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Platelet-derived growth factor promotes survival of rat and human mesencephalic dopaminergic neurons in culture

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Summary. The effect of two isoforms of platelet-derived growth factor (PDGF), PDGF–AA and PDGF–BB, was tested on dissociated cell cultures of ventral mesencephalon from rat and human embryos. PDGF–BB but not PDGF–AA reduced the progressive loss of tyrosine hydroxylase- (TH)-positive neurons in rat and human cell cultures. The mean number of TH-positive cells in the PDGF–BB-treated rat culture was 64% and 106% higher than in the control cultures after 7 and 10 days in vitro, respectively. Corresponding figures for human TH-positive neurons were 90% and 145%. The influence of PDGF–BB was specific for TH-positive neurons and not a general trophic effect, since no change of either total cell number or metabolic activity was found. In PDGF–BB-treated cultures of human but not rat tissue the TH-positive neurons had longer neurites than observed in control or PDGF–AA-treated cultures. These data indicate that PDGF–BB may act as a trophic factor for mesencephalic dopaminergic neurons and suggest that administration of PDGF–BB could ameliorate degeneration and possibly promote axonal sprouting of these neurons in vivo.

Key words: Platelet-derived growth factor – Dopaminergic neurons – Cell culture – Parkinson's disease – Rat – Human

Introduction

In Parkinson's disease, there is a progressive degeneration of mesencephalic dopamine- (DA)-producing neurons. The etiology of Parkinson's disease is not known, but it has been suggested that a lack of trophic factor(s) might be the underlying cause of this disorder (Appel 1982). Over the past few years a large number of studies have been carried out in order to identify factors that can promote survival and neurite outgrowth of mesencephalic DA neurons. Hypothetically, administration of such factors in vivo might delay the progression of the disease and could also improve the symptomatic relief exerted by intrastriatal grafts of fetal mesencephalic DA neurons in patients (Lindvall et al. 1992).

Cell culture experiments have shown that the survival of mesencephalic dopaminergic neurons can be promoted by several purified growth factors such as acidic and basic fibroblast growth factor (aFGF, bFGF; Ferrari et al. 1989; Engele and Bohn 1991), brain-derived neurotrophic factor (BDNF; Hyman et al. 1991; Knüsel et al. 1991), epidermal growth factor, and insulin and insulin-like growth factors (Knüsel et al. 1990). Much interest has been focussed on BDNF and bFGF, which enhance DA uptake and the number of neurite-bearing cells in cultures of embryonic rat mesencephalon (Ferrari et al. 1989; Knüsel et al. 1990, 1991; Hyman et al. 1991). BDNF can also protect DA neurons from the neurotoxic action of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in vitro (Hyman et al. 1991). The effects of BDNF and bFGF after administration in vivo are less clear. No significant increase in the number of mesencephalic dopaminergic neurons surviving a transection of the nigrostriatal pathway was observed after injection of BDNF into the lesion area, the substantia nigra or the lateral ventricle (Hefti et al., to be published). Furthermore, BDNF administration into the lateral ventricle or directly into the implant did not influence the survival of TH-positive neurons in mesencephalic dopaminergic grafts or their axonal outgrowth in the DA-denervated striatum (Sauer et al. 1992). Gelatin foam (Gelfoam) implants containing bFGF and stereotaxically injected aFGF have been reported to increase the density of TH-positive fibers in the surrounding host striatum of MPTP-treated mice (Date et al. 1990; Otto and Unsicker 1990). However, only minor effects of bFGF were shown on sprouting from intrastriatal grafts of embryonic rat dopaminergic neurons (Steinbusch et al. 1990).

Platelet-derived growth factor (PDGF) is a potent mitogen as well as a chemotactic factor for mesenchymally derived cell types (Heldin and Westermark 1990;

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Raines et al. 1990). It occurs as three isoforms, PDGF–AA, PDGF–BB, and PDGF–AB, constituting disulfide-linked homo- or heterodimers of two related polypeptides, the A- and B-chains. PDGF molecules exert their biological effect via cross-linking of cell surface receptors. There are two forms of PDGF receptors, the PDGF α-receptor and the PDGF β-receptor. Receptor specificity restricts ligand-binding of the PDGF A-chain only to the α-receptor, whereas the PDGF B-chain can bind either to the α- or the β-receptor (Hart et al. 1988; Heldin et al. 1988). Therefore, the signalling process initiated by PDGF will depend both on the isoforms of PDGF and on the cell surface receptor expression.

PDGF A- and B-chains have recently been identified in neurons throughout the entire brain (Sasahara et al. 1991; Yeh et al. 1991). Higher levels of mRNAs encoding the PDGF α- and β-receptors are found in the developing brain than in the adult (Reddy et al. 1992). The PDGF α-receptor was shown to be expressed by oligodendrocyte-type 2 astrocytes, whereas the PDGF β-receptor was located on neurons (Smits et al. 1991; Pringle et al. 1992). Survival and neurite outgrowth were promoted by PDGF–BB but not by PDGF–AA in neuronal cultures from newborn rat brains (Smits et al. 1991). Binding studies revealed a high level of PDGF β-receptors and the absence of PDGF α-receptors in these neuronal cultures. Thus, different isoforms of PDGF may specifically act as a regulatory and/or trophic factor for both neurons and glia (Richardson 1991).

The present experiments were performed to test the hypothesis that PDGF might act as a trophic factor for mesencephalic DA neurons. Cell cultures of ventral mesencephalic tissue from rat and human embryos were examined for the effects of PDGF–AA and PDGF–BB on cell survival and neurite outgrowth. Further experiments were conducted to distinguish influences on total cell survival from specific effects on TH-positive, presumed dopaminergic, neurons.

Materials and methods

Cell cultures

Ventral mesencephalon was dissected from 14-day-old rat embryos (E14) or from cadaveric human embryos. The human tissue was obtained from routine suction abortions with the approval of the Research Ethical Committee at the Medical Faculty of the University of Lund. The age of the human material, as assessed by ultrasound, was 6–8 weeks postconception (crown-rump length 18–25 mm). Pooled tissue was incubated in 0.1% trypsin/0.05% DNase (Worthington Biochemical Corporation and Sigma DN–25, respectively) at 37° C for 20 min, rinsed four times in 0.05% DNase, and mechanically dissociated using a 1-ml Gilson pipette. The tissue was then centrifuged at 600 rpm for 5 min and the pellet resuspended in Dulbecco's minimal essential medium (DMEM; Gibco). The cell number in this suspension was counted with a trypan blue dye exclusion method and 100000 cells/cm^2 were plated onto four-well chamber slides (Nunc, USA) precoated with 10 μg/ml poly-L-lysine (Sigma) and 2.5 μg/ml merosin (Chemicon). Cells were incubated overnight in DMEM + 10% fetal calf serum (FCS) 1 ml/well at 5% CO_2, 37° C, and 95% humidity. After 24 h in vitro (0–24 h = 1 day in vitro, DIV), culture medium was changed to serum-free N2 medium (Bottenstein et al. 1979), consisting of DMEM/ Ham's F12 (1:1) mixture (Gibco) supplemented with 10 mM $NaHCO_3$, 2 mM *l*-glutamine, 110 mg/ml sodium pyruvate, 100 μg/ml transferrin, 20 ng/ml insulin, 100 μM putrescine, 20 nM progesterone, 30 nM sodium selenite, and no antibiotics. Cells were grown for 2, 7, and 10 DIV with or without human recombinant PDGF–AA (30 ng/ml) or PDGF–BB (30 ng/ml; Östman et al. 1989), and the culture medium was replaced every 3rd day.

For rat mesencephalic tissue, TH immunocytochemistry and microscopic evaluation of the survival of TH-positive neurons were performed in six independent culture experiments at 2, 7, and 10 DIV, each condition tested comprising quadruplicate wells. The methylthiazoyl tetrazolium (MTT)-assay (see below) was performed in parallel cultures in the same number of wells. Total cell number was assessed at 2, 7, and 10 DIV in two representative rat cultures in quadruplicate wells.

Due to limited amounts of human mesencephalic tissue, the effect of PDGF–BB on the survival of TH-positive neurons was only tested in two cultures grown for 7 DIV and in four cultures grown for 10 DIV (each culture comprising quadruplicate wells). For each time point, the MTT assay could be performed in one parallel culture in quadruplicate wells. PDGF–AA was tested in two cultures at 7 DIV and in three cultures at 10 DIV. The MTT assay was only performed in one PDGF–AA-treated culture at 7 DIV. Total cell number was counted in two representative human cultures from 7 and 10 DIV in control and PDGF–AA- and PDGF–BB-treated cultures.

Smears of embryonic ventral mesencephalic tissue

Smears were produced from rat cells in three different experiments. After the mesencephalic tissue had been incubated in trypsin/DNase, rinsed and dissociated as discussed above, 5 μl of the cell suspension was spread out on poly-L-lysine-coated microscope slides. The smears were air-dried for 2–4 h and then stored at −20° C until processed for TH immunocytochemistry.

TH immunocytochemistry

Cultures and smears were rinsed once with phosphate buffer (PB, 0.2 M, pH 7.4), fixed with 4% paraformaldehyde for 15 min at room temperature and washed three times with PB. They were then quenched with 3% H_2O_2 for 7 min, washed three times with PB, and preincubated with 5% normal swine serum (NSS) and 0.1% Triton X–100 in PB for 1 h at room temperature. Cultures were incubated with the primary TH antibody (1:500; Pel Freeze)/2% NSS and 0.1% Triton X–100 in PB overnight at 4° C. Following three washes with 0.1% Triton X–100 in PB, the cultures were incubated for 1 h at room temperature with biotinylated swine-anti-rabbit antibody (1:200) diluted in 0.1% Triton X–100 in PB. The cultures were washed three times with PB. Labelling was visualized by incubation with Vectastain ABC Elite kit for 30 min at room temperature and finally with 0.05% 3,3′-diaminobenzidine/0.03% H_2O_2 for 3–6 min.

Microscopic analysis

The survival of DA neurons was quantified by counting all TH-positive neurons per well in a blind manner with respect to the individual culture conditions. In two representative cultures of both rat and human mesencephalic tissue the longest TH-positive neurite/neuron among 100 neurons/well at 10 DIV was measured with a reticule grid. This grid is subdivided into 100 squares (0.5 mm × 0.5 mm) and was used at × 200 magnification. In the same cultures total cell number was counted in ten randomly chosen microscopic fields per well at × 200 magnification. The total cell number and number of TH-positive cells in the smears were counted simultaneously in ten randomly chosen fields.

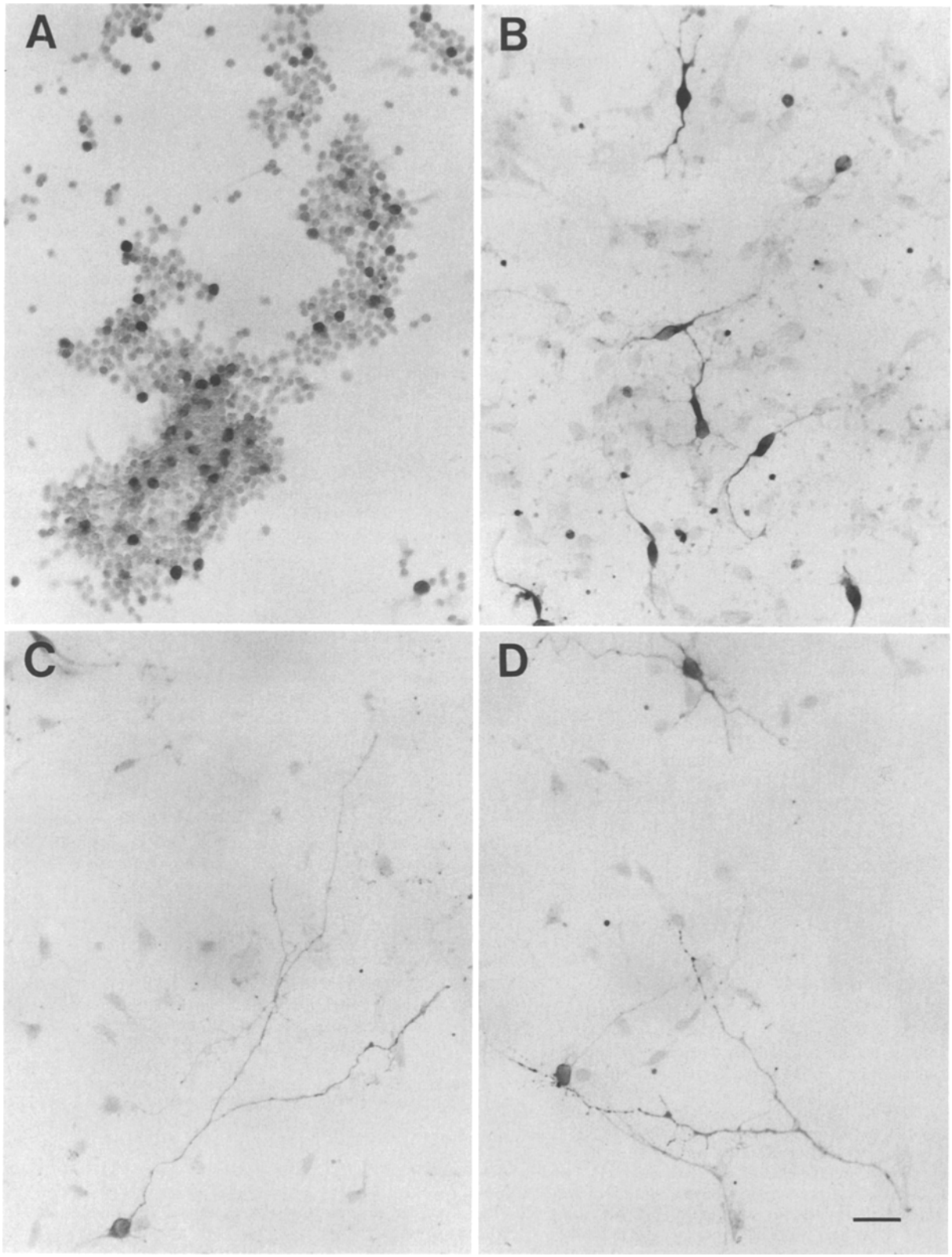

Fig. 1A–D. Brightfield photomicrographs of a cell smear (**A**) and of cell cultures (**B–D**) prepared from ventral mesencephalic tissue of 14-day-old rat embryos and immunostained for tyrosine hydroxylase (TH). **B–D** Representative examples of the number and appearance of the TH-positive neurons cultured under serum-free conditions for 2 (**B**), 7 (**C**) and 10 days (**D**). *Scale bar* 50 μm

MTT assay

To obtain further information about total cell number and viability of the cell culture a modified version of the MTT assay was performed (Manthorpe et al. 1986; Nikkhah et al. 1992). This assay is based on the reduction of the tetrazolium salt MTT to a blue product, formazan, by enzymes only present in metabolically active cells. Briefly, cell cultures were incubated with 0.2 mg/ml MTT (Sigma) for 4 h at 37° C. The supernatant was completely removed and replaced with 200 µl dimethyl sulfoxide (DMSO). After a 5-min incubation on a rotatory shaker the coloured extract was transferred to a 96-well plate and absorbance was measured at 550/620 nm (test/reference wavelength) using a spectrophotometer.

Statistical analysis

Results are expressed as mean ± SEM of the cell cultures comprising quadruplicate wells. For statistical evaluation of effects on cell survival, data were subjected to one-way analysis of variance (ANOVA) and Scheffé post-hoc test. Group comparison of the neurite outgrowth classes was performed using a Chi-square test followed by ANOVA and Scheffé post-hoc test for single classes.

Results

Effects of PDGF-AA and PDGF-BB on rat mesencephalic neurons

Assessment of survival and differentiation of TH-positive neurons was performed at 2, 7, and 10 DIV. The appearance of TH-positive neurons in control cultures at these time points in serum-free medium (SFM) is illustrated in Fig. 1 (B–D). At 2 DIV there were numerous TH-positive neurons with short processes. At 7 DIV their number had decreased dramatically, but the remaining neurons exhibited longer neurites and more extensive branching patterns. Only a few TH-positive neurons survived until 10 DIV.

Cell counting in smear preparations of the cell suspension prior to seeding revealed that 8–10% of the total cell number were TH-positive neurons (Fig. 1A). Since 200000 cells were plated per well, this would give approximately 16000–20000 TH-positive neurons per well. At 2 DIV the number of TH-positive neurons had already dropped to 1402 ± 81 per well, which represents only about 8–10% of the initial number of TH-positive neurons (Fig. 2A).

This decline continued to 483 ± 14 TH-positive neurons per well at 7 DIV and 273 ± 17 TH-positive neurons per well at 10 DIV, as estimated in one representative culture ($n = 4$ wells; Fig. 2A). In cultures treated with PDGF-BB but not with PDGF-AA the loss of TH-positive neurons was significantly reduced (Fig. 2A) at 7 and 10 DIV. Addition of PDGF-AA and -BB did not influence the total number of cells (Fig. 2B) or the overall metabolic activity as shown by the MTT assay (Fig. 2C). From 2 to 10 DIV the total number of cells in the cultures decreased by about 30%. The decline in the number of TH-positive neurons over this period was much more pronounced, acounting to about 80% in the control cultures.

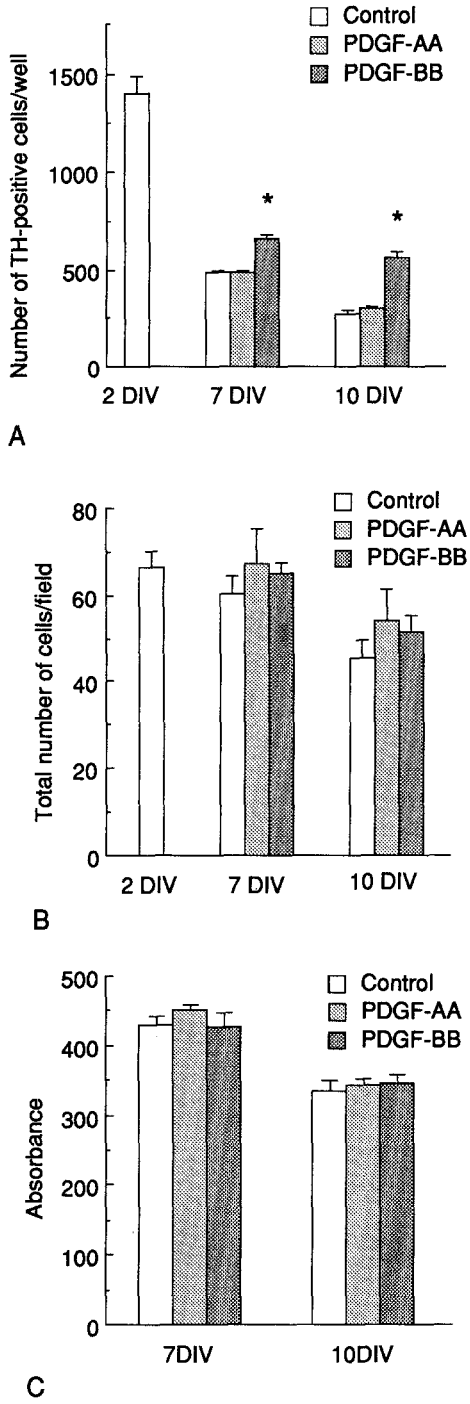

Fig. 2A–C. Effects of platelet-derived growth factor-AA *(PDGF-AA)* and *PDGF-BB* on the mean number of tyrosine hydroxylase-*(TH)*-positive neurons per well (**A**) and mean total cell number per observation field (**B**) at 2, 7, and 10 days in vitro *(DIV)* in a representative rat mesencephalic cell culture. The metabolic activity (C) was measured by the methylthiazoyl tetrazolium (MTT) assay at 7 and 10 DIV in parallel. (Means ±SEM, quadruplicate wells). * denotes statistically significant differences (one-way ANOVA and post-hoc Scheffé, $P < 0.001$) from the control culture in serum-free medium at the corresponding time points

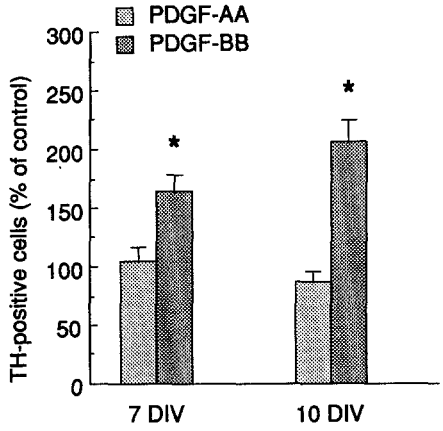

Fig. 3. Effects of platelet-derived growth-factor-AA *(PDGF–AA)* and *PDGF–BB* on the number of tyrosine hydroxylase- *(TH)*-positive neurons in a series of six rat mesencephalic cultures grown for 7 and 10 days in vitro *(DIV)*. The survival of TH-positive cells for each treatment is expressed in percentage of control cultures in serum-free medium at the corresponding time point. *Bars* represent the mean percentage (± SEM) of the survival of TH-positive cells (quadruplicate wells in each culture). * denotes statistically significant difference (one-way ANOVA and post-hoc Scheffé, $P < 0.002$) from the control cultures

Figure 3 summarizes the results after addition of PDGF–AA and –BB to six independent rat ventral mesencephalic cell cultures. The value for each culture represents the mean of quadruplicate wells. In PDGF–BB-treated cultures the number of TH-positive neurons was 64% and 106% higher than in the control (SFM) at 7 and 10 DIV, respectively. In contrast, PDGF–AA had no effect on the survival of TH-positive neurons at either time point. No significant effect on neurite outgrowth of rat TH-positive mesencephalic neurons could be detected after addition of either PDGF–AA or PDGF–BB as assessed in two cultures ($n = 4$ wells per culture; data not shown).

In two cultures of rat and one culture of human ventral mesencephalic cells the addition of PDGF–AA and –BB caused extensive proliferation of a fibroblast-like cell type. In these cultures there was a significantly higher total cell number and metabolic activity after addition of PDGF–AA and PDGF–BB than in the control. They also showed a more pronounced decrease in number of TH-positive neurons with time (up to 30–50%) and no effect of PDGF–BB on the number of TH-positive neurons was found at 7 and 10 DIV. The

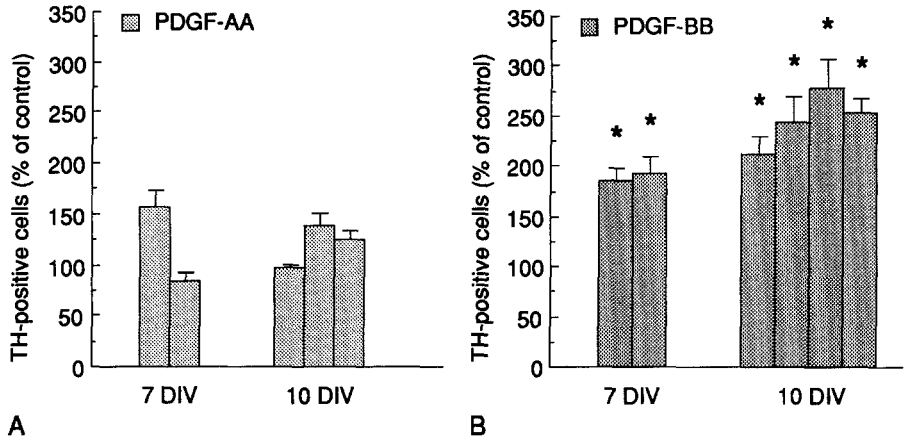

Fig. 4A, B. Effects of platelet-derived growth factor-AA *(PDGF–AA)* **(A)** (five cultures) and *PDGF–BB* **(B)** (six cultures) on the number of tyrosine hydroxylase-*(TH)*-positive cells in human mesencephalic cultures. The survival of TH-positive cells for each culture is expressed in percentage of the corresponding control culture in serum-free medium at the same time point. Each *bar* represents the result of an independent culture (quadruplicate wells) at 7 or 10 days in vitro *(DIV)*. * denotes statistically significant differences (one-way ANOVA and post-hoc Scheffé, $P < 0.01$) from the control culture

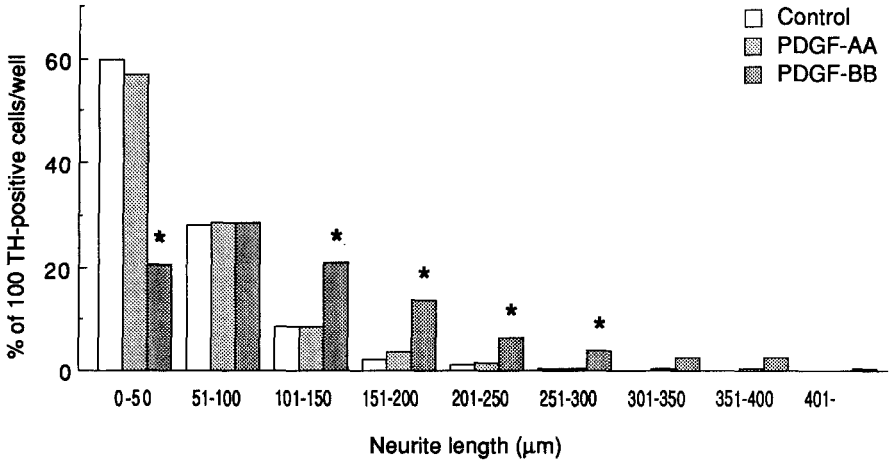

Fig. 5. Effect of platelet-derived growth factor-AA *(PDGF–AA)* and *PDGF–BB* on the length of the longest neurite on human tyrosine hydroxylase- *(TH)*-positive neurons at 10 days in vitro *(DIV)*. The neurite length has been plotted in different classes for a random sample of 100 TH-positive cells per well (quadruplicate wells) from two representative cultures. * denotes statistically significant differences as seen in cultures treated with PDGF–BB for the single classes of neurite length (one-way ANOVA and post-hoc Scheffé, $P < 0.05$) compared with control cultures in serum-free medium

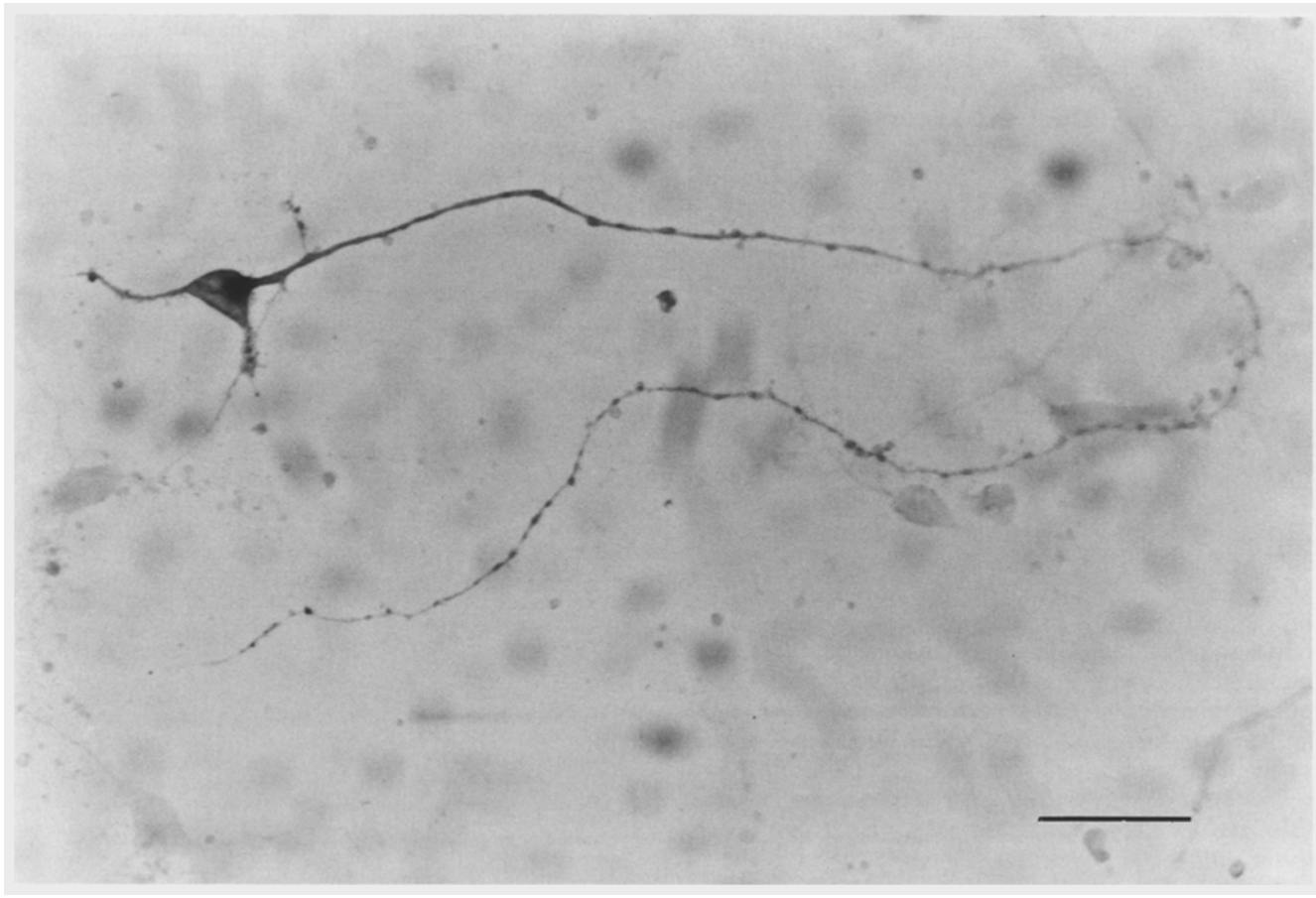

Fig. 6. Example of the extensive neurite outgrowth from a human mesencephalic tyrosine hydroxylase-positive neuron in a platelet-derived growth factor-BB-treated culture grown for 10 days in vitro. *Scale bar* 50 μm

results from these cultures were not included in the further analysis.

Effects of PDGF–AA and PDGF–BB on human mesencephalic neurons

Consistent with the data obtained from rat neurons, there was no significant effect of PDGF–AA on the number of human TH-positive neurons (Fig. 4A) at 7 or 10 DIV as assessed in five independent cultures.

In the PDGF–BB-treated cultures the number of surviving neurons was higher than in controls, by 90% at 7 DIV and by 145% at 10 DIV (Fig. 4B). There was no significant difference between control and PDGF–AA and PDGF–BB-treated cultures in the overall cell survival as revealed by total cell count and MTT assay (data not shown).

In human cultures supplied with PDGF–BB, the neurite outgrowth from TH-positive cells was more pronounced than in control cultures (Chi-square test, $P<0.001$). At 10 DIV about 90% of the TH-positive neurites in control or PDGF–AA-containing cultures were shorter than 100 μm, whereas in the PDGF–BB-treated cultures 51% of the TH-positive neurons extended their neurites beyond 100 μm (Fig. 5). An example of a human TH-positive neuron from a culture treated with PDGF–BB is given in Fig. 6.

Discussion

The present data indicate that the progressive cell loss in cultures of both rat and human mesencephalic TH-positive, presumed dopaminergic, neurons can partly be prevented by addition of PDGF–BB. In contrast, PDGF–AA did not influence cell survival. The action of PDGF–BB was specific for TH-positive neurons and not a general trophic effect, since no change of either total cell number or metabolic activity was found. However, it could not be excluded that also cell types other than the TH-positive cells, present in low numbers, were stimulated by PDGF–BB. In PDGF–BB-treated cultures of human, but not rat, mesencephalic tissue the TH-positive neurons had longer neurites than observed under serum-free conditions or in cultures treated with PDGF–AA. Three cultures were excluded due to PDGF-dependent overgrowth of a fibroblast-like cell type, most likely originating from mesenchymal tissue contaminating the preparation.

Taken together, our observations suggest that PDGF–BB acts as a neurotrophic factor for mesencephalic dopaminergic neurons in culture. These results extend the recent demonstration of a neurotrophic activity of PDGF–BB on early postnatal rat brain cultures (Smits et al. 1991) by indicating a functional effect of PDGF–BB on a specific neuronal population. In con-

trast, in a recent report by Engele and Bohn (1991) PDGF did not influence either dopaminergic or glial cell survival. This discrepancy might depend on the fact that the effect of each PDGF isoform was not studied separately. It could also be explained by the lower PDGF concentrations used by Engele and Bohn (1991; up to 8 ng/ml compared with 30 ng/ml in the present study).

The neurotrophic activity of PDGF–BB as shown for cultured embryonic dopaminergic neurons may increase our understanding about the functional role of PDGF isoforms also in the developing and adult central nervous system in vivo. PDGF B-chain immunoreactivity has been found in the substantia nigra of nonhuman primates (Sasahara et al. 1991) and functional PDGF β-receptors have been demonstrated in this region in newborn rats with gradual decline with age (Smits et al. 1991). These findings are in accordance with the hypothesis that PDGF–BB exerts a trophic action on mesencephalic DA neurons also in vivo. Furthermore, in a parallel study (A. Smits et al., in preparation) we have found that within grafts of ventral mesencephalon from rat fetuses, implanted into the DA-depleted adult rat striatum, many cells express high levels of PDGF. The immunostaining in the graft gradually decreases, whereas in the surrounding host striatum PDGF-positive glial cells become more numerous. In view of the results of the present study, it seems possible that these changes in PDGF levels in both graft and host tissue could be important for the survival and differentiation of grafted embryonic DA neurons.

The finding of TH-positive neurons with longer neurites in PDGF–BB-treated cultures of human mesencephalic neurons could be explained by increased survival of this particular population of cells. However, it may also suggest that PDGF–BB is able to stimulate neurite outgrowth in addition to its effect on cell survival. The lack of any effect on neurite outgrowth in rat cultures could be related to the stage of development of the dopaminergic neurons when exposed to PDGF–BB. Possibly, the neurite-outgrowth promoting effect of PDGF–BB occurs only during a restricted time period, at a developmental stage tested with human, but not rat, tissue in the present study. It also remains to be examined further as to whether there are species-specific modifications of the PDGF isoforms, their receptors, or their receptor-mediated effects, as only human, but not rat, recombinant PDGF was investigated in the present study.

Whether the effect of PDGF–BB results from a direct action on dopaminergic neurons or requires the presence of other cell types in the culture is not known. The trophic effect of bFGF on mesencephalic DA neurons in vitro has been proposed to be indirect (Knüsel et al. 1990; Engele and Bohn 1991), mediated via glia cells, whereas BDNF probably acts directly on these neurons (Hyman et al. 1991). Using serum-free growth conditions, as in the present experiments, the expected number of glial fibrillary acidic protein (GFAP)-positive cells in low-density cultures lies below 1% (Ferrari et al. 1989), which makes an indirect, glia-mediated effect less likely here. However, it remains to be shown whether PDGF receptors are localized on the dopaminergic neurons themselves, which would support a direct trophic effect of PDGF.

In conclusion, the effect of PDGF–BB on the survival of TH-positive neurons in culture, as indicated by the present study, raises the possibility that PDGF–BB may influence the development and maintenance of mesencephalic dopaminergic neurons also in vivo. If so, exogenous administration of PDGF–BB could provide a new approach to counteract neural degeneration and to stimulate axonal sprouting from intrinsic and grafted dopaminergic neurons. It seems highly warranted to test these hypotheses by in vivo experiments in animal models of Parkinson's disease.

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References

Appel SH (1982) Neurotrophic factors and diseases of aging: an approach to ALS, parkinsonism, and Alzheimer's disease. In: Comparative pathobiology of major age-related diseases, current status and research frontiers. Liss, New York, pp 411–423

Bottenstein P, Sato GH (1979) Growth of a rat neuroblastoma cell line in serum-free supplemented medium. Proc Natl Acad Sci USA 76:514–517

Date I, Notter MFD, Felten SY, Felten DL (1990) MPTP-treated young mice but not aging mice show partial recovery of the nigrostriatal dopaminergic system by stereotaxic injection of acidic fibroblast growth factor (aFGF). Brain Res 526:156–160

Engele J, Bohn MC (1991) The neurotrophic effects of fibroblast growth factors on dopaminergic neurons in vitro are mediated by mesencephalic glia. J Neurosci 11:3070–3078

Ferrari G, Minozzi M-C, Toffano G, Leon A, Skaper SD (1989) Basic fibroblast growth factor promotes the survival and development of mesencephalic neurons in culture. Dev Biol 133:140–147

Hart CE, Forstrom JW, Kelly JD, Seifert RA, Smith RA, Ross R, Murray MJ, Bowen-Pope DF (1988) Two classes of PDGF receptor recognize different isoforms of PDGF. Science 240(10):1529–1531

Heldin C-M, Westermark B (1990) Platelet-derived growth factor: mechanism of action and possible in vivo function. Cell Regul 1:555–566

Heldin C-M, Bäckström G, Östman A, Hammacher A, Rönnstrand L, Rubin K, Nistér M, Westermark B (1988) Binding of different dimeric forms of PDGF to human fibroblasts: evidence for two separate receptor types. EMBO J:1387–1393

Hyman C, Hofer M, Barde Y-A, Juhasz M, Yancopoulos GD, Squinto SP, Lindsay RM (1991) BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. Nature 350:230–232

Knüsel B, Michel PP, Schwaber SS, Hefti F (1990) Selective and nonselective stimulation of central cholinergic and dopaminergic development in vitro by nerve growth factor, basic fibroblast growth factor, epidermal growth factor, insulin and the insulin-like growth factors I and II. J Neurosci 10:558–570

Knüsel B, Winslow JW, Rosenthal A, Burton LE, Seid DP, Nikolics K, Hefti F (1991) Promotion of central cholinergic and dopaminergic neuron differentiation by brain-derived neuro-

trophic factor but not neurotrophin 3. Proc Natl Acad Sci USA 88:961–965

Lindvall O, Widner H, Rehncrona S, Brundin P, Odin P, Gustavii B, Frackowiak R, Leenders KL, Sawle G, Rothwell JC, Björklund A, Marsden CD (1992) Transplantation of fetal dopamine neurons in Parkinson's disease: one-year clinical and neurophysiological observations in two patients with putaminal implants. Ann Neurol 31:155–165

Manthorpe M, Fagini R, Skaper SD, Varon S (1986) An automatic colorimetric microassay for neurotrophic factors. Brain Res Dev Brain Res 25:191–198

Nikkhah G, Tonn JC, Hoffmann O, Kraemer HP, Darling JL, Schönmayr R, Schachenmayr W (1992) The MTT assay for chemosensitivity testing of human tumors of the central nervous system. J Neurooncol 13:297–307

Östman A, Bäckström G, Fong N, Betsholtz C, Wernstedt C, Hellman U, Westermark B, Valenzuela P, Heldin C-H (1989) Expression of three recombinant homodimeric isoforms of PDGF in *Saccharomyces cerevisiae*: evidence for difference in receptor binding and functional activities. Growth Factors 1:271–281

Otto D, Unsicker K (1990) Basic FGF reverses chemical and morphological deficits in the nigrostriatal system of MPTP-treated mice. J Neurosci 10 (6):1912–1921

Pringle NP, Mudhar HS, Collarini EJ, Richardson WD (1992) PDGF receptors in the rat CNS: during late neurogenesis, PDGF α-receptor expression appears to be restricted to glial cells of the oligodendrocyte lineage. Development (in press)

Raines EW, Bowen-Pope DF, Ross R (1990) Peptide growth factors and their receptors. In: Sporn MB, Roberts AB (eds) Handbook of experimental pharmacology, vol 95/I. Springer, Berlin Heidelberg 95:I, pp 173–262

Reddy UR, Pleasure D (1992) Expression of platelet-derived growth factor (PDGF) and PDGF receptor genes in the developing rat brain. J Neurosci Res 31:670–677

Richardson WD (1991) PDGF in neurons. Curr Biol 1:162–164

Sasahara M, Fries JWU, Raines EW, Gown AM, Westrum LE, Frosch MP, Bonthron DT, Ross R, Collins T (1991) PDGF B-chain in neurons of the central nervous system, posterior pituitary, and in a transgenic model. Cell 64:217–227

Sauer H, Fischer W, Nikkhah G, Brundin P, Björklund A (1992) Brain-derived neurotrophic factor enhances function rather than the survival of intrastriatal mesencephalic grafts (abstract). 15th Annual Meeting of the European Neuroscience Association, Munich, Germany

Smits A, Kato M, Westermark B, Nistér M, Heldin C-H, Funa K (1991) Neurotrophic activity of platelet-derived growth factor (PDGF): rat neuronal cells possess functional PDGF β-type receptors and respond to PDGF. Proc Natl Acad Sci USA 88:8159–8163

Steinbusch HW, Vermeulen RJ, Tonnaer JA (1990) Basic fibroblast growth factor enhances survival and sprouting of fetal dopaminergic cells implanted in the denervated rat caudate-putamen: preliminary observations. Prog Brain Res 82:81–86

Yeh H-J, Rult KG, Wang Y-X, Parks WC, Snider WD, Duel TF (1991) PDGF A-chain gene is expressed by mammalian neurons during development and in maturity. Cell 64:209–216