Vol. 122, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 18, 1984 Pages 230-236

STIMULATION OF PLASMINOGEN ACTIVATOR IN OSTEOBLAST-LIKE CELLS BY BONE-RESORBING HORMONES

J.A. Hamilton*, S.R. Lingelbach*, N.C. Partridge and T.J. Martin

*University of Melbourne, Department of Medicine
Royal Melbourne Hospital, Melbourne, Australia

University of Melbourne, Department of Medicine
Repatriation General Hospital, Heidelberg, Australia

Received May 28, 1984

SUMMARY: Hormonal control of plasminogen activator (PA) was studied in clonal rat osteogenic sarcoma cells which are phenotypically osteoblast, and in osteoblast-rich rat bone cell cultures. The bone-resorbing hormones (parathyroid hormone, prostaglandin E_2, epidermal growth factor and 1,25-dihydroxyvitamin D_3) stimulated PA activity in both cell types. The relative efficacies of vitamin D metabolites and of prostanoids reflect their relative potencies as stimulators of bone resorption.

The evidence (1) that collagenase is synthesized primarily in osteoblasts of mouse bone in response to parathyroid hormone (PTH) led to the hypothesis that osteoblast production of collagenase might be an initiating event in bone resorption, leaving mineral to be phagocytosed by monocytes and osteoclasts (1). Collagenase produced by bone in vitro has been shown to be almost entirely in the latent form (1,2) requiring activation, for example by other proteolytic enzymes. Plasmin, the protease formed after the activation of plasminogen by plasminogen activator (PA), can activate latent collagenase (3) and degrade connective tissue directly (4). Furthermore, the activity of the PA/plasmin proteolytic enzyme system has been correlated in a number of cases with connective tissue turnover and remodelling (5). Functional receptors or responses for the major bone-resorbing hormones have been demonstrated in osteoblasts (6-9). It is reported here that the major bone-resorbing hormones

0006-291X/84 $1.50

stimulate the PA activities of the rat osteogenic sarcoma cell line, UMR 106-01, and of osteoblast-rich calvarial cells.

METHODS

The UMR 106-01 cell line was derived from a clonal osteogenic sarcoma line, UMR 106 (7,10) and has very similar properties. The parent line is enriched in the osteoblast phenotype, including adenylate cyclase responsiveness to PTH and prostaglandins, high alkaline phosphatase activity and a cytosol receptor for 1,25-dihydroxyvitamin D_3 (1,25 $(OH)_2D_3$), and is believed to be a suitable model for the study of hormonal regulation of the osteoblast (6-10). Experiments reported in this paper with subclone UMR 106-01 were carried out on cells between the 7th and 20th passages. Normal osteoblast-rich populations of cells were prepared from newborn rat calvaria as described (6) and used at the first subculture passage.

All cells were cultured in Eagles MEM containing non-essential amino acids, Hepes (20 mM), gentamycin (80 mg/l) and 5% fetal bovine serum (FBS) (Flow Laboratories). Cells were plated at 2 x 10^4 cells/0.2 ml in ^{125}I- fibrin-coated wells of 96-well tissue culture plates prepared as previously described (11). After incubation for 20 h in complete medium, the cultures were washed twice with phosphate buffered saline, pH 7.4 (PBS), and placed in Eagles' MEM containing 0.1% bovine serum albumin (BSA) (fatty acid free) (Sigma Chemical Co.) in the presence or absence of human plasminogen (8 µg/ml) (12) to measure the plasminogen-dependent fibrinolytic (or PA) activity. As indicated, BSA was replaced in some experiments, by 5% acid-treated FBS, from which the plasminogen had been removed by affinity chromatography (12,13); this procedure also allows the plasminogen-dependence of the fibrinolytic activity to be assessed, the acid treatment removing protease inhibitors from the FBS (14). Aliquots (50 µl) of the supernatant medium were taken from triplicate cultures at 20 h and assayed for solubilized radioactivity as a measure of the fibrinolytic activity (11). The activity is expressed as a percentage of the total cpm released from the plate by trypsin (60-80,000 total cpm per well for the experiments in this paper).

Sources of prostaglandins, vitamin D metabolites, synthetic human PTH (hPTH (1-34), epidermal growth factor, and bovine PTH (1-84) were as previously described (6-10).

RESULTS

The PA activity of the UMR 106-01 cells treated with several agents is illustrated in Fig. 1. There was PA activity in the untreated cells, which was increased considerably by PGE_2 (10^{-7}M) and by synthetic hPTH (1-34) (3 x 10^{-8}M), and to a lesser extent by epidermal growth factor (EGF) (1.7 x 10^{-9}M). EGF was no more active at 1.7 x 10^{-8}M than at 1.7 x 1^{-9}M but showed some activity even at 1.7 x 10^{-10}M (data not shown). PTH, PGE_2 and EGF all stimulate bone resorption in organ cultures of rat or mouse bone,

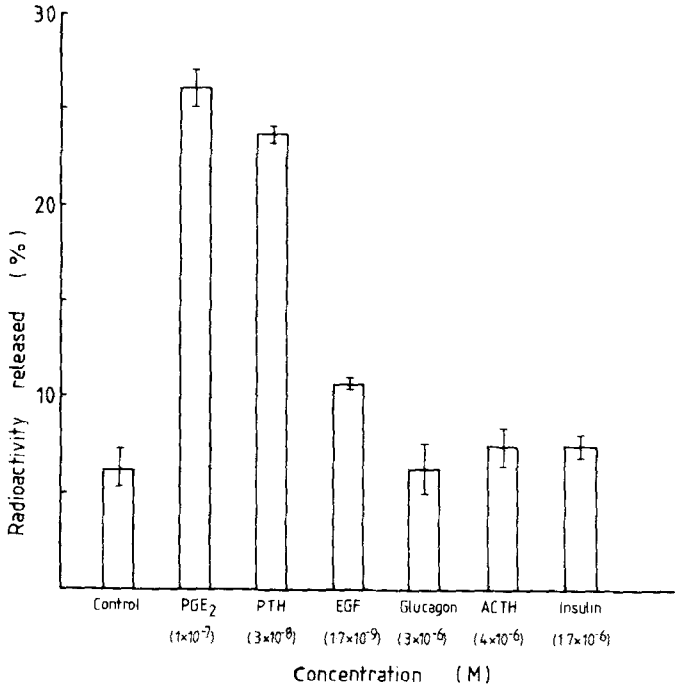

Figure 1 PA activity of UMR 106-01 cells in the presence of bone-resorbing hormones. Cells were plated and PA activity measured as in Methods with the medium containing BSA. The plasminogen-independent fibrinolytic activity was < 5% of the plasminogen-dependent (i.e. PA) activity. Mean values ± SEM are reported for a typical experiment.

and the osteogenic sarcoma cells possess receptors and/or specific responses for all of them (6-10). On the other hand, glucagon, adrenocorticotrophin (ACTH) and insulin, which do not resorb bone, had no effect on PA activity even at high concentrations.

PGE_2, which is the most potent bone resorbing prostanoid (15), was the most active of the prostanoids tested in elevating the PA activity (Fig. 2a). Data from a representative experiment are presented; in other experiments, 6-keto PGF_1 and 13,14-dihydro-15 keto-PGE_2 were inactive at 10^{-7}M. $1,25(OH)_2D_3$ is also a potent bone-resorbing hormone (16), and it too stimulated the PA activity of the UMR 106-01 cells at quite low concentrations (Fig. 2b). The effects are shown also of 25-hydroxyvitamin D_3 ($25(OH)D_3$), 24 R,25-dihydroxyvitamin D_3 ($24R,25(OH)_2D_3$), and

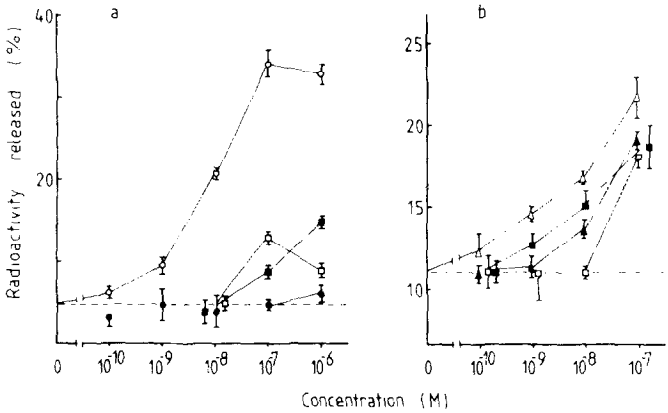

Figure 2 PA activity of UMR 106-01 cells in the presence of prostaglandins and vitamin D_3 analogues. The same experimental protocols and data presentation as in Fig. 1. For Fig. 2a, the following prostaglandins were tested: PGE_2 (○), $PGF_{2\alpha}$ (●), 6-keto-$PGF_{1\alpha}$ (□) and 13,14-dihydro-15-keto-PGE_2 (■). For Fig. 2b, the following vitamin D_3 analogues were tested in a separate experiment: $1,25(OH)_2D_3$ (△), $1,24,25(OH)_2D_3$ (■), $25(OH)D_3$ (▲) and $24,25(OH)_2D_3$ (□). Control PA values for untreated cells are denoted as (---) in the two experiments. The plasminogen-independent fibrinolytic activities in both experiments were < 5% of the plasminogen-dependent activities.

1, 24,25 tri-hydroxyvitamin D_3 ($1,24,25\ (OH)_3D_3$). The relative potencies of vitamin D metabolites in stimulating the PA activity of the osteosarcoma cells resemble closely their *in vitro* bone resorbing activities (16) and their ability to bind to the $1,25(OH)_2D_3$ receptor (7). Kinetic studies indicate that the elevated PA activity in response to PTH, PGE_2 and $1,25\ (OH)_2D_3$ was first detected between 4 and 8 hours at 37°C (data not shown).

The above studies were carried out with the UMR 106-01 tumour cells. In Fig. 3, it is shown that osteoblast-rich calvarial cells also respond to low concentrations of PTH, PGE_2, and $1,25(OH)_2D_3$ with an increase in PA activity. EGF was also stimulatory in the concentration range 1.7×10^{-11}M to 1.7×10^{-9}M (data not shown). The PTH used for the experiment in figure 3a was bovine PTH (1-84) but similar results were obtained with hPTH (1-34).

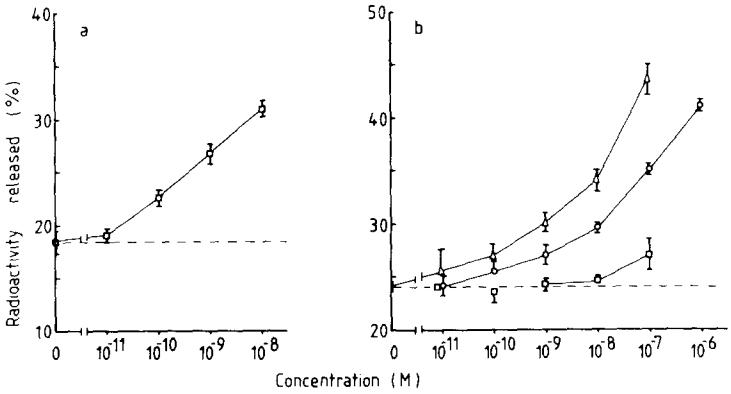

Figure 3 PA activity of osteoblast-rich calvarial cells in the presence of bone-resorbing hormones. Osteoblast-rich calvarial cells were plated and the PA activity measured as in Methods, with medium containing acid-treated, plasminogen-depleted FBS. Means ± SEM are reported for the following treatments: a, bovine PTH (1-84); b, PGE_2 (o), $1,25(OH)_2D_3$ (Δ), $24,25(OH)_2D_3$ (□).

DISCUSSION

The above results show that bone-resorbing hormones increase the PA activity of osteoblast-like cell populations at concentrations comparable with those that cause bone resorption in vitro. The hormones studied in this work have different mechanisms for their initial actions. PTH and PGE_2 act by stimulating adenylate cyclase (17), the active vitamin D metabolite acts as a steroid hormone, and EGF through a cell surface receptor and non-cyclic AMP-dependent pathways. The similar effects of these three classes of agents on PA production by osteoblast-like cells indicates that at some later point their cellular actions may converge. These mechanisms are currently under investigation.

Thus osteoblast-like cells can be included among several cellular systems where PA activity can be modulated by hormones (5,14,18). In a number of these cases a correlation has been found between the expression of PA activity and the processes of connective tissue turnover and remodelling and cell migration.

Examples of these are granulosa cells at the time of ovulation (19), breast cells during resorption (20), and trophoblasts at the time of implantation (21). Plasmin can degrade cartilage (4) and basement membrane (22) as well as activate latent collagenase. The latter property has been demonstrated, for example, in the conditioned medium from cultures of rheumatoid tissue (3). A similar activation could occur in bone, and provide a basis for the proposed collagenase contribution to resorption (1). Virtually all of the collagenase activity in media from bone explants appears to be in a latent form (1,2), and therefore requires activation, presumably by another proteinase. Activation by locally-generated plasmin, whose activity in turn would be determined by the regulation of PA levels in the bone cells by the appropriate hormonal stimulus, might achieve this. Moreover, plasmin could act on the noncollagenous matrix or tissue components before mineral removal and collagen degradation, and might complete the degradation of collagen which has been cleaved into large fragments by specific collagenase. Such a proposal has been considered previously for the involvement of lysosomal enzymes (23) ; however, neutral proteinases, such as plasmin, which function optimally at the pH of the extracellular milieu, are likely appropriate contributors to these extracellular events.

The above findings are consistent with a role for osteoblasts in contributing to resorption of bone matrix under the control of various hormones (1). Such a role would complement that proposed previously (24), in which the osteoblast is suggested to control the function of the osteoclast and thus have a pivotal role in the control of the bone resorption process.

Acknowledgement This work was supported by grants from the National Health and Medical Research Council of Australia and the Anti-Cancer Council of Victoria.

REFERENCES

1. Sakamoto, S., and Sakamoto, M. (1982) J. Periodont. Res. 17, 523-526.
2. Vaes, G.(1971) Biochem. J. 123, 23P-24P.
3. Werb, Z., Mainardi, M.D., Vater, C.A., and Harris, E.D.Jr. (1977) New Eng. J. Med. 296, 1017-1023.
4. Lack, C.W., and Rogers, H.J. (1958) Nature (Lond) 182, 948-949.
5. Mullins, D.E., and Rohrlich, S.T. (1983) Biochim. Biophys. Acta 695, 177-214.
6. Partridge, N.C., Alcorn, D., Michelangeli, V.P., Kemp, B.E., Ryan, G.B., and Martin T.J. (1981) Endocrinology 108, 213-219.
7. Partridge, N.C., Frampton, R.J., Eisman, J.A., Michelangeli, V.P., Elms, E., Bradley, T.R., and Martin, T.J. (1980) FEBS Lett. 115, 139-142.
8. Livesey, S.A., Kemp, B.E., Re, C.A., Partridge, N.C., and Martin T.J. (1982) J. Biol.Chem. 257, 14983-14988.
9. Ng, K.W., Partridge, N.C., Niall, M., and Martin T.J. (1983) Calc. Tissue. Int. 35, 298-303.
10. Partridge, N.C., Alcorn, D., Michelangeli, V.P., Ryan, G. B., and Martin T.J. (1983) Cancer Res. 43, 4308-4314.
11. Hamilton, J.A., Zabriskie, J.B., Lachman, L.B., and Chen, Y-S. (1982) J. Exp. Med. 155, 1702-1718.
12. Deutsch, D.G., and Mertz, T. (1970) Science 170, 1095-1096.
13. Hamilton, J.A., and Slywka, J. (1981) J. Immunol. 126, 851-855.
14. Vassalli, J-D., Hamilton, J.A., and Reich, E. (1976) Cell 8, 271-281.
15. Raisz, L.G., and Martin T.J. (1984) In "Bone and Mineral Research" ed. W.A. Peck (Publ. Elsevier) Vol. 2, 286-310.
16. Raisz, L.G., Trummel, C.L., Holick, M.F., and DeLuca, H.F. (1972) Science 175, 768-769.
17. Klein, D.C., and Raisz, L.G. (1971) Endocrinology 89, 818-826.
18. Strickland, S., and Beers, W.H. (1976) J. Biol. Chem. 251, 5694-5702.
19. Beers, W.H., Strickland, S., and Reich, E. (1975) Cell 6, 387-394.
20. Ossowski, L., Beigel, D., and Reich, E. (1979) Cell 16, 929-940.
21. Strickland, S., Reich, E., and Sherman, M.J. (1976) Cell 9, 231-240.
22. Beers, W.H. (1975) Cell 6, 379-386.
23. Eilon, G., and Raisz, L.G. (1978) Endocrinology 103, 1969-1975.
24. Rodan, G.A., and Martin, T.J. (1981) Calc. Tissue Int. 33, 349-351.