INTERACTIONS OF PARATHYROID HORMONE AND PLASMA MEMBRANES FROM RAT KIDNEY

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SUMMARY: The initial interaction between parathyroid hormone and isolated purified membrane preparations from rat kidney is probed with the use of a highly tritiated, biologically active derivative of parathyroid hormone, [^{3}H] acetamidino-parathyroid hormone. A method for assaying the binding of the labeled parathyroid hormone to these membranes at nanogram per milliliter concentrations has been demonstrated utilizing this method. The binding described is not reduced by pre-incubations with large excess quantities of other polypeptide hormones, but is qualitatively abolished by pre-incubation with smaller quantities of unlabeled parathyroid hormone. The oxidized, biologically inactive labeled parathyroid hormone does not bind to these membranes.

Hormone-cell surface interactions have been demonstrated *in vitro* for a number of polypeptide hormones (1-8). Specific binding assays employing biologically active, highly labeled hormones and purified membrane preparations (6), or whole cell preparations (2) have provided a direct means of examining these hormone-receptor interactions. This to date has not been possible with parathyroid hormone mainly because attempts to prepare a biologically active, highly labeled derivative of PTH have been unsuccessful. Thus, direct examination of the hormone-receptor interaction between PTH and its known target organs, kidney and bone, has awaited the development of a new labelling technique for PTH.

Recently we have prepared a tritiated derivative of PTH (acetamidino PTH) and found it to be biologically active (9). Preparations of this material in

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Abbreviations used are: PTH, parathyroid hormone; [^{3}H]PTH, tritiated acetamidino parathyroid hormone; ACTH, adrenocorticotropic hormone, OX-[^{3}H]PTH, oxidized, tritiated acetamidino parathyroid hormone; Na_2EDTA, ethylenediamine tetraacetic acid, disodium salt.

which 80% of the ten free amino groups in the hormone are labeled show a dose-response relationship virtually identical to that found for the native hormone in the classical serum calcium elevation assay of Munson (15,16).

This paper describes the specific binding of labeled PTH to isolated membrane fractions from a PTH target tissue, rat kidney.

MATERIALS AND METHODS. Highly purified, bovine parathyroid hormone (1800-2200 Units/mg protein) was purchased from Wilson Laboratories and further purified by a modification of the method of Keutman et al. (10). The tracer, tritiated acetamidino parathyroid hormone, was prepared as described previously (9). The specific activity of the hormone was 1.0×10^6 dpm per microgram. The biologically inactive, oxidized [^{3}H]PTH was prepared by addition of 50 µg of [^{3}H]PTH to a solution of 150 mM H_2O_2 and 50 mM acetic acid in a total volume of 1 ml. The solution was incubated for one hour at 37° C. and the reaction terminated by addition of 5 µg of catalase (Sigma). The oxidized, labeled parathyroid hormone (OX-[^{3}H]PTH) was then separated from the reaction mixture by gel-filtration column chromatography on Bio-Gel P-30. This material was inactive in the serum calcium assay.

Purified plasma membrane preparations of rat kidney were prepared according to the method of Fitzpatrick et al. (11), as modified by Shriver et al. (12)

Interaction of the labeled hormone with the plasma membrane preparations was assayed by the following method. A 0.5 ml sample of the membranes, prepared as a suspension (600 µg/ml), was incubated for five minutes with 10 ng of [^{3}H]PTH. These incubations were performed at 0° C. to suppress the hormone-inactivating system present in such broken cell preparations (13). The incubation sample was then applied to a 30 X 0.9 cm column (Pharmacia) packed with Bio-Gel P-30, 50-100 mesh (Bio-Rad Laboratories) and chromatographed in a 0.25 M sucrose, 70 mM imidazole, 1 mM Na_2EDTA buffer. One ml fractions were collected in a Gilson refrigerated fraction collector set at 3° C. Each fraction was then assayed for protein concentration and total radioactivity. Protein concentration was determined by method of Lowry (14), using crystalized

bovine serum albumin as a standard. A 0.5 ml aliquot of each fraction was removed, solubilized in NCS tissue-solubilizer (Amersham/Searle), and counted by liquid scintillation on a Picker Nuclear Liquimat. The efficiency of the counting was established and results converted to dpm.

Competition studies were performed, where indicated, by pre-incubating the membrane sample with 100-fold or 500-fold excess quantities of unlabeled insulin (Sigma),*ACTH, glucagon (Sigma), and PTH for five minutes at 0° C., prior to the addition of, and incubation with, the [^{3}H]PTH.

RESULTS AND DISCUSSION. Figure 1 indicates the results obtained when a 300 µg

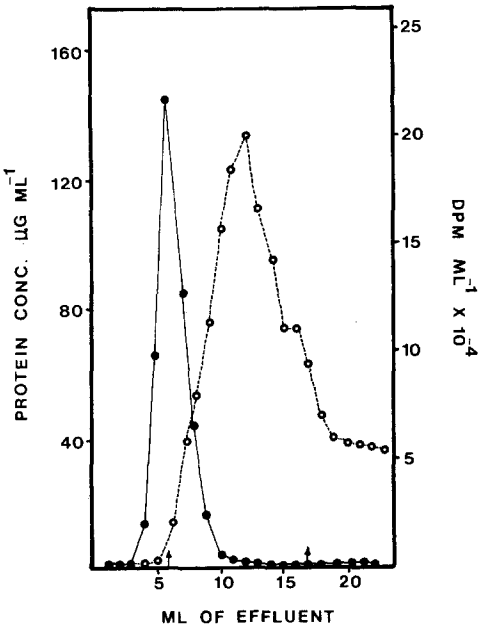

FIGURE 1. Bio-Gel P-30 gel filtration chromatography of a 300 µg sample of kidney plasma membranes (●—●), and 10 µg of [^{3}H]PTH (○--○), each chromatographed separately. Chromatography and assays were performed as described in the text.

*Kindly supplyed by Dr. Jerome Kowal, Dept. of Medicine, Case Western Reserve University.

sample of purified membranes and a 10 µg sample of [^{3}H]PTH are chromatographed separately on Bio-Gel P-30. This figure illustrates that the membranes eluted in the void volume on this column, while [^{3}H]PTH is significantly retarded, thus providing a mechanism for separation of "bound" and "free" hormone. In addition, it should be noted that the Bio-Gel columns, under the conditions specified, exhibit a degree of affinity for the free [^{3}H]PTH as demonstrated by marked tailing of the [^{3}H]PTH profile. This interaction is more pronounced with lower amounts of hormone, leading to peak broadening and somewhat irregular elution patterns (See e.g. Fig. 2). Such elution profiles are not indicative

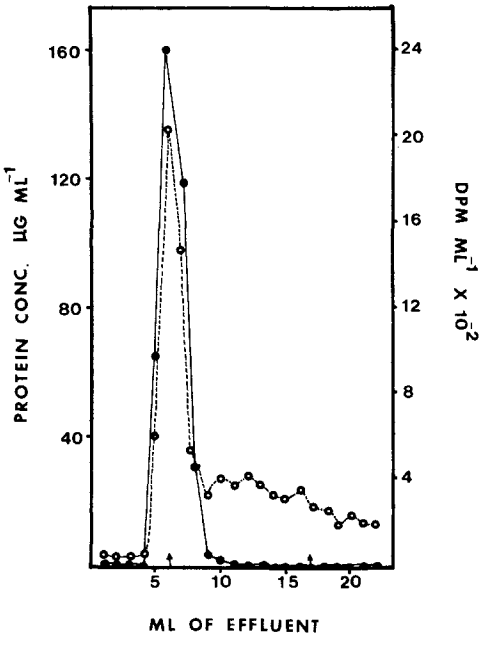

FIGURE 2. Bio-Gel P-30 gel filtration chromatography of a 300 µg sample of kidney plasma membranes incubated for 5 minutes at 0° C with 10 ng of [^{3}H]PTH prior to chromatography. Results from the assay of protein (●—●) and radioactivity (○-○) are shown. Chromatography and assays were performed as described in the text.

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of heterogeneity in the hormone preparation, since larger amounts of the [^{3}H]PTH elute as a sharp, single peak with some tailing, rather than as several distinct peaks.

Figure 2 indicates the results obtained when the same membranes are incubated for 5 minutes at 0° C. with 10 ng of [^{3}H]PTH, and then chromatographed on Bio-Gel P-30. The majority of the eluted radioactivity now appears in the void volume with the membranes. Furthermore, the peak is sharp and symmetrical with the protein peak, strongly suggesting that the ^{3}H is now associated with the membranes. Competition studies performed as described above, with 500-fold excess pre-incubations of glucagon, insulin and ACTH did not reduce the subsequent binding of the [^{3}H]PTH as indicated in Figure 3.

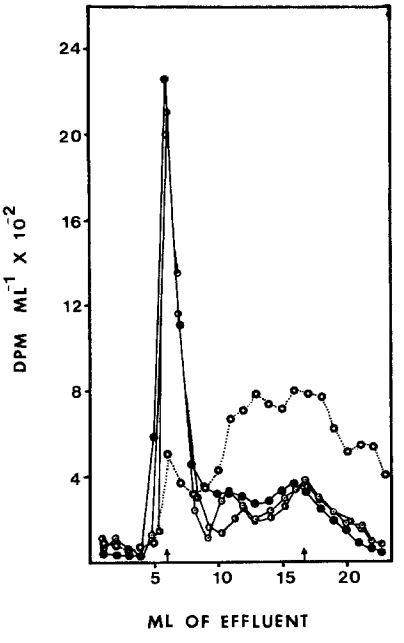

FIGURE 3. Bio-Gel P-30 gel filtration chromatography of a 300 µg sample of kidney plasma membranes pre-incubated with either 5 µg ACTH (◑—◑), 5 µg insulin (⊙—⊙), 5 µg glucagon (●—●), or 1 µg unlabeled PTH (○···○) and subsequently incubated with 10 ng [^{3}H]PTH prior to chromatography. Chromatography and assays were performed as described in the text.

However, pre-incubations of the membranes with 100-fold excess of cold PTH over [^{3}H]PTH, qualitatively abolished the subsequent binding of the [^{3}H]PTH, as shown in Figure 3.

Treatment of PTH with peroxide appears to selectively oxidize methionine

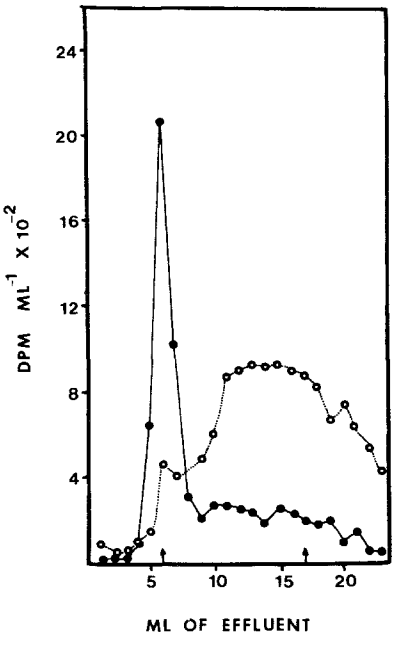

FIGURE 4. Bio-Gel P-30 gel filtration chromatography of a 300 µg sample of kidney plasma membranes incubated for 5 minutes at 0° C with either 10 ng of [^{3}H]PTH (●—●), or 10 ng of OX-[^{3}H]PTH (○···○). Chromatography and assays were performed as described in the text.

in the hormone. Since reduced Met-8, is critical for biological activity, the effect of oxidation on binding was investigated. Figure 4 compares the interaction of the membranes with: (1) 10 ng of [^{3}H]PTH, and (2) 10 ng of OX-[^{3}H]PTH. It is quite clear that the oxidized hormone binds poorly, if at all. This observed inability of the oxidized, labeled PTH to bind to kidney plasma membranes may well explain the biological inactivity of this molecule (15).

Thus, based on the observations relayed in this communication, it can be concluded that a specific interaction of [^{3}H]acetamidino-parathyroid hormone and this kidney membrane preparation can be demonstrated in vitro. This interaction occurs within an order of magnitude of the reported physiological, circulating concentration of PTH, and may well provide the means

for investigating the interaction of PTH with its renal receptor, at the molecular level. It is also of interest that this association and the displacement by cold PTH occurs at levels of hormone which are much lower than those necessary to activate adenylate cyclase *in vitro* (8). Thus, this is the first reported *in vitro* system which operates at physiological levels of PTH.

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