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Insulin-like growth factors act synergistically with basic fibroblast growth factor and nerve growth factor to promote chromaffin cell proliferation

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ABSTRACT **We have investigated the effects of insulin-like growth factors (IGFs), basic fibroblast growth factor (bFGF), and nerve growth factor (NGF) on DNA synthesis in cultured chromaffin cells from fetal, neonatal, and adult rats by using 5-bromo-2′-deoxyuridine (BrdUrd) pulse labeling for 24 or 48 h and immunocytochemical staining of cell nuclei. After 6 days in culture in the absence of growth factors, nuclear BrdUrd incorporation was detected in 30% of fetal chromaffin cells, 1.5% of neonatal cells, and 0.1% of adult cells. Addition of 10 nM IGF-I or IGF-II increased the fraction of BrdUrd-labeled nuclei to 50% of fetal, 20% of neonatal, and 2% of adult chromaffin cells. The ED_{50} value of IGF-I- and IGF-II-stimulated BrdUrd labeling in neonatal chromaffin cells was 0.3 nM and 0.8 nM, respectively. In neonatal and adult chromaffin cells, addition of 1 nM bFGF or 2 nM NGF stimulated nuclear BrdUrd incorporation to approximately the same level as 10 nM IGF-I or IGF-II. However, the response to bFGF or NGF in combination with either IGF-I or IGF-II was more than additive, indicating that the combined effect of the IGFs and bFGF or NGF is synergistic. The degree of synergism was 2- to 4-fold in neonatal chromaffin cells and 10- to 20-fold in adult chromaffin cells compared with the effect of each growth factor alone. In contrast, the action of bFGF and NGF added together in the absence of IGFs was not synergistic or additive. IGF-II acted also as a survival factor on neonatal chromaffin cells and the cell survival was further improved when bFGF or NGF was added together with IGF-II. In conclusion, we propose that IGF-I and IGF-II act in synergy with bFGF and NGF to stimulate proliferation and survival of chromaffin cells during neonatal growth and adult maintenance of the adrenal medulla. Our findings may have implications for improving the survival of chromaffin cell implants in diseased human brain.**

The chromaffin cells of the adrenal medulla are derived from the neural crest and are ontogenetically related to sympathetic neurons. In the rat, chromaffin-cell precursors invade the primordial adrenocortical structure about embryonic day 14 where they differentiate into mature chromaffin cells (1). Chromaffin-cell division in the rat occurs mainly during fetal and neonatal development, whereas proliferation of adult chromaffin cells is very low (2–5). Little is known about the factors that regulate the growth of chromaffin cells. Postnatally, basic fibroblast growth factor (bFGF) is present in bovine and rat adrenal medulla (6, 7), and bFGF stimulates the proliferation of neonatal rat chromaffin cells (8, 9). It is not known whether nerve growth factor (NGF) is present in the adrenal medulla, but specific high-affinity NGF binding sites have been demonstrated in chromaffin cells from neonatal rats (10), and NGF acts as a mitogen on cultured chromaffin cells from neonatal and adult rats (11, 12). In addition, NGF and bFGF stimulate transdifferentiation of cultured chromaffin cells into a sympathetic neuron-like phenotype and increase cell survival and catecholamine synthesis (8, 9, 13–15). In adult rats, chromaffin-cell replacement decreases after denervation of the adrenal medulla, suggesting that neurogenic stimuli can regulate chromaffin-cell proliferation (16).

Insulin-like growth factor I and II (IGF-I and IGF-II, respectively) act as neurotrophic factors in various parts of the developing and adult nervous system (17). IGF-II mRNA and immunoreactivity have been demonstrated in adult human and rat adrenal medulla (18–21). Human and bovine chromaffin cells express the IGF-I receptor tyrosine kinase that mediates the action of both IGF-I and IGF-II (22–24), and IGF-I stimulates catecholamine synthesis and secretion by cultured bovine chromaffin cells *in vitro* (22, 25–27). IGF-I and IGF-II stimulate the proliferation of rat PC12 pheochromocytoma cells (28, 29), but it is not known whether IGFs stimulate the division of normal chromaffin cells.

Implantation of chromaffin cells from the autologous adrenal medulla into the brains of patients with Parkinson disease has been used, but the dopamine cells of transplants did not survive and sustained clinical improvement was not obtained (30, 31). One reason for the poor survival of chromaffin-cell transplants may be lack of growth factors in the striatum, which has a low content of NGF and IGF-II (32–34). Administration of NGF to chromaffin-cell grafts in animal models with Parkinson disease resulted in increased cell survival (35, 36), suggesting that treatment with growth factors may improve the viability of intrastriatal chromaffin cell implants.

The present study was initiated to answer two questions: (*i*) whether IGF-I and IGF-II stimulate proliferation of chromaffin cells from fetal, neonatal, and adult rat adrenal medulla and (*ii*) whether IGFs interact with bFGF and NGF in stimulating cell division and survival of chromaffin cells. We used isolated chromaffin cells from fetal, neonatal, and adult rats in primary culture and identified proliferating chromaffin cells by double immunocytochemical staining of nuclei for 5-bromo-2′-deoxyuridine (BrdUrd) incorporated in DNA and of cytoplasm for tyrosine hydroxylase. Our data show that IGF-I and IGF-II stimulate chromaffin-cell division and act synergistically with bFGF and NGF on proliferation and survival of neonatal and adult chromaffin cells.

MATERIALS AND METHODS

Chemicals. Human IGF-I and IGF-II were purchased from Amersham. Human insulin and human bFGF were gifts from

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Abbreviations: bFGF, basic fibroblast growth factor; BrdUrd, 5-bromo-2′-deoxyuridine; FCS, fetal calf serum; IGF, insulin-like growth factor; NGF, nerve growth factor.

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Novo-Nordisk, Bagsvaerd, Denmark. Murine 2.5S NGF and monoclonal antibody to tyrosine hydroxylase were from Boehringer Mannheim. Collagenase, poly(D-lysine), bovine serum albumin (Cohn fraction V), progesterone, putrescine, Na_2SeO_3, transferrin, and BrdUrd were from Sigma. Trypsin was from Difco. Dulbecco's modified Eagle's medium (DMEM), RPMI 1640 medium, fetal calf serum (FCS), horse serum, glutamine, penicillin, and streptomycin were from Biological Industries, Tel Aviv. Texas red-conjugated goat polyclonal antibody to mouse IgG was from Accurate Chemicals and fluorescein isothiocyanate-conjugated mouse monoclonal antibody to BrdUrd was from Becton Dickinson. Culture dishes were from Costar.

Cell Culture. Primary cultures of chromaffin cells were established from rat embryos on fetal day 18–20, newborn rats on postnatal day 7 or 8, or adult female Wistar rats of 3–6 months old as described by Unsicker *et al.* (37). The adrenal medulla was dissected from the cortex and pooled in Mg^{2+}- and Ca^{2+}-free Hanks' balanced salt solution on ice. The tissue was incubated at 36°C for 40 min in 1 ml of 0.2% collagenase followed by two 15-min incubations in 1 ml of 0.125% trypsin. After trituration, isolated cells were washed with 1 ml of horse serum to inactivate trypsin. Cells ($0.5–1 \times 10^4$ cells) were plated on glass coverslips (12 mm) or in 24-well plastic culture dishes coated with poly(D-lysine) and collagen prepared from rat tail (38). Cells were cultured for 2 days in DMEM/RPMI 1640 medium, 1:1 (vol/vol), with 20% (vol/vol) horse serum, glutamine, penicillin, and streptomycin at 37°C in a humidified atmosphere of 10% CO_2/90% air. Chromaffin cells were identified by immunostaining for cytoplasmic tyrosine hydroxylase and constituted 70–85% of the cells in cultures from neonatal and adult rats and 30–50% of the cells in cultures from fetal rats.

Immunocytochemistry. Cells on glass coverslips were washed once with phosphate-buffered saline (PBS) and fixed 8 min with methanol on ice. DNA was denatured 20 min with 1.5 M HCl followed by three PBS washes. The cells were preincubated 30 min at 20°C in PBS with 10% horse serum and washed three times in PBS. The cells were incubated sequentially for 60 min at 20°C with three antibodies diluted in PBS/2% horse serum/0.5% Tween 20 and washed three times in PBS after each incubation. The cells were incubated first with mouse monoclonal antibody to tyrosine hydroxylase diluted 1:300, second with Texas red-conjugated goat polyclonal antibody to mouse IgG diluted 1:300, and finally, with fluorescein isothiocyanate-conjugated mouse monoclonal antibody to BrdUrd diluted 1:25 (39). The coverslips were mounted in PBS with 90% (vol/vol) glycerol and examined by fluorescence microscopy.

Assay of DNA Synthesis. Cells on glass coverslips were washed once with DMEM/RPMI 1640 medium and cultured in this medium with addition of 0.4% bovine serum albumin and N1 supplement [N1 = 20 nM progesterone/0.1 mM putrescine/30 nM Na_2SeO_3/60 nM transferrin; N1 supports the survival of chromaffin cells and sensory neurons in culture (37, 40)]. Insulin was omitted from the N1 supplement to avoid crossreaction with the IGF-I receptor. IGF-I, IGF-II, insulin, NGF, or bFGF was added for 6 days with one medium shift on day 3. Control experiments showed that only 2–3% of added IGF-I, IGF-II, or insulin tracers were degraded after 3 days of culture (data not shown). BrdUrd (5 μM) was added during the final 24 or 48 h of the experiment. DNA synthesis in chromaffin cells was demonstrated by double immunocytochemical staining of cytoplasmic tyrosine hydroxylase and nuclear BrdUrd. Chromaffin cells with BrdUrd-labeled nuclei, alone or in clusters of two to eight cells, were counted until 200–400 tyrosine hydroxylase-positive cells had been examined. The data are expressed as the percentage of tyrosine hydroxylase-positive cells with BrdUrd-labeled nuclei.

Assay of Cell Survival. Neonatal chromaffin cells on plastic culture dishes were cultured in medium with 0.4% albumin and N1 supplement without insulin. After 2, 4, or 6 days of culture in the presence of IGF-II, bFGF, or NGF, the cells were washed twice in Hanks' balanced salt solution. Chromaffin cells adhering to the dish were counted in two orthogonal sections of each dish (≈5% of the total area) using phase-contrast microscopy. Neonatal chromaffin cells were identified by their characteristic morphology [i.e., phase-bright rounded or polygonal cells with a large round nucleus and occasionally bearing processes or being flattened after addition of bFGF or NGF (11, 15, 37)]. More than 90% of the chromaffin cells adhering to the culture substratum were viable as estimated by nigrosin blue exclusion.

Assay of Neuronal Differentiation. Neonatal chromaffin cells on glass coverslips were cultured in medium with 0.4% albumin and N1 supplement without insulin and exposed to NGF, bFGF, or IGF-II for 6 days. After washing in PBS, the cells were immunostained for tyrosine hydroxylase and examined by immunofluorescence microscopy. The percentage of neurite-bearing chromaffin cells was determined by examination of 200–400 tyrosine hydroxylase-positive cells.

RESULTS

Stimulation of DNA Synthesis of Chromaffin Cells by IGF-I, IGF-II, and Insulin. Chromaffin cells from fetal, neonatal, or adult rats were cultured 6 days in the absence or presence of 10 nM IGF-I, 10 nM IGF-II, 10 nM insulin, or 20% FCS and

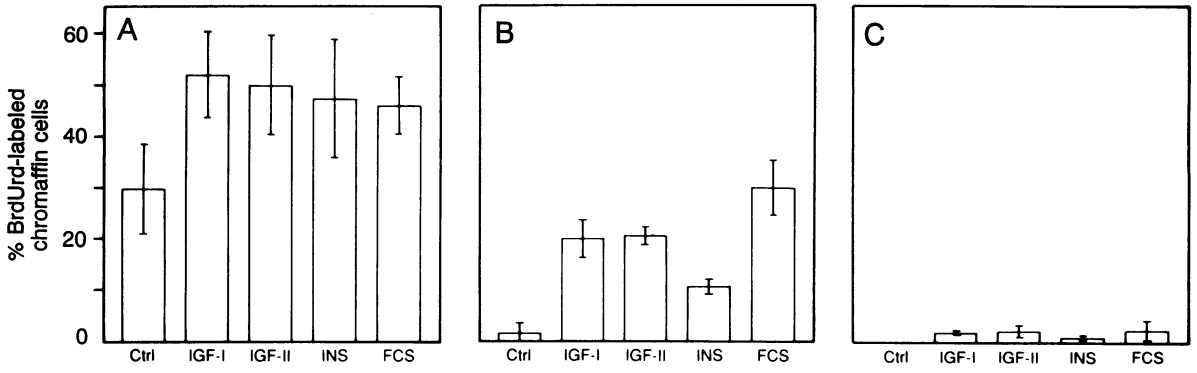

FIG. 1. Effect of IGF-I, IGF-II, insulin, and FCS on the DNA synthesis of chromaffin cells from fetal (*A*), neonatal (*B*), and adult (*C*) rats. Cells were cultured for 6 days in the presence of 10 nM IGF-I, 10 nM IGF-II, 10 nM insulin, or 20% FCS and pulse-labeled with BrdUrd for the final 48 h. After fixation the cells were stained with antibodies to tyrosine hydroxylase and BrdUrd and examined by fluorescence microscopy. The percentage of tyrosine hydroxylase-positive cells with BrdUrd-labeled nuclei was determined. Data are mean ± SD of five experiments. Ctrl, control.

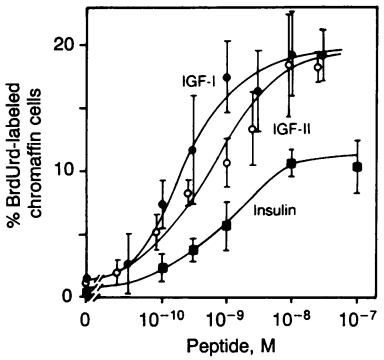

FIG. 2. Concentration dependence of IGF-I-, IGF-II-, and insulin-stimulated DNA synthesis in neonatal rat chromaffin cells. Cells were cultured for 6 days in the presence of IGF-I (●), IGF-II (○), or insulin (■) at concentrations from 30 pM to 100 nM and pulse-labeled with BrdUrd for the final 48 h. The percentage of tyrosine hydroxylase-positive cells with BrdUrd-labeled nuclei was determined. Data are mean ± SD of four experiments.

pulse-labeled with BrdUrd during the final 48 h in culture. Fig. 1 shows that 30% of fetal chromaffin cells incorporated BrdUrd under basal conditions. Addition of IGF-I, IGF-II, insulin, or FCS increased the fraction of BrdUrd-labeled fetal chromaffin cells to 50%. Basal incorporation of BrdUrd in neonatal chromaffin cells was seen in 1.5% of the cells, and IGF-I or IGF-II increased the BrdUrd labeling to 17% of the cells. Insulin was only half as efficient as IGFs, whereas FCS had a slightly larger effect. Only 0.1% of adult chromaffin cells incorporated BrdUrd in the absence of growth factors, and in the presence of IGF-I or IGF-II, the fraction of BrdUrd-labeled cells was increased to 1.8% and 2.8%, respectively. FCS was as efficient as IGFs whereas insulin had a lower effect.

The effect of IGF-I and IGF-II on BrdUrd labeling of neonatal rat chromaffin cells was dose-dependent with the same maximal effect (Fig. 2). The concentration of IGF-I and IGF-II giving a half-maximal effect (EC_{50}) was 0.3 nM and 0.8 nM, respectively. Insulin stimulated DNA synthesis with an EC_{50} value of 1 nM and a lower maximal effect than IGFs. We conclude that IGF-I, IGF-II, and insulin stimulate DNA synthesis in cultured chromaffin cells from fetal, neonatal, and adult rats and exert their mitogenic effect in the physiological concentration range.

Synergistic Effect of IGFs and bFGF or NGF on DNA Synthesis and Proliferation of Chromaffin Cells. bFGF at a maximally stimulating concentration of 1 nM (9) increased the fraction of BrdUrd-labeled neonatal chromaffin cells to the same level of 10% as 10 nM IGF-II after 6 days in culture and pulse labeling with BrdUrd for the final 24 h (Fig. 3*A*). Addition of bFGF plus IGF-II increased the fraction of BrdUrd-labeled cells to 45%, which is significantly higher than an additive effect, indicating that the two growth factors act in synergy. Furthermore, NGF at a maximally stimulating concentration of 2 nM (11) acted synergistically with 10 nM IGF-II in neonatal chromaffin cells since the BrdUrd incorporation was increased by NGF plus IGF-II to 40% compared with the effect of the two growth factors alone of 20% and 10%. In contrast, the effect of bFGF plus NGF was neither additive nor synergistic.

In adult chromaffin cells, the synergistic effect of bFGF and IGF-II was more pronounced (Fig. 3*B*). Upon stimulation with either growth factor alone for 6 days and a final 24-h BrdUrd pulse, these cells showed a very low response of ≈1% of the cells being labeled with BrdUrd, but in the presence of bFGF plus IGF-II ≈20% of the adult chromaffin cells had incorporated BrdUrd. The combined effect of NGF and IGF-II on adult chromaffin cells was also clearly synergistic as 10% of nuclei were BrdUrd-labeled compared with the effect of 1% to each growth factor alone. bFGF and NGF added together in the absence of IGF-II showed no additive or synergistic effect. In both neonatal and adult chromaffin cells, 10 nM IGF-I showed the same synergy with bFGF or NGF as was observed with IGF-II (data not shown).

Measurement of the time course of the effects of IGF-II and bFGF on DNA synthesis in neonatal chromaffin cells showed that the synergistic stimulation of BrdUrd incorporation occurred 2–3 days after the addition of growth factors and reached a maximal level that was constant from 4 to 6 days (Fig. 4). In adult chromaffin cells, the synergistic stimulation of DNA synthesis by IGF-II and bFGF was seen after 5 days (data not shown).

To assess whether BrdUrd incorporation in DNA is followed by cell division, neonatal chromaffin cells were stimulated 6 days with 10 nM IGF-II plus 1 nM bFGF and pulse-labeled with BrdUrd for 3 h. The cells were either fixed immediately or washed twice to remove BrdUrd and cultured for 2 additional days in the presence of growth factors before fixation. In cultures fixed immediately after the BrdUrd pulse on day 6, >80% of labeled chromaffin cells were singlets. However, BrdUrd-labeled chromaffin cells were predominantly doublets or quadruplets 2 days after the BrdUrd pulse on day 8 (data not shown). Since the cell density in the cultures was very low, we assume that doublets and quadruplets of BrdUrd-labeled chromaffin cells arise from cell division and not from aggregation. These data suggest that IGF-II- and bFGF-stimulated DNA synthesis is followed by cell division. In conclusion, our data show that IGF-I and IGF-II can interact with bFGF plus NGF to stimulate DNA synthesis and proliferation of neonatal and adult chromaffin cells in a synergistic manner.

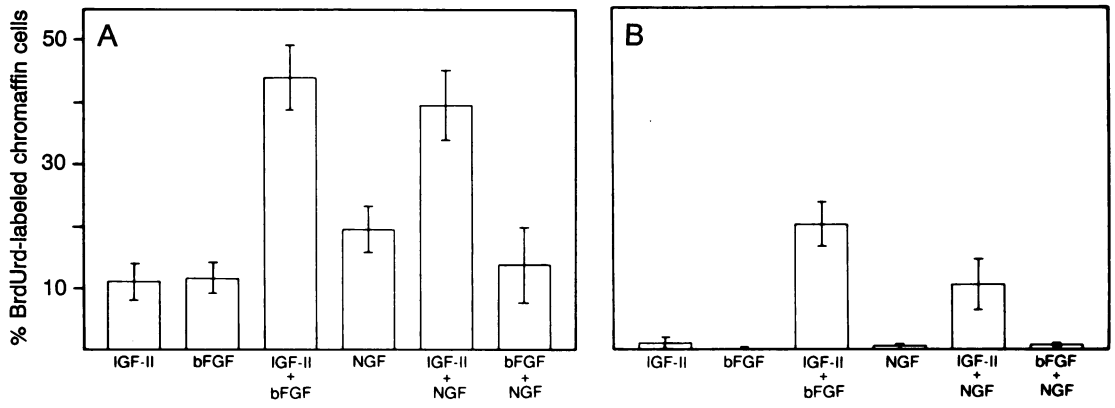

FIG. 3. Stimulation of DNA synthesis in neonatal (*A*) and adult (*B*) rat chromaffin cells by IGF-II, bFGF, and NGF. Cultures were treated for 6 days with 10 nM IGF-II, 1 nM bFGF, 2 nM NGF, or in various combinations and pulse-labeled with BrdUrd for the final 24 h. The percentage of tyrosine hydroxylase-positive cells with BrdUrd-labeled nuclei was determined. Data are mean ± SD of four experiments.

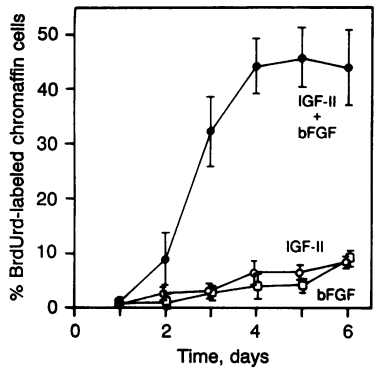

FIG. 4. Time course of IGF-II- and bFGF-stimulated DNA synthesis in neonatal rat chromaffin cells. Cultures were treated with 10 nM IGF-II (○), 1 nM bFGF (□), or in combination (●) for 1–6 days and pulse-labeled with BrdUrd for the final 24 h. The percentage of tyrosine hydroxylase-positive cells with BrdUrd-labeled nuclei was determined. Data are mean ± SD of three experiments.

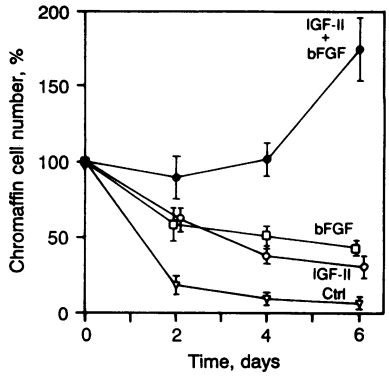

FIG. 5. Effect of IGF-II and bFGF on survival of neonatal rat chromaffin cells. Cells were treated with 10 nM IGF-II (○), 1 nM bFGF (□), or in combination (●). Cells cultured in the absence of growth factors served as control (▽). After 2, 4, or 6 days, the cells were washed and the number of viable chromaffin cells attached to the dish was determined and expressed as percent of the initial value. Data are mean ± SD of three experiments.

IGF-II, bFGF, and NGF Are Survival Factors for Chromaffin Cells. In the absence of growth factors, the number of viable neonatal chromaffin cells attached to the culture dish declined rapidly, reaching 7% of its initial value after 6 days of culture (Fig. 5). In the presence of 10 nM IGF-II or 1 nM bFGF, the chromaffin cell number declined at a slower rate and after 6 days the percentage of surviving cells was 30–40% of the initial cell number. Addition of IGF-II plus bFGF completely prevented the decline in chromaffin cell number after 2–4 days and reversed it to 175% of the initial value after 6 days of culture. This effect may be attributed partly to increased cell survival and partly to increased cell proliferation (see Fig. 4). Addition of 2 nM NGF alone increased the fraction of surviving chromaffin cells after 6 days to 20% (±2%, $n = 4$) of its initial value, whereas NGF plus IGF-II increased the chromaffin cell number to 105% (±15%, $n = 4$) after 6 days in culture (data not shown).

Exposure to either NGF or bFGF for 6 days induced neuronal transdifferentiation of 11% (±2%, $n = 4$) and 2% (±1%, $n = 4$) of neonatal rat chromaffin cells, respectively, as demonstrated by others (8, 9, 13–15). IGF-II alone did not elicit neurite formation beyond basal values (<0.1%). However, the fraction of neurite-bearing cells induced by NGF or bFGF was reduced by IGF-II to 6% (±2%, $n = 4$) and 0.5% (±0.2%, $n = 4$), respectively (data not shown). In conclusion, IGF-II acts as a survival factor and mitogen for neonatal chromaffin cells without induction of neuronal differentiation, and the effect of IGF-II is potentiated by bFGF or NGF.

DISCUSSION

Mitogenic Effect of IGF-I and IGF-II. In this report we describe the mitogenic effect of IGF-I and IGF-II on chromaffin cells from fetal, neonatal, and adult rats *in vitro*. We employed a serum-free culture system to avoid interference from IGFs and IGF-binding proteins in serum. The responsiveness to IGF-I and IGF-II differed significantly with the developmental stage, being highest in neonatal and lowest in adult chromaffin cells. The dose–response relationship of IGFs in neonatal chromaffin cells corresponds to that observed in other cell types, and the fact that IGF-I is more potent than IGF-II suggests that the effect is mediated by the IGF-I receptor (41). The mitogenic effect of insulin on the chromaffin cells may also be mediated by the IGF-I receptor that binds insulin with low affinity ($K_i \approx 0.1$–1 μM). However, the relatively high potency of insulin ($ED_{50} \approx 1$ nM) and a maximal response that is distinct from that of the IGFs may indicate that insulin is acting through the insulin receptor present on chromaffin cells (23).

Synergistic Actions of IGFs and bFGF or NGF. The finding of synergy between IGFs and bFGF or NGF in stimulation of DNA synthesis in neonatal and adult rat chromaffin cells suggests that divergent signals at the receptor level may converge in the signaling pathway leading to DNA synthesis and cell division. Furthermore, the nonadditive effects of bFGF and NGF may indicate that their receptors activate similar signals. The IGF-I receptor tyrosine kinase is distinct from the bFGF receptor and NGF receptor tyrosine kinases in that it phosphorylates the insulin receptor substrate 1, which functions as an initial common signaling molecule in IGF-I and insulin signaling (42, 43). Apart from this difference, activation of the IGF-I, bFGF, and NGF receptors leads to stimulation of phosphatidylinositol 3-kinase and Ras GTP binding (42–47), and these signaling molecules may represent the points of convergence in the mitogenic signal pathway.

Previous studies have shown that NGF and bFGF induce an initial mitogenic response in neonatal and adult rat chromaffin cells and rat PC12 cells that is followed by neuronal differentiation and growth arrest (8, 9, 11, 12, 48, 49), whereas IGF-I and IGF-II stimulate proliferation of PC12 cells only and are not capable of inducing a neuronal phenotype (28, 29). This is confirmed by our finding that IGFs did not induce neuronal differentiation of chromaffin cells in contrast to bFGF and NGF, suggesting that the signals leading to neuronal differentiation are different from those leading to cell division.

Chromaffin Cell Survival. The increased neonatal chromaffin cell number after 2 days in the presence of IGF-II seems mainly to be due to increased cell survival as IGF-II-stimulated proliferation was first seen after 3–4 days. Improved cell viability may in part be a consequence of the actions of IGF-II on cellular metabolism, such as increased glucose and amino acid uptake and stimulation of protein synthesis (41). The stimulatory effects of bFGF and NGF on chromaffin cell survival *in vitro* have previously been demonstrated (8, 9, 14, 15). In our study the effect of IGF-II on chromaffin cell survival was additive with the effect of bFGF or NGF and the decline in cell number was fully prevented after the first 2–4 days of culture. IGF-II added with bFGF even reversed the decline to an increase in chromaffin cell number after 6 days due to stimulation of cell proliferation.

Chromaffin Cell Proliferation *in Vivo*. Our observation that IGF-I and IGF-II are mitogens for fetal and neonatal rat chromaffin cells *in vitro* and act in synergy with NGF and bFGF may indicate that these factors are involved in the development of the adrenal medulla. Biosynthesis of IGF-I,

IGF-II, NGF, or bFGF has not been demonstrated in the fetal or early neonatal adrenal medulla (6, 50, 51), but the growth factors may originate from the circulation or innervation.

Immunoreactive bFGF appears at postnatal day 8 in an increasing fraction of rat chromaffin cells and is confined to the noradrenergic subpopulation of chromaffin cells in the adult rat (6). In the adult bovine adrenal medulla, the majority of chromaffin cells are bFGF-immunoreactive (7). IGF-II is synthesized by the adult human adrenal medulla (18–20), but the expression is apparently confined to a subpopulation of 1–10% of the chromaffin cells (21). Thus, IGF-II and bFGF may stimulate replacement of chromaffin cells in adult mammals in an autocrine or paracrine manner.

The scarcity of mitotic figures in adult rat chromaffin cells *in vivo* has led to the dogma that adult chromaffin cells are postmitotic (2) and more recent work has confirmed that adult rat chromaffin cells rarely divide *in situ* (3–5). However, adult rat chromaffin cells are not irreversibly postmitotic as they reveal a growth potential comparable to that of neonatal chromaffin cells when exposed to the synergistic action of IGF-I or IGF-II and bFGF or NGF in culture. In a recent study, Tischler *et al.* (12) demonstrated that NGF is a potent inducer of proliferation and neuronal differentiation for adult rat chromaffin cells *in vitro* in agreement with our findings. The apparent postmitotic state of adult chromaffin cells *in situ* may be induced by inhibitory factors in the adrenal gland. Thus, corticosterone and pituitary adenylate cyclase-activating polypeptide 38 inhibit IGF-II- and bFGF-stimulated growth of cultured neonatal and adult chromaffin cells from 75% to 95% (M.F., unpublished observation).

Implantation of Chromaffin Cells in Brain. Brain implants of chromaffin cells have been used as dopaminergic donors in patients with Parkinson disease, but the long-term benefit has been hampered by poor survival of the implanted cells (30, 31). The approach has been abandoned and replaced by transplantation of human fetal ventral mesencephalic cells that seems to give better results (52, 53). Administration of NGF to brain implants of chromaffin cells in experimental animals has led to improved cell survival (35, 36). Based on our data a combination of IGFs, NGF, and bFGF may be more effective than each growth factor alone in improving the survival of the chromaffin cell implants. Thus, implantation of autologous adrenal medullary tissue in diseased human brain should be reconsidered as it may be advantageous over transplantation of heterologous fetal tissue regarding tissue accessibility, immunotolerance, and medical ethics.

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