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Activity of human, bovine and porcine platelet-derived growth factor in a radioreceptor assay with human placental membrane protein

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Radioiodinated human platelet-derived growth factor (^{125}I-PDGF), and unlabeled human (Hu), bovine (Bo), and porcine (Po) PDGF were used to study PDGF receptors in human term placental membrane preparations. The binding of ^{125}I-Hu-PDGF was inhibited by unlabeled preparations. Half-maximal occupancy of the binding sites occurred at 7–9 nM of Hu-, Bo- or Po-PDGF. Scatchard analysis of the binding data indicated a single class of receptors having a K_d value of about $1.1–1.2 \times 10^{-10}$ M. Thus, the sensitive radioreceptor assay does not detect any species specificity of PDGF.

Platelet-derived growth factor; Membrane protein; Radioreceptor assay; (Human placenta)

1. INTRODUCTION

Platelet-derived growth factor (PDGF) is a protein of M_r 30000, composed of two disulphide-linked subunit chains. The dimer structure of PDGF appears to be important for its biological effects. Human (Hu) PDGF exists as a heterodimer composed of A and B chains of approximately equal size [1]. The B chain is almost identical to part of the v-*sis* oncogene product of simian sarcoma virus (SSV) [2]. Porcine (Po) PDGF exists as a homodimer of B chains [3]. The structure of bovine (Bo) PDGF is not clear as yet [4].

PDGF is a potent mitogen for mesenchymal cells [5,6]. It has been shown that PDGF, like other tissue hormones, exerts its biological effects through interaction with specific, high-affinity receptors associated with its target cells. Recently, the structure of the receptor for PDGF has been described. It contains a single stretch of predominantly hydrophobic amino acids (transmembrane region), an amino-terminal extracellular domain (the ligand-binding region) and an intracellular domain carrying the enzymatic phosphotransferase activity [7]. At 10^{-9} M or less, PDGF binding to the receptors stimulates replication of fibroblasts, glial, endothelial and other connective tissue cells in vitro [8–12]. Here, we show that preparations of human term placenta express high-affinity membrane protein receptors binding Hu-, Bo- and Po-PDGF.

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2. MATERIALS AND METHODS

Hu-PDGF and Bo-PDGF were purified as described [4,13]. The final products were 90% pure as estimated from analytical SDS-polyacrylamide gel electrophoresis. Po-PDGF was purified using ion-exchange chromatography on DEAE-Sephadex A-50 and CM-Sephadex C-50,

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gel filtration on Sephadex G-100 and chromatography on poly(U)-Sepharose. It was 50% pure after the last step of purification (Narczewska and Czyrski, unpublished). Hu- and Bo-PDGF stimulated Balb/c 3T3 and human embryonic fibroblast proliferation in cultures at concentrations of 0.5–1.5 ng/ml (0.05–0.15 nM).

Purified Hu-PDGF (approx. 500000 mitogenic units/ml protein [13]) was labeled with ^{125}I according to Hunter and Greenwood [14]. Briefly, 5 μg PDGF was labeled with 0.5 mCi ^{125}I. ^{125}I-PDGF was separated from unbound radioactivity on a Sephadex G-25 column as described by Heldin et al. [8]. The specific activity was approx. 15000 cpm per ng protein.

Gel electrophoresis of ^{125}I-PDGF was performed according to Laemmli [15], using a gel concentration of 10%. After slicing, gels were assayed for radioactivity in a gamma spectrometer. The mobilities of the M_r markers were determined by protein staining.

Human placental membranes were isolated from fresh-frozen term human placenta by differential centrifugation as described by Hock and Hollenberg [16].

Placental membrane aliquots (200 μg protein) were incubated for 2 h at 37°C with gentle shaking in binding medium (phosphate-buffered saline, PBS; pH 7.2) containing various concentrations of unlabeled Hu-, Bo- or Po-PDGF. The total volume of the binding assay mixture was 200 μl. Experiments were performed in the presence of 0.1% bovine albumin. After 2 h labeled Hu-PDGF (200000 cpm) was added and the binding was continued under the same conditions. Experiments were terminated by rapid centrifugation of the incubation mixture followed by three washes of the pellets with ice-cold PBS containing 1% bovine albumin. Nonspecific binding to membrane protein, estimated from the amount of binding in the presence of a 50-fold molar excess of unlabeled Hu-, Bo- or Po-PDGF, was usually between 5 and 10% of the total binding.

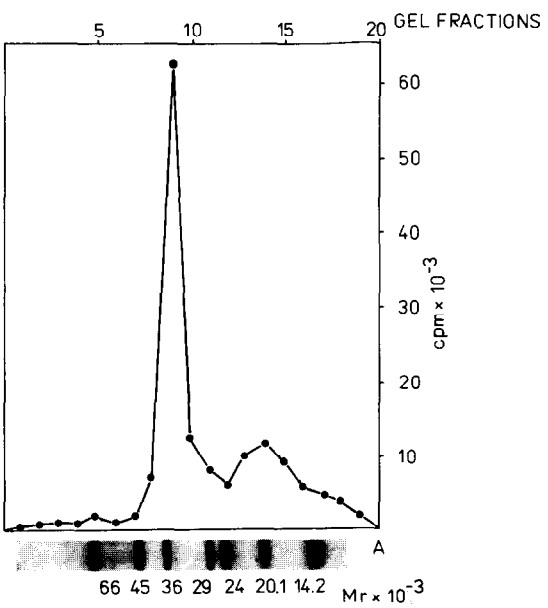

Fig.1. Electrophoresis of ^{125}I-PDGF used as a tracer in the radioreceptor assay (RRA). PDGF was labeled with ^{125}I (Institute of Atomic Energy, Świerk, Poland; 4.32 GBq/ml). 100000 cpm was applied to analytical SDS-polyacrylamide gel electrophoresis under non-reducing conditions. Standard proteins from Serva were used.

3. RESULTS AND DISCUSSION

All fractions obtained after chromatography of

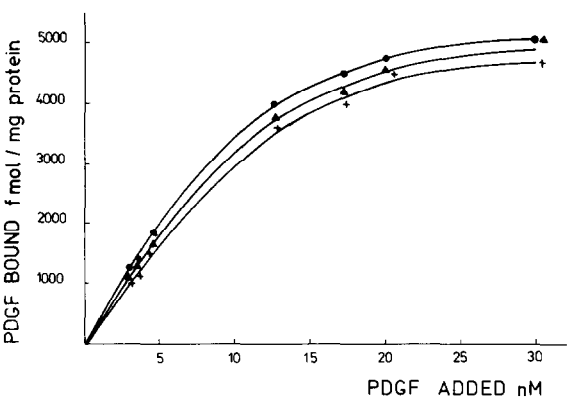

Fig.2. Concentration dependence of ^{125}I-labeled PDGF binding to human placental membrane protein preparations. Membrane proteins were incubated for 2 h at 37°C with various amounts of unlabeled (●) Hu-, (▲) Bo- or (+) Po-PDGF and 15 ng ^{125}I-Hu-PDGF as described in section 2. After washing and centrifugation the membrane-associated radioactivity was determined in a gamma spectrometer (Beckman 4000). Binding has been plotted with correction for the nonspecific binding, which was taken as radioactivity bound in the presence of a 50-fold molar excess of unlabeled Hu-, Bo- or Po-PDGF.

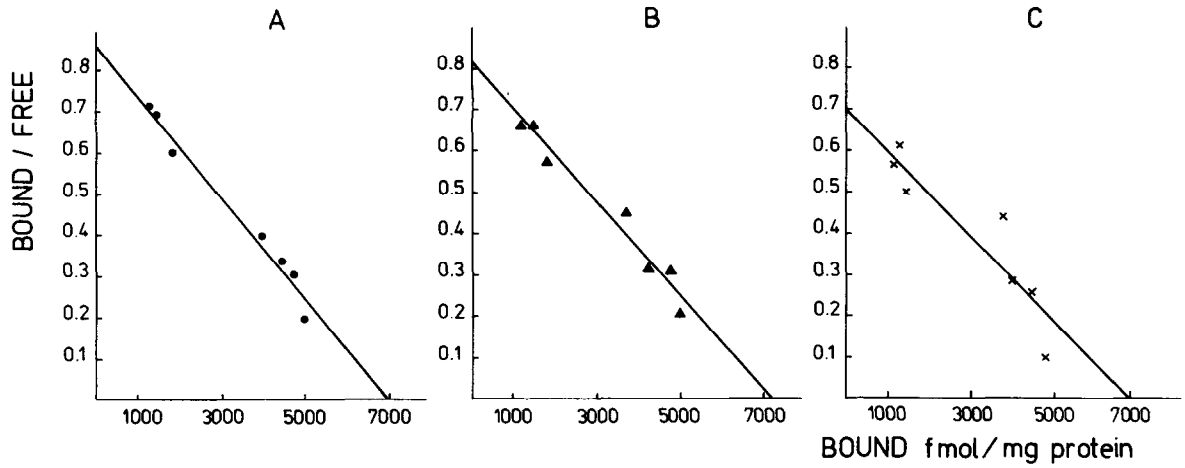

Fig.3. Scatchard plot of the results shown in fig.2, illustrating the binding of ^{125}I-labeled Hu-PDGF to human placental membrane receptors. Preincubation with unlabeled: Hu-PDGF (A), Bo-PDGF (B), Po-PDGF (C).

labeled PDGF on the Sephadex G-25 column were subjected to polyacrylamide gel electrophoresis. We have found that the fractions containing a protein of M_r about 34000 are able to bind to the placental membrane receptors. Fig.1 shows an electrophoresis run of ^{125}I-Hu-PDGF which has been used as tracer in our experiments.

Incubation of the placental membrane proteins with various concentrations of Hu-, Bo- or Po-PDGF demonstrated a saturable binding reaction (fig.2). A Scatchard plot [17] of the binding data indicated a single class of receptors. The apparent K_d values were 1.2×10^{-10} M for Hu- and Po-PDGF and 1.1×10^{-10} M for Bo-PDGF. 1 mg of the receptor protein was shown to bind approx. 7000 fmol PDGF (fig.3A–C).

Binding of ^{125}I-Hu-PDGF was a saturable reaction. Scatchard [17] analysis at equilibrium showed only one class of high-affinity binding sites. The binding of ^{125}I-Hu-PDGF to receptors was inhibited by unlabeled Hu-, Bo- or Po-PDGF. The results suggest that the term human placenta is a rich source of PDGF receptors for the estimation of free PDGF in blood preparations, tissue fluids, cell extracts, etc. Anionic fractions of the platelet lysate containing transforming growth factor β [4,18] or human interferon α did not compete with ^{125}I-Hu-PDGF for receptor occupancy even at large excess of the preparations (not shown).

We could not determine which cells of the placenta possess the receptors for PDGF. The receptors are probably expressed on the surface of different cells as suggested by others [10–12] but their structure appears to be similar.

The placental membrane receptors do not discriminate between Hu-, Bo- or Po-PDGF.

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