ENZYMATIC MODIFICATION OF BOVINE AND OVINE PARATHYRIN RECEPTOR

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1. Introduction

Receptor assay using labelled parathyrin (PTH) allowed so far only binding studies in the unphysiological μg-range [1–3]. This is probably due to chemical and conformational changes of the labelled hormone with consequent rapid degradation at the receptor site. Labelling of peptide hormones with the most commonly used isotope ^{125}J even under 'mild conditions' grossly alters the physicochemical state of the respective hormones. Receptor studies using ^{125}J labelled hormones reflect a physicochemical behaviour of these hormones at the receptor site but not necessarily a physiological function inherent to the unlabelled hormone. We have described recently a new method of a receptor assay using labelled antibodies instead of labelled hormone [4–7] with a true sensitivity of 1000–2000 fg of bovine (bPTH)/100 μg renal plasma membrane protein. Herein we present data on characteristics of the PTH receptor using enzyme treatment.

2. Materials and methods

The labelled-antibody-membrane-assay (LAMA) was performed as described [4,5]. Antibody AVII-3 (kindly provided by Dr. Bouillon. Rega Institute Leuven, Belgium) was labelled with ^{125}J after extraction onto UG-bPTH-Immunoadsorbent (ImAd). UG-bPTH refers to bPTH (Sephadex G-75 purified bPTH from Hormon-Chemie, München) chromatographed on Ultrogel AcA 54 (LKB, Gräfelfing), column size 2.5 × 100 cm, to yield highly purified bPTH (1500-2000 U/mg). bPTH standard was the preparation 71/324 from the MRC, Mill Hill, London, exhibiting high biological activity [5]. In all studies with LAMA a constant concentration of bPTH (12.5 ng/ml) was used. Chick kidney plasma membranes (from 18–20 weeks old animals) were prepared following essentially the technique of Fitzpatrick et al. [8], membrane purity was controlled by electron microscopy and protein was estimated by a biuret-method (Boehringer, No. 15899). Membranes were stored at −60°C and were found to be stable (bPTH binding and preservation of adenylate cyclase activity) for at least 2 months. Bovine membranes were prepared from homogenates of freshly free dissected renal cortex at 4°C. Trypsin (Cat. No. 15330), phospholipase A (Cat. No. 15057), phospholipase D (Cat. No. 15345), Neuraminidase (Cat. No. 15434) and Galactosidase (Cat. No. 15079) were from Boehringer Mannheim. Duplicates of 150 μg renal plasma membrane protein were treated with different concentrations of the respective enzyme dissolved in Albano-buffer [4] as indicated. After incubation over 40 min at 37°C membranes were washed twice in Albano-buffer [4] and LAMA performed as described. For control membranes were incubated in buffer only before assay. Results are expressed as percentage increase of receptor bound hormone detected by labelled antibody over background (plasma membranes in the absence of bPTH standard addition with and without prior enzyme treatment) ∓ SEM. Enzyme treated membranes did not alter background binding of labelled antibody apart from trypsin due to protein denaturation. bPTH control binding in untreated membranes was set as 100% [4,5].

3. Results and discussion

Cuatrecasas has recently summarized studies on the enzymatic modification of insulin and glucagon receptors [9]. Such treatment indicates the importance of distinct membrane constituents to bind hormones. Our results on enzyme treatment of bovine and ovine renal plasma membranes (Fig.1) suggest species related structural differences of the cell surface. Trypsin at low concentrations reduced and in higher concentrations abolished bPTH binding to both ovine and bovine membranes. This is in agreement with Chase who has recently reported on the selective proteolysis of the receptor for PTH whereas the receptor for calcitonin and prostaglandin E_1 was not altered [10]. These results were, however, derived from PTH mediated increase of 3,5'-cAMP in renal cortex in contrast to the direct effect

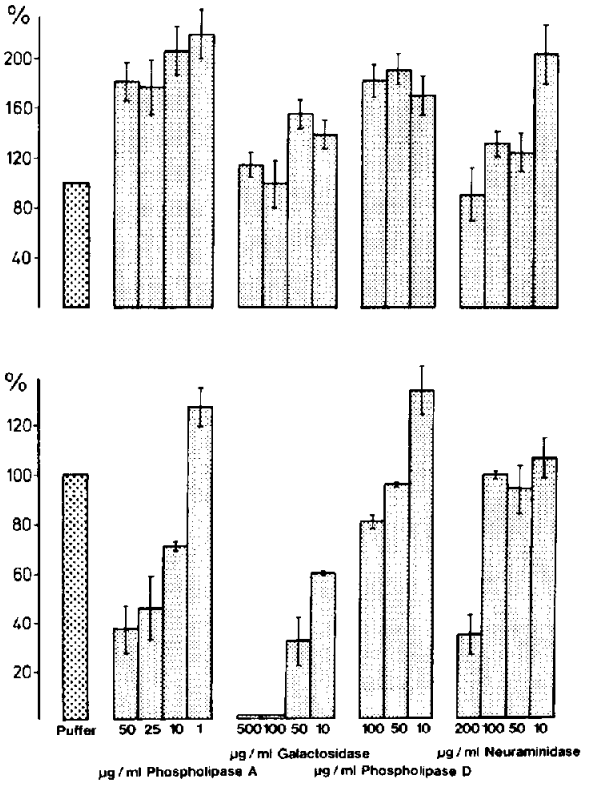

Fig.1. Effect of pretreating ovine (upper part of the figure) and bovine plasma membranes (lower part) with increasing concentrations of enzymes.

on binding demonstrated by our results. Similar effects of trypsin have been noted on insulin and glucagon receptors. Phospholipase A in low concentration of 1.0 µg/ml increased binding of bPTH to bovine membranes by 27% but much more to ovine membranes, by 80%. 10–50 µg/ml of phospholipase A diminished binding in bovine membranes drastrically, whereas in ovine membranes binding was still increased from 70–230% over control without prior enzyme treatment. The effect of phospholipase D treatment shows an essentially similar pattern to that of phospholipase A in membranes of both species. Therefore, the bovine receptor for bPTH bears a strong resemblance to the glucagon receptor in liver membranes whereas the ovine receptor for bPTH resembles the insulin receptor. Phospholipase treatment of ovine membranes exposes new bPTH binding sites which are probably freely exposed to the aqueous environment. In bovine membranes, however, phospholipids form an essential constituent of the receptor to maintain the binding conformation as emphazised recently for glucagon by Rubalcava and Rodbell [11].

Galactosidase in concentrations from 10–50 µg/ml increases bPTH binding to ovine membranes, but higher doses had no effect, whereas in bovine membranes galactosidase treatment causes a profound fall in hormone binding. Galactose groups and hence glycoproteins may be of crucial importance in the bovine bPTH receptor similar to the insulin receptor, but galactose seems only a minor constituent of the glycoprotein composition in ovine membranes. Neuraminidase has only in the high concentrations of 200 µg/ml a depressive effect of bPTH binding in bovine receptor indicating that gangliosides are not critical in the glycolipid structure. Again, a similarity to the insulin receptor is evident in contrast to ovine membranes where neuraminidase increases binding mainly at low concentrations. Our results clearly show that the structural requirement and the overall conformation of the bPTH receptor is quite different between ovine and bovine species. The glycoprotein nature of bovine receptor and the critical importance of phospholipids for bPTH binding are evident. This result may possibly be relevant in the interpretation of species differences described for the interaction of bPTH with receptor preparations from different species [12–14].

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References

[1] Heath, D. A. and Aurbach, G. (1974) Excerpta Medica International Congress Series No. 346, Elsevier Amsterdam p. 159.
[2] Sutcliffe, H. S., Martin, I. J., Eisman, J. A. and Pilczyk, R. (1973) Biochem. J. 134, 913.
[3] Di Bella, F. P., Donsa, I. P., Miller, S. S. and Arnaud, C. D. (1974) Proc. Natl. Acad. Sci. USA 71, 723.
[4] McIntosh, C. H. S. and Hesch, R.-D. (1975) Biochim. Biophys. Res. Commun. 64, 376.
[5] McIntosh, C. H. S. and Hesch, R.-D. (1976) Biochem. Biophys. Acta 426, 535.
[6] Jüppner, H. and Hesch, R.-D. (1976) Biochim. Biophys. Res. Commun., in press.
[7] Hesch, R.-D. and Jüppner, H. (1976) Vth ICE, Hamburg, abstract.
[8] Fitzpatrick, D. J., Davenport, G. R., Forte, L. and Laudon, E. J. (1969) J. Biol. Chem. 244, 3561.
[9] Cuatrecasas, P. (1974) Ann. Rev. Biochem. 43, 169.
[10] Chase, L. (1975) Endocrinology 96, 70.
[11] Rubacalva, B. and Rodbell, M. (1973) J. Biol. Chem. 248, 3831.
[12] Martin, I. J., Vakakis, N., Eisman, J. A., Livesey, S. J. and Treagear, G. W. (1974) J. Endocr. 63, 369.
[13] Chu, L. L. H., Forte, L. R., Anast, C. S. and Cohn, D. V. (1975) Endocrinology 97, 1014.
[14] Goltzman, D., Peytremann, A., Callahan, E. N., Segre, G. V. and Potts, Jr., J. (1976) J. Clin. Invest. 57, 8.