Vol. 141, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 26, 1986 Pages 67-72

BRAIN-DERIVED GROWTH FACTOR IS A CHEMOATTRACTANT FOR FIBROBLASTS AND ASTROGLIAL CELLS

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Received September 15, 1986

SUMMARY. Bovine brain-derived growth factor (BDGF), a 16-17 kDa protein with biochemical properties resembling brain-derived acidic fibroblast growth factor (acidic FGF) and endothelial cell growth factor, was found to have potent chemotactic activity for bovine ligament fibroblasts, human skin fibroblasts and rat astroglial cells, maximal at 100-200 pg/ml. The chemotactic activity was completely blocked by protamine sulfate (5 ug/ml), an inhibitor of receptor-binding and mitogenic activity of BDGF. BDGF did not stimulate migration of human monocytes. These results indicate that the effects of BDGF 'in vivo' might extend to mesenchymal cell recruitment.

Bovine brain-derived growth factor (BDGF) is a potent mitogen for a variety of cell types including endothelial cells, fibroblasts, osteoblasts, glial cells, chondrocytes, smooth muscle cells and epithelial cells (1-3). The chemical and physical properties of BDGF suggest that it is either identical to, or closely resembles, brain-derived acidic fibroblast growth factor (1,4-6) and endothelial cell growth factor (7). In contrast, BDGF differs from pituitary FGF in chemical, physical and immunological properties, although it does compete with pituitary FGF for the same receptor on the surface of responsive cells (3,8). BDGF elutes from heparin-Sepharose as two distinct components, A and B, which differ in molecular weight, 16,000 and 17,000 respectively, but which are indistinguishable by isoelectric point, receptor binding characteristics, mitogenic activity and immunoreactivity with anti-BDGF-A serum (3). The BDGF receptor appears to be a 135 kDa protein associated with protein

0006-291X/86 $1.50

tyrosine kinase activity (9). The physiological function of BDGF is unknown. The specific localization of BGDF in neurons (10) and its wide spectrum of activity suggest that BDGF may have an important role in neural tissue.

In the present report we show that BDGF exerts chemotactic activity for fibroblasts and astroglial cells. The activity is expressed maximally at approximately 10 pM and is blocked by protamine sulfate, an inhibitor of BDGF binding in responsive cells (3).

MATERIALS and METHODS

Cell Preparations: Human skin fibroblasts were obtained from explants of skin obtained at surgery and fetal bovine ligament fibroblasts were prepared from explants of fetal bovine ligamentum nuchae (11,12). Astroglial cells, which had been obtained from one day-old rat cerebral cortical tissue by the procedure of McCarthy and de Vellis (13), were provided by Dr. Joseph W. Yang, Washington University School of Medicine, St. Louis, MO. Human mononuclear leukocytes were prepared from peripheral blood of healthy volunteers by Ficoll-Hypaque separation (14).

Preparation of BDGF: BDGF-A was purified as previously described (3). The BDGF-A preparation was homogeneous based on the criteria of SDS polyacrylamide gel electrophoresis and analytical isoelectric focusing.

Chemotaxis Assay: Chemotactic activity was determined in a multi-blind well apparatus (30 wells) using a double membrane method, as previously described (12,15,16). The lower compartment of each well was filled with 240 ul of the test solution, or control medium. The upper and lower compartment of each well were separated by either an 8 um (fibroblasts and astroglial cells) or a 5 um (monocytes) micropore polycarbonate membrane (Nucleopore Corp., Pleasanton, CA.) that was overlaying a 0.45 um micropore cellulose nitrate membrane (Millipore Corp., Bedford, MA.) To facilitate cell attachment, the polycarbonate membranes were presoaked in poly-L-lysine. The upper compartment of each well was filled with 350 ul of Dulbecco's modified Eagle's minimum essential medium (The Basic Cancer Center, Washington University Medical Center, St. Louis, Mo.) containing human serum albumin (American Red Cross Blood Services, Wash., D.C.) 0.1% (w/v) and either 1.2×10^5 cells per ml (fibroblasts or astroglial cells) or 2.5×10^6 cells per ml (mononuclear leukocytes). The apparatus was incubated at 37°C, in 5% carbon dioxide-95% air for either 6 hours (fibroblasts and astroglial cells) or 2 hours (monocytes), then disassembled, after which each membrane pair was removed and stained with hematoxylin. Under high dry magnification (x400), cells that had migrated to the interface between the two membranes or to the surface of the lower membrane were counted. Five fields were counted on each membrane. Each experiment was done in triplicate. Cell migration, reported as net cell migration, was calculated by subtracting from total cell migration the migration occurring when the lower compartment contained only control media. Platelet-derived growth factor (PDGF), isolated as previously described (17), at 30 ng/ml, or fibrinopeptide B (Bachem, Torrance CA.) at 10^{-9} M (18), was used as a positive control for experiments with fibroblasts and astroglial cells; formyl-methionyl-leucyl-phenylalanine (Sigma Chemical Corp., St. Louis, MO.) at 10^{-8} M was the positive control

for experiments involving monocytes. To test whether protamine sulfate would affect the chemotactic activity of BDGF, protamine sulfate (Sigma), 5 ug per ml, was included with BDGF in some experiments.

RESULTS AND DISCUSSION

Fetal bovine ligament fibroblasts have been used in our laboratory for studies of chemotactic activity of PDGF and other ligands (12,16,18). The optimal concentration of PDGF for fibroblast chemotaxis is close to, or identical with, the Kd of PDGF binding to its receptor, 1 nM. For this reason, the chemotactic activity of BDGF was examined at concentrations close to the Kd of BDGF binding to its receptor in responsive cells, 10-20 pM. As shown in Figure 1, BDGF did stimulate cell migration in this concentration range, and the optimal concentration, 200 pg/ml ($\sim$ 12 pM), was almost identical to the Kd of BDGF binding to 3T3 cells (3). The number of cells that migrated in response to BDGF at 200 pg/ml was similar to the number that we have previously observed at the optimal concentrations of PGDF and human fibrinopeptide B (16,18).

To determine whether fibroblast migration to BDGF was directed ("chemotactic") or random ("chemokinetic"), checkerboard analysis was performed. As shown in Table 1, fibroblasts migrated only when the concentration of BDGF in the lower compartment exceeded that in the upper compartment, indicating that migration of fibroblasts to BDGF is in

Table 1. The chemotactic activity of BDGF for fibroblasts

	Upper compartment			
	BDGF (pg/ml)			
	0	50	100	200
Lower compartment				
BDGF (pg/ml)				
50	27* $\pm$ 2.8	5 $\pm$ 1.5	6 $\pm$ 2.1	8 $\pm$ 1.4
100	43 $\pm$ 3.1	27 $\pm$ 2.3	6 $\pm$ 1.7	6 $\pm$ 2.1
200	61 $\pm$ 3.3	50 $\pm$ 2.2	34 $\pm$ 2.1	9 $\pm$ 3.2

* cells per high power field, $\pm$ S.E.M.

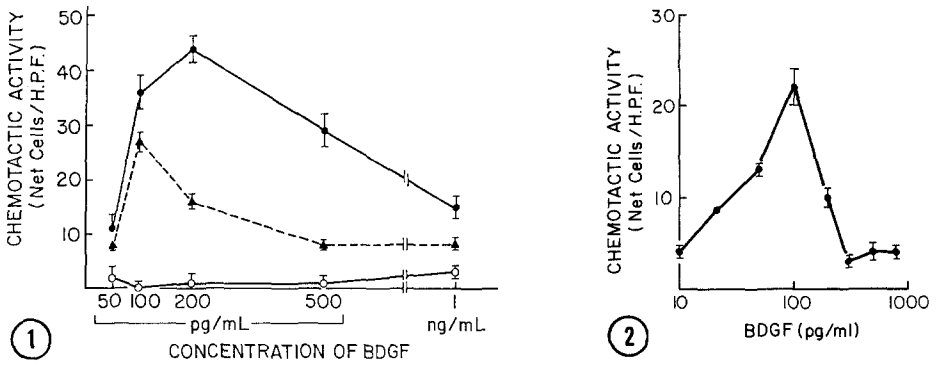

Figure 1. Effect of brain-derived growth factor (BDGF) upon the migration of fibroblasts. BDGF was added to the lower compartments of modified Boyden chambers at the concentrations shown. Fibroblasts, either from fetal bovine ligament (●) or from human skin (▲), were added to the upper compartments. Also shown is the effect of adding protamine, 5 ug/ml, to BDGF in studies with ligament fibroblasts (O). Positive controls using PDGF, 30 ng/ml, or fibrinopeptide B, 10^{-9} M, yielded 40 - 60 cells per H.P.F., as previously reported (16,18). Mean and standard errors.

Figure 2. Astroglial cell migration stimulated by BDGF. The experimental procedure was as described in the legend of Figure 1, except rat astroglial cells were used for the assay. Mean and standard errors.

response to a concentration gradient. Chemokinesis was not observed as fibroblast migration was not stimulated when the the concentration of BDGF was the same in the upper and lower compartments.

The mitogenic activity of BDGF appears not to be species specific (3,8). It was not surprising therefore that BDGF exhibited chemotactic activity toward human skin fibroblasts with an optimal concentration at 100 pg/ml (Figure 1).

Protamine has been shown to be a potent inhibitor of BDGF binding to receptors and BDGF mitogenic activity in responsive cells (3). Figure 1 shows that protamine at 5 ug/ml also inhibits the chemotactic activity of BDGF suggesting that BDGF mediates fibroblast chemotaxis through interaction with BDGF cell surface receptors. The inhibition of BDGF chemotactic activity by protamine is not due to a toxic effect of this compound on cell migration since at this concentration protamine has no effect on the chemotactic activity of PDGF for bovine ligament fibroblasts.

BDGF is a potent growth factor for astroglial cells (3). We therefore investigated the chemotaxis of astroglial cells to BDGF. As shown in Figure 2, BDGF stimulated the migration of astroglial cells, and

the optimal concentration of BDGF for stimulation of migration was found to be 100 pg/ml.

We have previously shown that PDGF is a potent chemotactic factor for neutrophils, monocytes and fibroblasts (15,16). Accordingly, the effect of BDGF on migration of human mononcytes was examined. It was found that BDGF expressed no chemotactic activity for monocytes (data not shown) over the concentration range tested for fibroblast chemotactic activity. The reason for lack of chemotactic activity for this cell type is not known. One possibility is that these cells lack BDGF receptors.

Recently, growth factors from bovine brain have been purified to apparent homogeneity by several laboratories (1,4,7). None of these factors has been reported to be chemotactic for mesenchymal cells although Terranova et. al. (19) have demonstrated chemotactic and chemokinetic activity of endothelial cell growth factor from bovine hypothalamus for bovine aortic endothelial cells, with an optimal concentration of 10^{-9} M. The present communication indicates that BDGF is extraordinarily active as a mesenchymal cell chemoattractant. The physiological function of BDGF is not yet known, but it is of interest to speculate that BDGF from neurons may recruit glial cells during normal development of the central nervous system and in responses to neuronal injury.

ACKNOWLEDGEMENTS

Supported by USPHS grants HL 29594 (R.M.S) and CA 38808 (J.S.H.), and a Grant in Aid, American Heart Association, Missouri affiliate (S.S.H.). J.S.H. is recipient of a Faculty Research Award from the American Cancer Society.

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