12 cells; however, its role in these processes is not clear.

This study is focused on an analysis of the influence of serpinb1a on the differentiation and apoptosis of neurons and neuron-like PC12 cells, as well as examination of the hypothesis that the studied factor may directly or indirectly regulate the activity of proteases that are involved in both processes, in particular, caspase-3.

MATERIALS AND METHODS

Neuronal cultures. The study was performed with cultured neocortical and hippocampal neurons from Wistar rats. One-day-old rat pups were decapitated under an ESCO LHC-5A1 laminar hood. The brain was isolated, the meninges were removed under a Leica EZ4D microscope, and the cortex or hippocampus were then dissected. The cortex (hippocampus) was minced and trypsinized in DMEM medium (Paneco, Russia). The cells were then triturated to obtain a cell suspension; after centrifugation, the cells were resuspended in 500 μL of Neurobasal medium (Invitrogen) supplemented with Glutamax (Invitrogen) and B27 (Invitrogen). The suspension was applied to poly-lysine-coated round 13 mm coverglasses at a concentration of 250000 cells per cm^2. The cells were cultured in 24-well plates at 37°C and 5% CO_2 in 1 mL of the same medium. Half of the medium was replaced once every 3 days.

Transfection of primary neuronal cell culture. On the third day in vitro (3 DIV), the primary neuronal cultures were transfected with the target plasmids that were used for virus packaging (see below). Transfection was performed using the Lipofectamine 2000 reagent (Invitrogen) following the manufacturer's protocol. A total of 1 μg of the serpinb1a-encoding or the control vector and 2.5 μL Lipofectamine2000 were used to transfect one well of a 24-well plate. Morphometric analysis was performed at 9 DIV.

Morphometric measurements of the length of neuronal dendrites. The vectors for the expression of serpinb1a and the control vector contained the gene for the green fluorescent protein (GFP); we used GFP fluorescence to evaluate the neuronal morphology. To enhance the GFP signal, we performed additional staining using antibodies to GFP (A11122, Invitrogen).

Cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 10 min at room temperature and then washed two times with PBS and treated with an 0.1% solution of the non-ionic detergent Triton X-100 in PBS for 15 min at room temperature. The cells were then incubated in blocking solution (5% normal goat serum dissolved in PBS-Tween) at room temperature for 1 hour. After blocking of nonspecific antibody binding, coverslips were incubated with primary anti-GFP antibodies (1 : 1000, A11122, Invitrogen) for 1 h at room temperature and washed with PBS-Tween three times for 10 min. We then performed staining with secondary antibodies (anti-rabbit IgG Alexa488, 1 : 1000, Invitrogen), after which the coverslips were washed three times with PBS-Tween for 10 min. The coverslips were then embedded in ProLong Gold antifade reagent (Invitrogen).

For morphometric studies, the cells were photographed using a CCD camera on a Keyence BZ-9000 microscope. Images of cells that express serpinb1a or the control vector were analyzed in ImageJ software using the NeuronJ plugin. Figure 1 illustrates the manner in which the lengths of the primary, secondary, and tertiary dendrites were measured.

Lentiviral suspensions with a high titer. The initial source of pure DNA that encodes rat serpinb1a was a

commercial EST-clone of OpenBiosystems (no. MRN1768-98080497 [7385870]). Specific amplification of DNA fragment was performed by PCR using the following primer pair:

forward 5'-TAT*TCTAGA*CAGCTAAGCAAGAGCCTTC-3'
reverse 5'-TTT*TCTAGA*CTTGCTGTCATCGTCTTTGTAGTCCATTGGGGAACAAACCCTGCCAA-3'.

The 3'-end of the obtained PCR product also contained the nucleotide sequence for synthesis of the Flag-epitope for the following detection of protein using antibodies to this epitope.

The initial vector for the packaging of lentiviruses pCSC-SP-PW-IRES_EGFP was kindly provided by A. Chen from Weitzman Institute (Israel). The pCSC_CMV-IRES-EGFP for pCSC-SP-PW-IRES_EGFP cassette was cloned into NotI sites in the pGEM-Tease vector (Promega).

The PCR product that carried the serpinb1a gene was cloned in pGEM-T-CMV-IRES-EGFP into XbaI sites. At the next stage, the CMV-Serpinb1a-IRES-EGFP cassette was cut from the pGEM-T_CMV-Seripnb1a-IRES-EGFP vector using NotI and inserted in pCSC-SP-PW-IRES_EGFP using the same restriction sites.

Packaging of lentiviral particles was performed using a standard protocol. HEK293T (Invitrogen) cells below the 20th passage were transfected via the calcium-phosphate method with three basic plasmids (7.8 μg pMDL, 4.2 μg pVSV, and 3 μg pREV per 100-mm Petri dish), which encode viral proteins, and pCSC-SP-PW-IRES_EGFP_Serpinb1a (12 μg per 100-mm Petri dish) contained a gene with the target protein. The supernatant was collected and filtered through a 0.45 μm filter; the viral particles were then precipitated by ultracentrifugation at 20000 *g* for 2 h at 4°C. The precipitate was resuspended in 300 μM OPTI-MEM and 5 μL aliquots were made and frozen at −70°C. Similarly, using a p156RRLsinPPTCMVGFPPRE empty backbone we obtained a suspension of viral particles for control cell line.

PC12 cells with a stable high level of serpinb1a expression. PC12 cells were cultured in DMEM supplemented with 10% fetal bovine serum (Invitrogen) and 2 mM L-glutamine (Paneko). To obtain transformed cultures, PC12 cells were passaged at a concentration of 50000 cells/well in 96-well plates. On the next day, the cultures were treated with 10, 1, and 0.1 μL of viral suspension with 10^9 infecting units/mL. The efficacy of transduction was evaluated using GFP expression 2 days after transduction. Effectively transduced cells after achievement of high density were then passaged into 24- and 6-well plates and 100-mm Petri dishes.

Induction of apoptosis in PC12 cells. PC12 cells that stably expressed serpinb1a and GFP or GFP alone were cultured at 37°C, 5% CO_2 in DMEM medium supplemented with 10% fetal bovine serum. To evaluate the activation of caspase-3, cells were passaged onto polylysine-coated wells of a 6-well plate at a density of 200000 cells/cm^2. On the next day, the medium was replaced with serum-free medium and in 16 h the cells were treated with staurosporine (STS, Sigma) at a concentration of 1 μM. Cells were collected in 0, 1, 4, 8, and 24 h after the addition of STS. The cells were frozen in liquid nitrogen and kept at −70°C.

Cell-viability assay. PC12 cells (that were transduced by viral particles that express GFP or serpinb1a) were passaged 1 day before the experiment in DMEM medium with serum in 100 μL (10^5 cells/mL) in the wells of a 96-well poly-lysine-coated plate. On the next day after passaging, STS at a final concentration of 0.1 μM was added to cells; control cells were treated with an equivalent amount of DMSO. In 24 h, the culture medium was aspirated and the cells were treated with 100 μL DMEM and a 10 μL solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT 0.5 mg/mL). The cells were incubated in a CO_2 incubator for 4 h until the development of intense staining. In 4 h, the culture liquid was aspirated and 100 μL DMSO was added. In 20 min, the absorption was measured on a Tecan Infinite M200 Pro plate spectrofluorometer at 540/690 nm.

The percentage of surviving cells was calculated using the following formula:

$$\frac{\text{absorption in wells with STS}}{\text{absorption in wells without STS}} \times 100\%.$$

We performed 18 independent experiments.

Measurements of protein concentration. Measurements of protein concentration was performed using a Bradford Assay Kit (Fermentas) or Pierce BCA Protein Assay Kit (Thermo Fisher) on a Tecan Infinite M200 Pro plate spectrofluorometer. Analysis of the data was performed using Tecan Magellan software.

Measurements of caspase-3 activity. To measure caspase-3 activity, a 25 μL sample was mixed on ice with 25 μL buffer for measurement of enzymatic activity (200 mM HEPES, pH 7.5, 200 μM Z-DEVD-AMC (Enzo Life Science), 20 mM DTT, 1 mM EDTA) in the wells of a 384-well Corning 384 Flatlack plate using a stepper pipette. Each sample was measured in two parallels. Measurements were performed on a Tecan Infinite M200 Pro plate spectrofluorometer; excitation was at 370 nm, and emission was measured at 430 nm at 37°C for 1 hour. The caspase-3 activity was measured using the kinetics of the accumulation of fluorescent 4-amino-7-methylcoumarine (AMC, Sigma) using the formula: activity ((μmol)/(min mg protein)) = ((fluorescence intensity (60 min) − fluo-

rescence intensity (0 min)) × 10)/(fluorescence intensity (10 μM) × 60 × protein concentration)).

Immunoprecipitation. To analyze the interaction of serpinb1a and caspase-3, we performed co-immunoprecipitation of these proteins. For this, PC12 cells that stably expressed serpinb1a and GFP or GFP alone were treated with 1 μM staurosporine for 24 h and then lysed in 1 mL buffer for immunoprecipitation (20 mM Tris-HCl, 137 mM NaCl, 1% NP-40, 2 mM EDTA, protease inhibitors (Roche, Complete-mini EDTA-free)) for 30 min at 4°C. The samples were then centrifuged at 12000 rpm for 15 min at 4°C. Supernatant was transferred to new vials and the protein concentration was measured in it. The samples were treated with 1 μg anti-Flag antibodies and incubated overnight on a shaker at 4°C. The samples were then treated with 20 mL of protein-A-sepharose preblocked with BSA and incubated for 4 h at 4°C on a shaker. The obtained complex was then washed three times with IP buffer for 10 min and then precipitated in sepharose and 50 μL Laemmli buffer was added to it. The samples were heated for 5 min at 95°C and then analyzed by Western blotting using a 12% polyacrylamide gel (PAAG).

Western blotting. To analyze serpinb1a expression via Western blotting, we obtained lysates of PC12 cells that stably expressed serpinb1a and GFP or GFP alone. For this, we made RIPA buffer (150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1 SDS, 50 mM Tris, pH 8.0) and mixed it with protease inhibitors (Complete-mini EDTA-free, Roche). The cells were lysed in 300 μL of RIPA buffer for 30 min on ice and then centrifuged at 10000 rpm at 4°C for 10 min. The supernatant was transferred to a new cooled vial. The protein concentration was measured and the volume that contained 50 μg of total protein was mixed with 2× Laemmli buffer and applied to PAAG.

Proteins were separated using electrophoresis in 10 or 12% PAAG and then transferred to a polyvinylidenfluoride membrane (PVDF) (100 mA, 40 min). Nonspecific binding was blocked by 5% skimmed milk in PBS overnight at +4°C; the membranes were incubated with primary αFlag antibodies (F1804, Sigma; 1 : 1000) or anticaspase-3 (Cell Signaling, 9661) for 1.5 h at room temperature and then washed three times for 10 min in 0.05% PBS-Tween. The membrane was then incubated with secondary antibodies conjugated with horseradish peroxidase (α-mouse or α-rabbit HRP) for 1 hour at room temperature and washed with 0.05% PBS-Tween; Pierce ECL Western Blotting Substrate (Thermo Scientific) was then applied to the membrane for 1 min. X-ray films were used to detect the chemiluminescent signal.

Reverse transcription and PCR (RT-PCR). The total pool of cell RNA was isolated from PC12 cells, cultured cortical neurons, and homogenates of the cortex of 30-day-old rats using the ExtractRNA reagent (Evrogen) in accordance with the manufacturer's protocol. To eliminate the DNA, the samples were treated with DNase 1 (Fermentas) in accordance with the manufacturer's recommendations. An MMLV RT kit (Evrogen) was used to synthesize cDNA; 0.5 μg RNA was taken into the reaction and cDNA synthesis was performed using random hexaprimers.

To establish the expression of serpinb1a, we performed PCR using the following primers (5'–3'):

forward: AGC CCA GCT CTC TAA GAC TT,
reverse: GAA CTC AGG AAG GAA ATT GTA GGT.

PCR products were analyzed using electrophoresis in a 1% agarose gel.

Data analysis. Statistical analysis of the data was performed using Statistica and Microsoft Excel software; the significance of the differences was determined using the Mann–Whitney criterion.

RESULTS

Serpinb1a as a modulator of processes that occur during cell death. Serpinb1a is a powerful inhibitor of serine and cysteine proteases that are actively involved in cell death. Hence, it is possible to hypothesize that serpinb1a has protective anti-apoptotic characteristics. To elucidate the possible protective role of serpinb1a in cell death, we performed experiments where apoptosis was induced by staurosporine (STS, 100 nM) in PC12 cells that overexpressed serpinb1a. At 24 h after the induction of cell death we evaluated the viability of experimental and control cells using the MTT test. In the control, the cell viability decreased in the presence of STS by approximately 40% (Fig. 2); however, in serpinb1a-overexpressing PC12 cells, we found a weak but significant increase in the number of cells that survived in the presence of STS (Fig. 2).

The obtained protective effect of serpinb1a overexpression suggests that serpinb1a may inhibit apoptotic proteases, in particular, caspase-3. To examine the possibility of an interaction between serpinb1a and caspase-3 we performed additional experiments, where we co-immunoprecipitated these proteins in lysates of both the control and straurosporine-treated PC12 cells. The subsequent analysis of precipitates using Western blotting showed the presence of a complex formed by serpinb1a and active caspase-3 (Fig. 3) in both the intact cells and the cells where we induced apoptosis. Thus, on the basis of these data it is possible to hypothesize that serpinb1a is one of the antiapop-

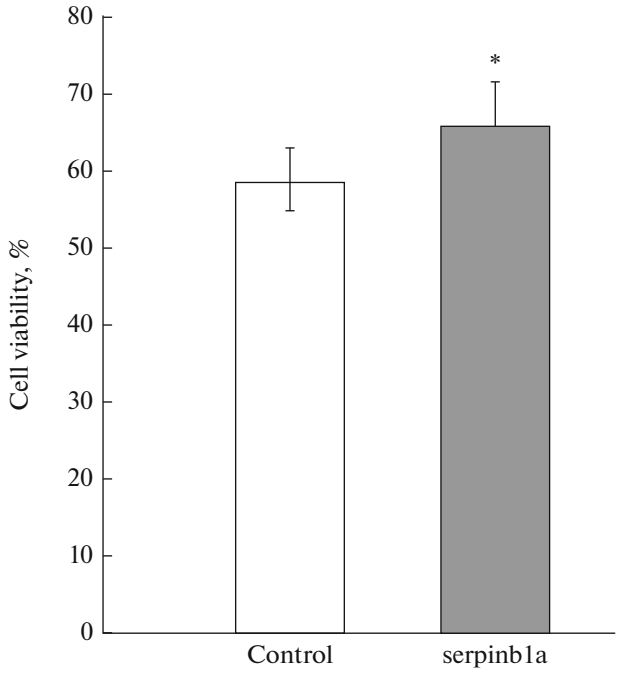

Fig. 2. The viability of PC12 cells in the control and after treatment with staurosporine (STS). Ordinate axis, cell viability in percent of control. The data are presented as the mean ± standard deviation. *Significant difference according to the Student's *t* test, $p < 0.05$.

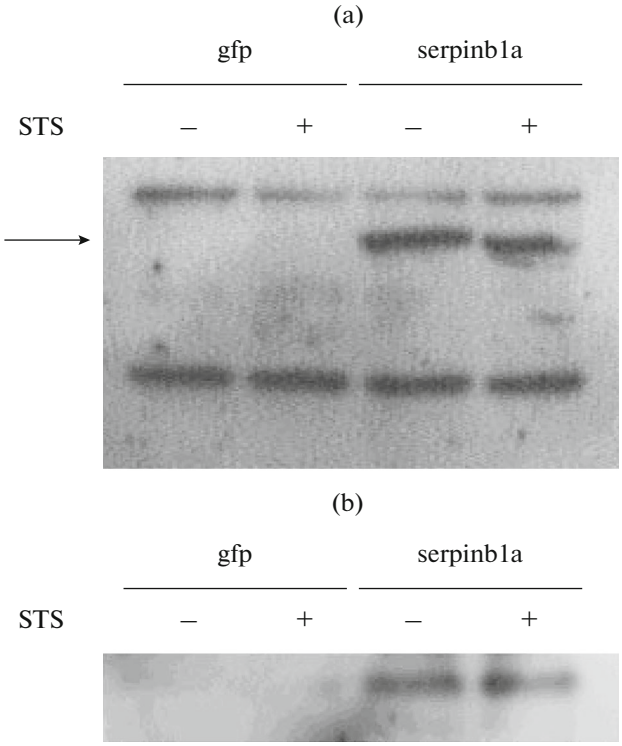

Fig. 3. Immunoprecipitation of serpinb1a and active caspase-3. (a) The result of immunoprecipitation with antibodies to the Flag epitope. The band that corresponds to serpinb1a is marked by an arrow. (b) Detection of active caspase-3 precipitated in a complex with serpinb1a.

totic factors that provide maintenance of cell viability due to direct interaction with caspase-3 and a decrease in the caspase-3-mediated proapoptotic effects.

Serpinb1a as a modulator of processes that occur during differentiation of non-neuronal cells and neurons. According to a number of studies, proteases are actively involved in the regulation of the differentiation of non-neuronal cells and neurons and control the growth of their cell outgrowths [1]. However, the question of the involvement of serpinb1a in the modulation of these processes is still open. To answer this question, in the first series of experiments we induced differentiation of control PC12 cells and serpinb1a-overexpressing PC12 cells by NGF (100 ng/mL) and evaluated the length of the cell outgrowths in the developing cultures in 24 and 48 h (NGF was present all of the time in the medium). Figure 4 shows that an increase in the expression of serpinb1a in the differentiating cells did not affect the length of the outgrowths 24 h after the addition of NGF; however, it led to a significant decrease in their length (by 25%) in 48 h. The results of our previous experiment suggest that serpinb1a may directly interact with caspase-3; hence, in our further study we evaluated the activity of a hypothetical target of serpinb1a, caspase-3, in the differentiating PC12 cells. We found that NGF-induced differentiation of PC12 cells is associated with a trend to an increase in the caspase-3 activity and this trend is retained for at least 48 h (Fig. 5).

Thus, the increase in the outgrowth length in the developing culture of PC12 cells coincides in time with the increase in the caspase-3 activity. The data we

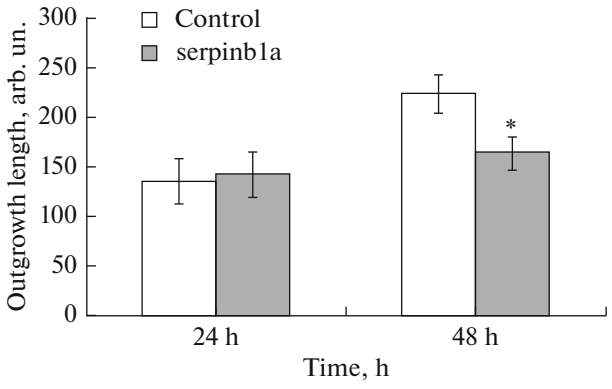

Fig. 4. Neuronal differentiation of PC12 cells that were transduced with viruses for the expression of GFP (control) and serpinb1a, at different stages of differentiation, in 24 and 48 h. The length of the outgrowths in control cells is significantly higher than in the experimental group at 48 h ($p < 0.05$, $n = 9$). The data are presented as the mean ± standard deviation. *Significant difference compared to the control at the same time point according to the Student's *t* test, $p < 0.05$.

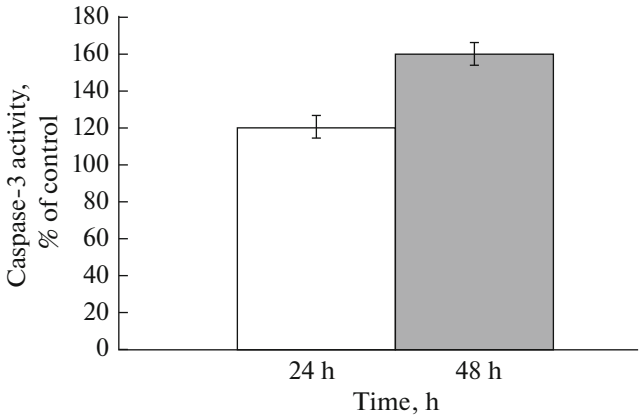

Fig. 5. NGF-induced differentiation of PC12 cells induces an increase in the caspase-3 activity in 24 h, which is enhanced in 48 h. Ordinate axis: an increase in the activity of caspase-3 compared to the control (100%).

obtained may point to a postponed modulatory role of serpinb1a with respect to one of its targets, active caspase-3, during the formation of outgrowths in differentiating PC12 cells.

NGF-induced differentiation of PC12 cells is frequently considered as a model of formation of neurites in neurons during their differentiation. The modulatory action of serpinb1a on the formation of PC12 outgrowths may suggest that serpin influences the formation of outgrowths in cultured hippocampal and cortical neurons. Hence, in the next experiments we evaluated the morphometric indices (average length of dendrites of different order and their average number per cell) in primary rat cortical and hippocampal cultures. We found that serpinb1a overexpression was accompanied by a significant shortening of secondary dendrites of hippocampal neurons compared to the control (Fig. 6a). Measurements of the average extension of neurites of cortical neurons did not reveal any differences compared to the control (Fig. 6b), although there was a trend to a decrease in the average dendritic length in the experimental group (not shown). This may be partly related to a decrease in the number of secondary dendrites per neuron, which was observed in cortical neurons transfected with serpinb1a (Fig. 6d). Analysis of the relationship between primary, secondary, and tertiary dendrites in hippocampal neurons did not reveal significant changes in

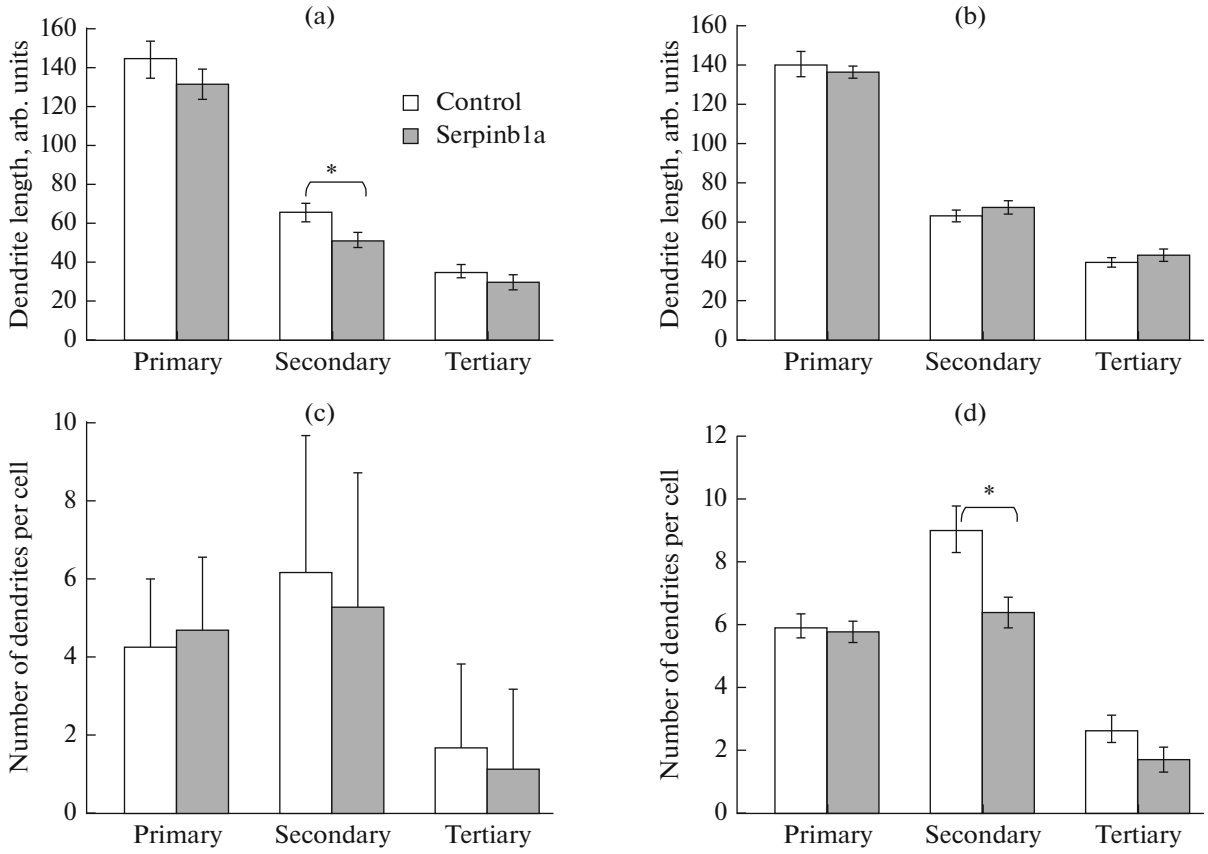

Fig. 6. The change in the average length of dendrites (a, b) and number of dendrites per cell (c, d) after an increase in serpinb1a expression in cultured hippocampal (a, c) and cortical (b, d) neurons. *, significant difference according to Mann–Whitney ($p < 0.05$).

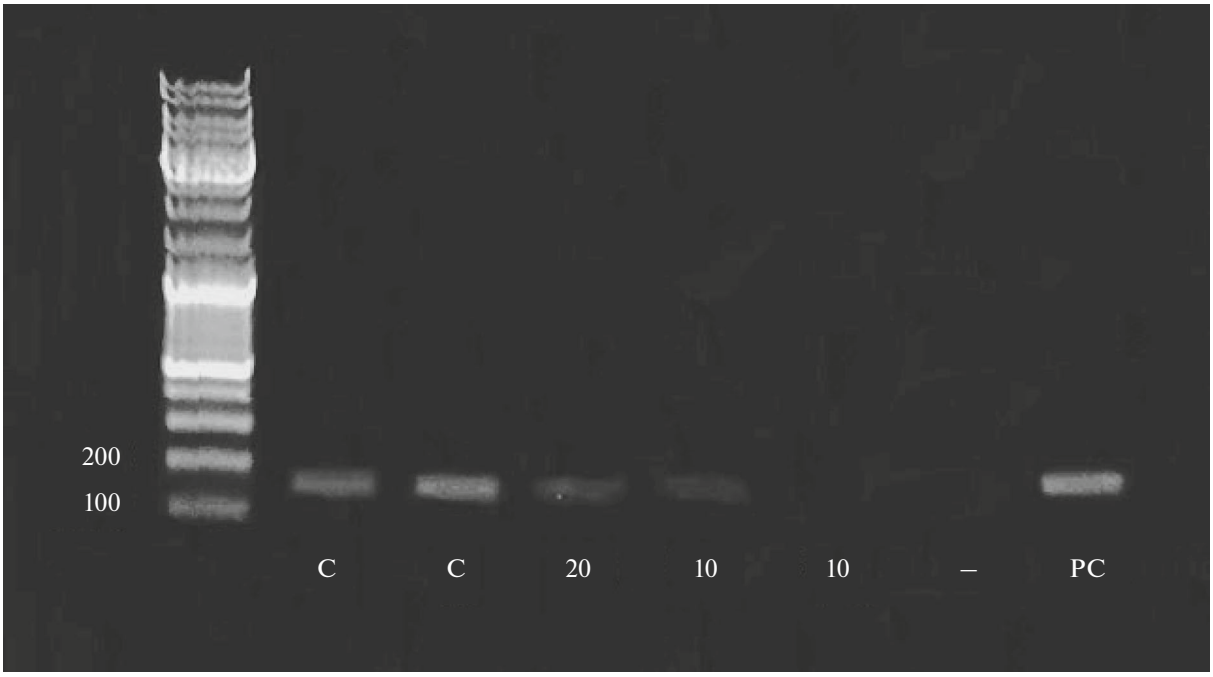

Fig. 7. An image of electrophoresis of DNA fragments obtained after PCR of serpinb1a. Designations: C, a fragment of the neocortex of 30-day-old rat; 20, a 20-day-old culture of cortical neurons; 10, a 10-day-old culture of cortical neurons; "–", negative control where the sample was replaced by water; PC, PC12 cells 18 h after induction of differentiation by NGF.

the control and serpinb1a-overexpressing cultures (Fig. 6c).

Presumably, the increase in the serpinb1a expression during transfection weakly influenced the growth of dendrites in neurons, because at this stage of culture development (9–10 days in vitro) the neurons had a high endogenous level of serpinb1a expression. However, analysis of serpinb1a mRNA expression in culture showed that at the 10th day in vitro serpinb1a was very weakly expressed in cultured cortical neurons, while by the 20th day, when the neuronal dendrites were almost formed, the serpinb1a expression became considerable (Fig. 7). Thus, the fact that serpinb1a overexpression weakly influenced neuronal morphology is not determined by the presence of endogenous serpinb1a. Moreover, it was found that in neurons, in contrast to PC12 cells, formation of outgrowths during differentiation is not accompanied by an increase in the serpinb1a mRNA level. In contrast, serpinb1a was observed only in relatively mature cultures (the 20th day in vitro) (Fig. 7).

DISCUSSION

Here, we studied the functional role of serpinb1a in the apoptosis and differentiation of PC12 cells. An increase in the expression of this factor during PC12 differentiation has been shown in a number of studies [5, 6]. However, the functional role of this increase in serpinb1a expression was not shown. We believe that serpinb1a may be a direct or indirect regulator of the activity of the apoptotic enzyme caspase-3, whose important role has been shown in both apoptosis and differentiation [1–3].

The role of serpinb1a in the regulation of processes that occur during cell death. The question of the molecular mechanisms of the influence of serpinb1a on the processes of cell apoptosis is still open. In these experiments, serpinb1a overexpression protected PC12 cells from staurosporine-induced apoptosis. We believe that the protective effect of serpin may be determined by the regulation of the caspase-3 activity, whose activity is known to be elevated during apoptosis. Cooley et al. [7] showed that serpinb1a does not directly influence caspase-3 activity in vitro; however, it is possible that serpinb1a may indirectly regulate the functioning of caspases by the suppression of other upstream proapoptotic factors (for example, the mitochondrial Omi/HtrA2 serine proteases). We found that serpinb1a can directly interact with active caspase-3 under normal condition and during induction of apoptosis, which suggests that serpinb1a may be a modulator of caspase-3 activity. However, it is still unclear whether serpinb1a modulates caspase-3 activity; answering this question will require additional experiments.

The role of serpinb1a in the regulation of processes that occur during differentiation of non-neuronal cells and neurons. It has been shown that inhibitors of serine proteases may be involved in processes that are not involved in cell death, cell differentiation, and control of growth of cell outgrowths [5, 6, 8, 9]. It has been

found that overexpressed serpinb1a suppressed the formation of outgrowths in PC12 cells during NGF-induced cell differentiation and this effect was observed only 48 h after the beginning of differentiation. NGF-induced differentiation of PC12 cells induced an increase in the serpinb1a level by itself during the first day [5, 6]; hence, it is possible that the additional increase in serpinb1a due to overexpression has no effect in 24 h. The level of serpinb1a in PC12 fell in approximately 24 h [6], which, at this stage of differentiation, is important for the functioning of some proteases. We believe that the effect of serpinb1a overexpression may develop due to influence on these proteases. According to our data, 48 h after NGF-induced cell differentiation caspase-3 activity remained at a high level, which suggests that it is necessary for differentiation. Serpinb1a may interact with this protease and presumably due to this it may modulate some processes that occur during differentiation, in particular, the formation of cell outgrowths. However, the role of caspase-3 or other proteases in the formation of outgrowths will require further experiments.

According to experiments performed with primary cultures of hippocampal neurons of rats, neuroserpin is present in cells of the nervous system and its overexpression regulates the shape of dendritic spines and increases the density of dendritic outgrowths [9]. According to the authors, neuroserpin may be considered as one of the potential regulators of cellular plasticity. Another representative of the serpin family, serpinb1a, is also expressed in nerve tissues [10], and, according to our data, in neuronal cultures. This raises the question about the involvement of serpinb1a in the regulation of cell characteristics in cultured neurons. Note that, according to the Allen Developing Mouse Brain Atlas, serpinb1a is absent in the mouse brain during embryogenesis and the first 3 weeks of postnatal ontogenesis and is found only in the brains of adult animals. Our data obtained in neuronal cultures also suggest that serpinb1a is expressed only in mature cultures. In other words, serpinb1a expression occurs in neuronal cells only at the stage when the process of active formation of neurites and synapses has finished. In our experiments, serpinb1a overexpression at early stages of culture formation has a relatively weak but significant influence on the formation of dendrites in the developing neurons of the hippocampus and neocortex. This suggests that at least the formation of dendrites in neurons does not include serpinb1a-dependent processes.

Thus, we found that serpinb1a overexpression influences the formation of outgrowths in PC12 cells and neurons differently: it inhibits the formation of outgrowths in PC12 cells and practically does not affect the dendritic tree in neurons. This means that the mechanisms of the formation of outgrowths in non-neuronal cells (PC12) and neurons are mediated by different signaling cascades.

CONCLUSIONS

Here, we showed that serpinb1a serves as one of the regulators of cell death and differentiation. In PC12 cells, serpinb1a had a weak antiapoptotic action. It was found that serpinb1a forms complexes with the active caspase-3 in both resting PC12 cells and cells that are undergoing apoptosis. Hence, we believe that the protective antiapoptotic action of serpinb1a may be mediated by its direct interaction with caspase-3.

We showed that serpinb1a decreases the intensity of outgrowth formation during NGF-dependent differentiation of PC12 cell; however, it weakly influences the formation of outgrowths in developing neuronal cultures of the hippocampus and neocortex. These data suggest that the formation of outgrowths in PC12 cells and cultured neurons are mediated by different cellular mechanisms.

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