INHIBITION OF PTH RECEPTOR BINDING AND PTH MEDIATED ADENYLATE CYCLASE ACTIVITY BY SOMATOSTATIN

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SUMMARY: This communication describes the inhibiting influence of Somatostatin on the binding of bovine parathyroid hormone (bPTH) to the receptor of a target organ using the labelled antibody method and a partially purified chicken renal membrane preparation with a high affinity for bPTH. bPTH binding to the receptor was diminished up to 47 % in the presence of Somatostatin Ala-1 (0.2 - 2000 ng/tube) while the modified Somatostatin Tyr-1 was without any effect. Similar inhibition of biological activity was also found in an adenylate cyclase assay with both peptides. Comparing the amino acid sequences of bPTH and Somatostatin Ala-1 two identical residues could be identified. Conclusion: Somatostatin and bPTH exhibit similar affinity for the ovine receptor because of the identical first amino acid which is essential for initial binding.

INTRODUCTION: Attemps have been made to discover the structural and conformational requirements of PTH for binding to specific receptor sides in the cell membrane of target tissues (1, 2, 3) and for stimulation of the adenylate cyclase with intact hormone and hormone fragments (4,5). Fragments of N-terminal bPTH having no biological activity were shown to inhibit bPTH stimulated adenylate cyclase. A minimum sequence of bPTH-(1-27) with biological potency was identified. For receptor binding the continous sequence from residue 3 to 27 was considered to be necessary (4, 5). To further elucidate these results we have applied our recently developed receptor assay (labelled antibody membrane assay LAMA (6, 7))which in contrast to other methods (1, 2, 3) avoids deleterious iodination of the hormone and allows the specific measurement of bPTH in the physiological pg-range.

MATERIALS AND METHODS:

Standard and labelled antibody

The standard preparation of bPTH (71/324) was generously provided by the MRC London. CMC-Immunoadsorbent (ImAd) was prepared according to Addison et al. (8). Iodination and elution of iodinated antibodies was as described recently (6, 7). The antibody against bovine PTH (antibody A-VIII) reacting primarily with the C-terminal region of bPTH was kindly supplied by Dr. Bouillon, Rega Institut, Leuven, Belgium. Highly purified bPTH (Calbiochem Lot No 400150) was used for the stimulation of AC. In LAMA Calbiochem PTH exhibited parallism to standard 71/324 and 25 % of its biopotency. Somatostatin Ala-1 and Tyr-1 were obtained from Serono, Freiburg, Germany. For the determinations of cAMP the "Cyclic AMP Assay Kit" was used following the instructions of the Radiochemical Centre, Amersham, England.

Preparation of Renal Plasma Membranes and Protein Estimations

Plasma membranes were prepared by the method of Fitzpatrick et al. (9). Kidneys from 15 weeks old chicken were rapidly removed after killing by cervical dislocation and immediately placed in ice-cold 0.25 M sucrose containing 1 mM EDTA. All subsequent procedures were carried out at 4°C. The kidneys were minced with scissors and homogenizied in a Potter Elvejhem teflon-glass homogenizer. Homogenization was carried out in 4 volumens of ice-cold 0.25 M sucrose + 1 mM EDTA. All subsequent procedures were as described by Fitzpatrick et al. (9). The final membrane pellets were homogenized in 50 mM Tris HCl buffer pH 7.4 containing 2 % human serum albumin (HSA) and stored at -60°C in multiple aliquots until use. Protein was measured by Biuret method (Boehringer Best. Nr. 15899, Mannheim, Germany).

Labelled antibody membrane assay (LAMA)

This assay has been described in detail elsewhere(6, 7). The incubation with bPTH or bPTH and Somatostatin together was carried out for 2 h at 24°C in a 50 mM Tris HCl buffer pH 7.4 containing 2 % HSA and began after addition of the membrane preparation (150 µg/tube). The second incubation with labelled antibody was for one hour at 24°C in the same buffer. Data are expressed as percentage increase (Control) of receptor bound hormone over unspecific binding (radioactivity bound to receptor in the absence of bPTH = Zero ± SEM). Control was set as 100 % and all results compared to this value. These conditions were found to be optimal using chicken membranes instead of bovine membranes described recently (6).

Adenylate Cyclase Assay

Adenylate cyclase was measured by a slightly modified method of Albano et al. (10). Assay mixture consisted of the following components in a final volume of 370 µl:

200 µl of a 92.5 mM Tris HCl buffer pH 7.4 containing 18.5 mM NaCl, 5.55 mM $MgCl_2$, 18.5 mM KCl, 11.1 mM Theophyllin and 3.7 mM ATP. 20 µl of an ATP-regenerating system containing creatine kinase (0.5 mg/0.1 ml) and creatine phosphate (0.3 mM),

test hormones or substances were added in a volume of 100 µl. The mixture was preincubated for 5 min at 37°C and the reaction started by addition the membrane preparation (~300 µg) with further incubation for 15 min at 37°C and termination by boiling for 3 min. All samples were stored at -20°C until assayed for cAMP.

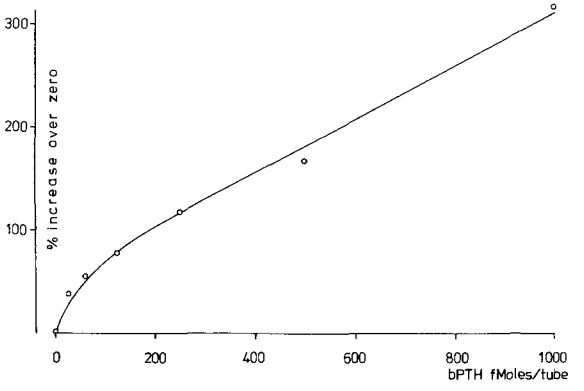

Fig. 1 Standardcurve for binding of bPTH to chicken renal plasma membranes under optimized conditions

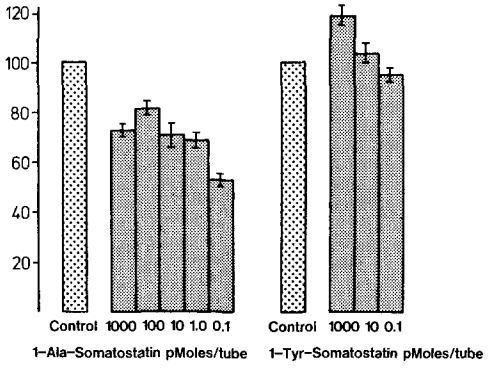

Fig. 2a, b The constant concentration of 265 fmol bPTH/tube was incubated with increasing amounts either of 1-Ala-Somatostatin (a) or 1-Tyr-Somatostatin (b)

RESULTS AND DISCUSSION: With chicken renal plasma membrane a highly sensitive standard curve for bPTH under optimized

assay conditions could be achieved over a range of 8.3 - 1060 fmol/tube (Fig. 1) in accord with the high affinity of these membranes for bPTH (7, 11). For subsequent experiments a constant concentration of 265 fmol/tube was used. Somatostatin Ala-1 (0.1 - 1000 pmol/tube) and bPTH were incubated with the membrane preparation (Fig. 2a). Compared to control tubes (bPTH incubated without Somatostatin) which showed an increase of bound radioactivity of 266 % over zero the decrease caused by Somatostatin Ala-1 was 47 - 19 %. Unphysiologically high concentrations of the inhibiting peptide were less effective than the lowest amount of 0.1 fmol/tube, nevertheless the decrease of radioactivity bound to the receptor was statistically significant ($p < 0.01$) at each concentration of Somatostatin Ala-1. Somatostatin Tyr-1 at the same concentrations did not inhibit the binding of bPTH to the membranes at all, but significant increase was seen at 1000 pmol/tube (Fig. 2b).

In contrast to the effect on receptor binding both Tyr-1 and Ala-1 Somatostatin had a similar effect on the activation of adenylate cyclase. Tab. 1 portrays the enzyme response to 2.0 nmol/tube bPTH and the dramatical decrease with various concentrations of Ala-1 and Tyr-1 Somatostatin. With Somatostatin Ala-1 maximal inhibition of 71 % was found when adding 20.0 nmol to the incubation medium compared to control tubes incubated with bPTH only. In the absence of exogenous bPTH even the basal levels of adenylate cyclase was diminished by 8 - 81 % in the presence of Somatostatin Ala-1. In contrast to the effect in LAMA Tyr-1 Somatostatin exhibited a smaller but definite reduction in bPTH stimulated adenylate cyclase, whereas the basal activity was not influenced. 2.0 nmol Somatostatin Ala-1 led to 9 % reduction of adenylate cyclase, whereas 10 nmol Somatostatin Tyr-1 were necessary to get the same reduction. In small concentrations Somatostatin Ala-1 and Tyr-1 led to a small but significant increase of bPTH stimulated adenylate cyclase.

Very little is known about initial steps in hormone action. Derivatives with antagonist properties were shown to be very useful to analyse the moleculare nature of the interactions between bPTH and renal cell membranes (4, 5). A minimal sequence

Table 1

addition	Adenylate cyclase activity (pmol cAMP/mg protein/15 min)	
	basal activity (testsubstances only)	stimulated cyclase (bPTH and test substances)
a) 2.0 nmol bPTH/tube + increasing amounts of Somatostatin Ala-1	135.2	452.5
0.2	125.6	476.7
2.00 nmol/tube	103.9	411.4
20.0	25.7	135.0
b) 2.0 nmol bPTH/tube + increasing amounts of Somatostatin Tyr-1	87.8	585.5
0.1	80.9	601.2
1.0 nmol/tube	85.1	563.3
10.0	75.9	527.2

Table 1a,b Basal and bPTH (2.0 nmol/tube) stimulated adenylate cyclase activity was measured after addition of increasing amounts either of 1-Ala-Somatostatin (a) or 1-Tyr-Somatostatin (b)

of bPTH was identified by the use of analogues devoid of biological activity in the adenylate cyclase assay but with the ability to displace intact hormone. Progressive shortening of the active bPTH-(1-34) from the COOH-terminus results in the peptide with the continuous sequence (1-27) which is the minimal lenght for stimulation of adenylate cyclase. The deletion of the first two residues of the N-terminus leads to a complete

loss of adenylate cyclase activation. These studies were performed using high concentrations of bPTH or derivatives in the µmol-range. The conditions used may, therefore, not be relevant under physiological circumstances. The application of our receptor assay (LAMA) allows, however, the study of interactions of bPTH competing substances at physiological concentrations of unlabelled hormone. The importance of the first amino acid on receptor binding could not yet be directly determined using measurement of adenylate cyclase activity. Our results indicate that Somatostatin Ala-1 competes significantly with bPTH at the receptor side at low concentrations. High concentrations of Somatostatin Ala-1 may be less effective due to steric hindrance. Thus the amino acid Alanine at position 1 is considered to be essential not only for activating of adenylate cyclase (4, 5) but also for receptor binding. The failure of Somatostatin Tyr-1 to diminish the binding of bPTH strengthens these conclusions convincingly.

In contrast to LAMA Somatostatin Tyr-1 has a definite but smaller inhibitory influence on bPTH stimulated adenylate cyclase activity compared to Somatostatin Ala-1. These results seem to indicate that in bPTH Alanine in position 1 may be of even more critical importance for specific receptor binding than for activation of adenylate cyclase. Moreover, receptor binding discriminates to a high extend between PTH of different species since hPTH (with Serine in position 1) exhibits lower affinity to this receptor as bPTH (12). Serine and Tyrosine are hydrophilic, Alanine is hydrophobic which may be important for conformational requirements. Only Somatostatin Ala-1 was able to reduce basal levels of adenylate cyclase activity indicating a more intrinsic effect on this enzyme in our particular membrane preparation. Since Somatostatin seems to be accumulated locally in the kidney (13) like for example in the pancreas a possible interaction at the membrane side may be of physiological importance.

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