GENERATION OF A CARBOXYL-TERMINAL FRAGMENT OF BOVINE PARATHYROID HORMONE BY CANINE RENAL PLASMA MEMBRANES

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SUMMARY

Incubation of ^{125}I-bovine parathyroid hormone with purified canine renal plasma membranes resulted in the generation of a carboxyl-terminal fragment of the labelled hormone. This fragment appears similar to that found previously in the human and bovine circulation and in the canine circulation after infusion of intact bovine parathyroid hormone.

Heterogeneity of parathyroid hormone (PTH)* in the circulation was initially suggested by Berson and Yalow (1). Habener and colleagues first demonstrated the presence of intact hormone and a carboxyl-terminal fragment of the hormone in the human and bovine circulation (2-4). Multiple forms of PTH in human serum were also found by Canterbury and Reiss (5), and Arnaud and colleagues (6). Since PTH appears to be secreted mainly in its intact 9500MW form (2), it is likely that the carboxyl-terminal fragment is a product of the peripheral metabolism of PTH. This concept has been confirmed by studies in which either unlabelled BPTH or ^{125}I-BPTH has been infused or injected into dogs or cows after which a carboxyl-terminal fragment of BPTH has appeared in the circulation (7-10). The site(s) of generation of this fragment has not been established.

In this report we present evidence that purified canine renal plasma membranes convert ^{125}I-BPTH to a carboxyl-terminal fragment similar to that previously described.

* Abbreviations: PTH, parathyroid hormone; BPTH, bovine parathyroid hormone; GPI, guinea pig anti-BPTH antiserum 1.

MATERIALS AND METHODS

Kidneys were obtained from mongrel dogs under sodium pentobarbital anesthesia and were immediately placed in 0.25M sucrose on ice. Purified renal plasma membranes were prepared by the method of Marx, Fedak, and Aurbach (11) with the exception that ultracentrifugation of homogenates of renal cortex and medulla was carried out on sucrose gradients with 7-9 steps in sucrose concentration. 0.5 ml aliquots of sucrose solutions were layered consecutively in a 5 ml centrifuge tube beginning with 43% sucrose and ending with 33% sucrose in 0.01M TRIS, 0.001M disodium EDTA, ph 7.5 (TRIS-EDTA) buffer. After centrifugation at 100,000xg for 90 minutes in a Beckman L3-50 ultracentrifuge, a band of turbidity was seen at the interface between 38 and 39% sucrose concentrations. This was the level of sucrose concentration at which the plasma membranes were found by Marx and colleagues (11). This material was aspirated and stored at -40°C. Protein content of this membrane preparation was determined by the method of Lowry et al.(12). Experiments with the membranes were carried out one to ten days after preparation.

Radioiodine-labelled BPTH (^{125}I-BPTH and ^{131}I-BPTH) was prepared by a modification of the method of Greenwood et al. (13). Purification of radioiodinated hormone was carried out by gel filtration on 0.9 x 60 cm columns of Bio-Gel P-10 (Bio-Rad, Richmond, California) prior to use in experiments (14).

To determine the effect of the plasma membranes on the BPTH molecule incubations of renal plasma membranes (10-240 μg protein), ^{125}I-BPTH (1.25-8 x 10^5 cpm) and 0.5 - 0.7% bovine serum albumin in 0.5 - 1 ml TRIS-EDTA were carried out at 25°C for 18 hours. Control incubations lacking plasma membranes were carried out under the same conditions. In other experiments plasma membranes

and ^{125}I-BPTH were incubated at 4°C, after heating the membranes at 56°C for 30 minutes, and in the presence of 1000 kallikrein inactivator units/ml of basic bovine pancreatic trypsin inhibitor (Trasylol, FBA Pharmaceuticals, New York), respectively. After the incubations ^{125}I-labelled hormone was separated from plasma membranes by layering the incubation mixture on 20% sucrose with 0.5% bovine serum albumin in TRIS-EDTA and then centrifuging for at least 30,000xg for 60 minutes. The upper layer in the centrifuge tube, containing the ^{125}I-labelled hormone, was aspirated and stored at -40°C.

The solutions containing the ^{125}I-labelled hormone were chromatographed on 0.9 x 60 cm columns of Bio-Gel P-10 and eluted with 0.05M sodium barbital and 1% blood bank plasma, ph 8.5. The columns were calibrated with intact ^{125}I-BPTH and in later experiments ^{131}I-BPTH was added to the solutions before chromatography to provide an internal standard of elution volume. 0.5 ml fractions were collected and counted on a Packard double channel gamma spectrometer with correction for channel cross-interference.

Immunochemical characterization of ^{125}I-BPTH before and after incubation with plasma membranes was carried out with a guinea pig anti-BPTH antiserum (GPI) which has been shown to recognize antigenic sites in both the amino-terminal and carboxyl-terminal regions of BPTH (4). Radioiodinated hormone was incubated with GPI alone, with GPI and an excess of the amino-terminal 34 amino acids of BPTH (6.1 ng/ml), and with GPI and an excess of the carboxyl-terminal 32 amino acids of BPTH (2.9 ng/ml), respectively. Separation of bound from free hormone was accomplished after three days with dextran-coated charcoal (14).

RESULTS

Figure 1 illustrates a typical elution pattern of ^{131}I-BPTH

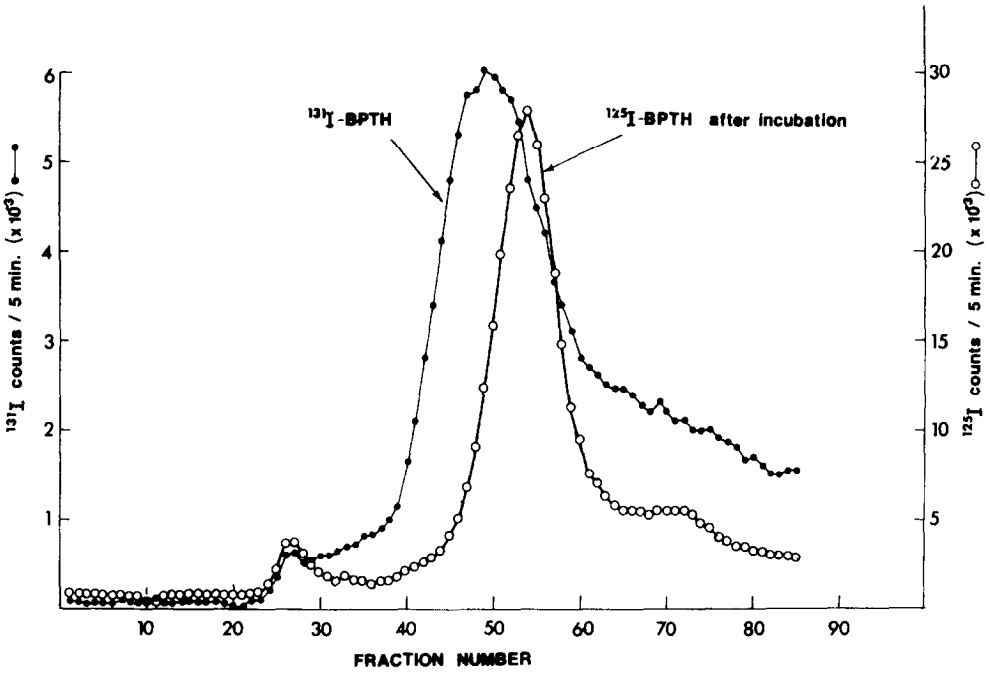

Figure 1. Gel filtration of ^{125}I-BPTH after incubation of 1.25 x 10^3 cpm with renal plasma membranes (240μg protein). ^{131}I-BPTH was added prior to chromatography.

(identical to intact ^{125}I-BPTH) and ^{125}I-BPTH after incubation with renal plasma membranes. The ^{125}I-BPTH after incubation consistently eluted 5 fractions after the intact ^{131}I-BPTH and ^{125}I-BPTH. This was noted after incubation with plasma membranes obtained from either renal cortex or medulla. ^{125}I-BPTH incubated in the absence of renal plasma membranes did not show a change in elution volume. Similarly there was no change in elution volume of ^{125}I-BPTH after incubation with plasma membranes at 4°C, after heating the membranes to 56°C for 30 minutes, or after incubation with basic bovine pancreatic trypsin inhibitor.

Figure 2 illustrates the immunochemical characteristics of ^{125}I-BPTH before and after incubation with renal plasma membranes. Binding of intact ^{125}I-BPTH to antiserum GPI was partially inhibited by both amino-terminal BPTH and carboxyl-terminal BPTH, whereas

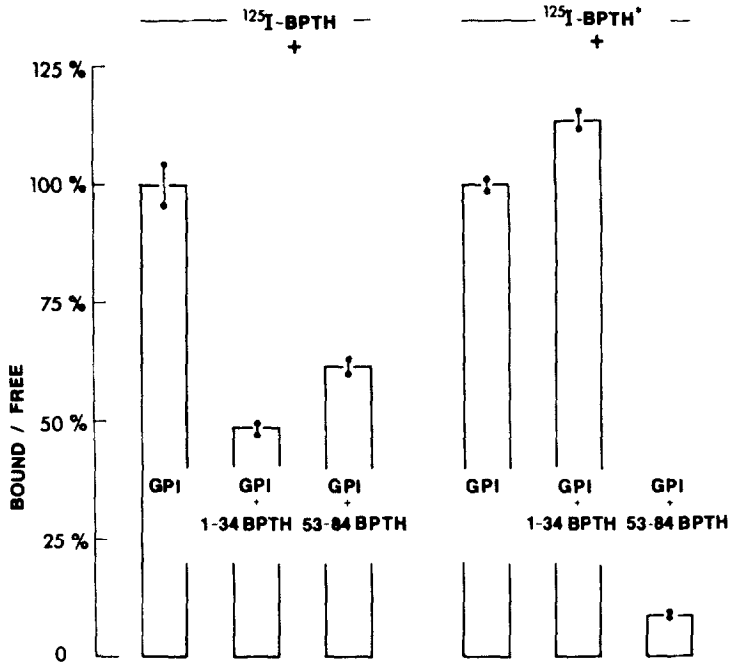

Figure 2. Suppression of binding of anti-serum GPI to ^{125}I-BPTH before and after incubation with renal plasma membranes (^{125}I-BPTH*) by amino-terminal (1-34) and carboxyl-terminal (53-84) fragments of BPTH. The duplicate incubations are represented by the dots connected by lines.

binding of labelled BPTH after incubation with renal plasma membranes is inhibited almost completely by carboxyl-terminal fragment and not at all by amino-terminal fragment.

DISCUSSION

In this study we have shown that incubation of ^{125}I-BPTH with canine renal plasma membranes results in a delay in elution of the labelled hormone on gel filtration and a loss of amino-terminal immunoreactivity, suggesting a cleavage of the hormone to a carboxyl-terminal fragment. By these two different methods, this fragment is indistinguishable from the approximately 7,000 dalton fragment first shown by Habener and colleagues (2) to be the principle circulating immunoreactive species of PTH in the

bovine species and in patients with hyperparathyroidism. The fragments have the same gel filtration patterns using identical methods and Habener et al (3) demonstrated absence of amino-terminal immunoreactivity by sequence-specific radioimmunoassays using the same reagents as in the present study. A similar fragment has also been found in the circulation of dogs after injection of unlabelled or radioiodine-labelled BPTH (7,8). The demonstrated temperature dependence and inhibition of the cleavage by a protease inhibitor are consistent with an enzymatic process.

The subcellular preparation which was used has been well characterized by marker enzymes and by electron microscopy, and consists almost exclusively of renal plasma membranes (11). This preparation has previously been demonstrated to contain receptors for PTH, calcitonin, and vasopressin (11, 15) and to degrade salmon calcitonin to forms not precipitable by trichloracetic acid.

Although the physiologic significance of these in vitro observations is not certain, it appears likely that the kidney is one site at which cleavage of PTH occurs in vivo and results in the release of a carboxyl-terminal fragment into the circulation. Since this fragment is not biologically active (16), it would appear to have no physiologic function in the circulation. The possibility of cleavage of the hormone at other sites and the fate of the amino-terminal portion of the molecule awaits further studies.

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