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CHARACTERIZATION OF THE PARATHYRIN RECEPTOR IN RENAL PLASMA MEMBRANES BY LABELLED HORMONE AND LABELLED ANTIBODY BINDING TECHNIQUES

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SUMMARY

The parathyrin receptor in renal cortex has been investigated by studying the binding of ^{125}I-labelled parathyrin, or of unlabelled parathyrin detected with ^{125}I-labelled antibodies, to a partially purified plasma membrane fraction. The kinetics of hormone uptake demonstrated a biphasic response in both systems at 22 °C but this phenomenon was not detectable at 37 °C. Specific displacement of lactoperoxidase labelled ^{125}I-labelled parathyrin occurred with 8 ng unlabelled bovine parathyrin. The apparent affinity constant was $2.3 \cdot 10^8$ M^{-1} and the apparent binding capacity of the membranes 1.25 pmol/mg protein. Using the labelled antibody technique the receptor showed maximal binding at pH 7.0–7.5. As little as 80 pg bovine parathyrin produced a significant increase in binding of labelled anti-bovine parathyrin antibody and saturation of binding sites was demonstrated at 2.5 pmol/mg protein. Oxidized hormone showed undetectable binding. Treatment of membranes with phospholipases A or D, or Trypsin greatly reduced subsequent hormone binding. Prior incubation of membranes with 1–34 synthetic parathyrin decreased the binding of intact hormone whereas gastrin, insulin and glucagon had no effect. Growth hormone and calcitonin slightly increased parathyrin binding.

INTRODUCTION

Parathyroid hormone (parathyrin) acts on the kidney to increase calcium reabsorption and phosphate excretion [1], and these effects are mediated through an adenylate cyclase system localized in the renal cortex [2–3]. In common with other polypeptide hormones [4–7] it is possible to demonstrate specific, displaceable binding of ^{125}I-labelled [8–10] or ^{3}H-labelled [11] parathyrin to plasma membranes from renal cortex. Nevertheless, owing to the lability of the methionine residue, which is critical for biological activity, even mild procedures such as lactoperoxidase labelling or ester conjugation are likely to modify the hormone, and this may account for the unphysiologically high levels of hormone necessary to cause displacement [8–11].

A labelled antibody method of detecting membrane bound hormone was developed to overcome the problem of producing biologically active labelled hormone [12]. This communication describes further characteristics of the hormone-membrane interaction as demonstrated by the binding of lactoperoxidase labelled ^{125}I-labelled parathyrin and of unlabelled hormone detected by labelled antibodies.

MATERIALS AND METHODS

Hormone preparations and antibodies

Parathyrin standard preparations: bovine parathyrin (71/324) and human parathyrin (approximately 4 % pure) were generously provided by the MRC (Mill Hill, London) and Prof. J. L. M. O'Riordan (Middlesex Hospital, Medical School, London), respectively. Synthetic human 1–34 parathyrin (Lot No. 14014) was obtained from Beckman, Palo Alto and bovine parathyrin (Lot No. 15045) from Wilson Laboratories, Illinois.

Antibodies: anti-bovine parathyrin (AS 262) and anti-bovine parathyrin cross reacting with human parathyrin (AS 201); both reacting primarily with the C-terminal region of the hormone, were obtained from the Medical Research Council (Mill Hill, London). Antibody A-VII was kindly supplied by Dr. R. Bouillon, Rega Institut, Leuven, Belgium. Anti-synthetic 1–34 human parathyrin was a generous gift of Prof. F. H. Dietrich, CIBA-Geigy, Basle and Dr. J. Fischer, Orthop. Klinik, Balgrist, Zürich.

Preparation of renal plasma membranes

Kidney-cortex plasma membranes were routinely prepared by the method of Fitzpatrick et al. [13]. Rat (4 to 5 month old) and chick (3 to 4 week old) kidneys were rapidly removed after killing by cervical dislocation and decapitation, respectively. Bovine and porcine kidneys were obtained from animals immediately after slaughter. In all cases the tissue was immediately placed on ice and processed as soon as possible. All subsequent procedures were carried out at 0–4 °C.

After removal of fat and renal capsule the cortex was carefully dissected from the medulla, minced with scissors and homogenized by hand in a Potter-Elevjhem teflon/glass homogenizer. Homogenization was carried out in 4 volumes of ice-cold 0.25 M sucrose in 10 mM Tris · HCl, pH 7.4, +1 mM EDTA. All subsequent procedures were as described by Fitzpatrick et al. [13]. The final membrane pellets were homogenized in 50 mM Tris · HCl, pH 7.4, containing $MgCl_2$ (3 mM), NaCl (10 mM) and KCl (10 mM) and stored in multiple aliquots in the same buffer.

Enzyme and protein estimations

Adenylate cyclase was assayed by the method of Albano et al. [14] except that an increase in the ATP-regenerating system was found to be necessary. Creatine kinase (5 mg/100 µl) was mixed with 150 µl of creatine phosphate (300 mM) and 20 µl of this was added to each incubation tube. Cyclic 3′,5′-adenosine monophosphate was measured by a binding protein assay [14–16]. Protein was measured by the method of Lowry et al. [17] using bovine serum albumin as the standard.

Preparation of immunoadsorbent and labelling of antibodies

Immunoadsorbents of bovine parathyrin (Wilson Laboratories, Illinois, Lot

No. 15045) and 1–34 synthetic human parathyrin (Beckman, Palo Alto, Lot No. 14014) were prepared by the method of Miles and Hales [18]. Antibody (20–50 μl) was extracted on to the respective immunoadsorbent and labelled with ^{125}I by the technique of Hunter and Greenwood [19]. The high affinity antibodies were then eluted and further purified as described by Addison et al. [20].

Labelled antibody membrane assay

The details of this assay have been described elsewhere [12] and unless otherwise stated incubation of hormone with membranes was carried out for 2 h at 20 °C and with antibody for 1 h at 20 °C. Data is expressed either as cpm bound (corrected for binding in the absence of hormone) or as percentage increase in bound radioactivity observed in the presence of parathyrin. All antibody incubations were carried out in NIRG buffer, pH 8.0 [12].

Labelling of parathyrin

The lactoperoxidase method of Marchalonis [21] as modified by Sutcliffe et al. [8] was used for labelling of bovine parathyrin with ^{125}I. To 5 μg of bovine parathyrin (Wilson Lot No. 15045) in 5 μl of 10 mM acetic acid were added 10 μl of 0.5 M phosphate buffer, pH 7.4, 1 mCi of $Na^{125}I$ in 10 μl, followed by 4 μg of lactoperoxidase (Calbiochem. San Diego, California) in 20 μl of 0.05 M phosphate buffer. After addition of 10 μl of 0.8 mM H_2O_2 the mixture was incubated for 20 min at room temperature. The reaction was terminated with 500 μl of 0.05 M phosphate buffer, pH 7.4+0.05 M 2-mercaptoethanol, and 5 mg of QUSO G32 (Philadelphia Quartz Co., U.S.A.) added. The hormone was eluted [8] and stored at −70 °C in multiple aliquots. On the day of use a sample was purified by gel-filtration on Biogel P 60 [8].

Binding of ^{125}I-labelled parathyrin to renal membranes

Plasma membranes (50 μl containing approximately 100 μg protein) were incubated for 30 min in polypropylene tubes (Sarstedt 46/6) with 50 μl ^{125}I-labelled parathyrin. Incubation buffer was 50 mM Tris · HCl pH 7.4 containing 2 % bovine serum albumin. Unlabelled bovine parathyrin (in incubation buffer) was added in 50-μl aliquots. After incubation at 22 °C the membranes were sedimented by centrifugation at 13 000 × *g* 1 min in a Hettich mikro rapid centrifuge (Hettich, Tuttlingen, Germany). The tips of the tubes, containing the pellet, were excised and counted in a gamma-counter. All binding data are the mean of triplicate samples and corrected for "non-specific binding" by subtracting from the total binding that amount bound in the presence of 1 μg bovine parathyrin.

Oxidation of parathyrin

Bovine parathyrin (100 ng) was incubated with hydrogen peroxide (0.15 M) and acetic acid (0.05 M) for 1 h at 37 °C. Catalase (5 μg) was then added and after a further 10 min at 22 °C the hormone was diluted with incubation buffer. As control, catalase was added before the hydrogen peroxide.

RESULTS

Renal cortex adenylate cyclase activity

The partially purified membranes (bovine or porcine) demonstrated a four-fold

TABLE I

RESPONSES OF BOVINE RENAL CORTEX ADENYLATE CYCLASE TO HORMONE ADDITION

Addition	Adenylate cyclase activity (nmol/mg protein/15 min)
A Control	0.204
Fluoride (10 mM)	1.87
Human parathyrin 1-34 (10 μg/ml)	0.290
Bovine parathyrin 1-34 (10 μg/ml)	1.94
Bovine parathyrin 1-84 (10 μg/ml)	2.19
B Control	0.190
Fluoride (10 mM)	1.54
Bovine parathyrin 1-84 (10 μg/ml)	1.73
Bovine parathyrin 1-84 (5 μg/ml)	1.48
Bovine parathyrin 1-84 (2.5 μg/ml)	1.06
Calcitonin M (10 μg/ml)	0.150
Calcitonin Porcine (10 μg/ml)	0.180

increase in specific activity compared to the homogenate. The enzyme was responsive to parathyrin and fluoride but not to calcitonin (Table I). Bovine parathyrin 1–84 (Wilson) and bovine parathyrin 1–34 showed similar levels of stimulation but human 1–34 parathyrin had very low biological activity. Maximum fluoride stimulation was obtained at a concentration of 10 mM.

Kinetics of binding of ^{125}I-labelled bovine parathyrin to renal plasma membranes

At 22 °C the binding of ^{125}I-labelled bovine parathyrin to porcine plasma membranes exhibited a biphasic response with a rapid uptake in the first two minutes, followed by apparent release and a further slower uptake which reached equilibrium after 30 min (Fig. 1). This phenomenon was reproducable in several assays. On raising

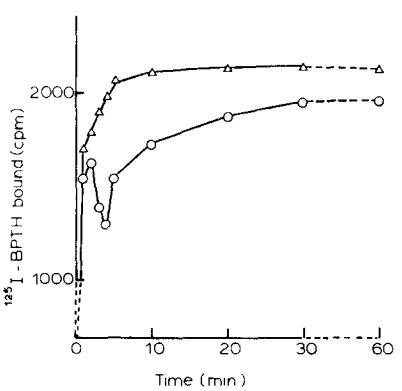

Fig. 1. Kinetics of binding of ^{125}I-labelled parathyrin to porcine renal cortex plasma membranes. ^{125}I-labelled bovine parathyrin (22000 cpm) was incubated with membranes for various time periods and the reaction terminated as described in Materials and Methods. Temperature of incubation: ○, 22 °C; △, 37 °C. BPTH, bovine parathyrin.

the temperature to 37 °C the initial phase of uptake was more rapid and equilibrium was essentially reached after 10 min. In addition, the release-reuptake phenomenon observed at 22 °C disappeared at the higher temperature. Similar results were obtained with bovine plasma membranes but in the case of rat membranes very low binding was detected and this could not be displaced with 1 μg of unlabelled bovine parathyrin. Binding of ^{125}I-labelled parathyrin (30 000 cpm) was linear over the range 10–200 μg membrane protein.

Displacement of bound ^{125}I-labelled bovine parathyrin

The ^{125}I-labelled bovine parathyrin bound to porcine renal membranes could be specifically displaced by unlabelled hormone (Fig. 2). However, although 8 ng bovine parathyrin caused significant displacement, a maximum of 60 % of bound ^{125}I-labelled bovine parathyrin could be displaced with 1 μg of unlabelled hormone. Scatchard analysis [22] of the data obtained from displacement studies revealed a single order of binding sites with the relatively low apparent affinity constant of $2.3 \cdot 10^8$ M^{-1}. The apparent binding capacity of the membranes was 1.25 pmol/mg protein.

Addition of insulin (10 μg) human calcitonin (10 μg), gastrin (10 μg), and glucagon (10 μg) caused no significant displacement of bound material. Incorporation of Ca^{2+} (1–10 mM), Mg^{2+} (1–10 mM), or EDTA (1–20 mM) into the buffer did not affect either binding or displacement. Heating of membranes to 100 °C for 1 min, treatment with HCl (0.1 M) or NaOH (0.1 M) reduced specific binding to less than 5 %.

Labelled antibody characterization of parathyrin binding to renal plasma membranes

Effect of pH. The influence of pH on hormone binding was investigated by incubating bovine parathyrin (Wilson) with bovine membranes for 30 min at 22 °C in buffer of pH between 5.5 and 10.0. The membranes were then washed in the respective buffer and further incubated with labelled antibody (AS 262) for 1 h at 22 °C. At pH values below 7.0 binding was greatly reduced and after treatment with dilute HCl (pH 3) there was no detectable binding. Maximum binding was observed at pH 7.0–

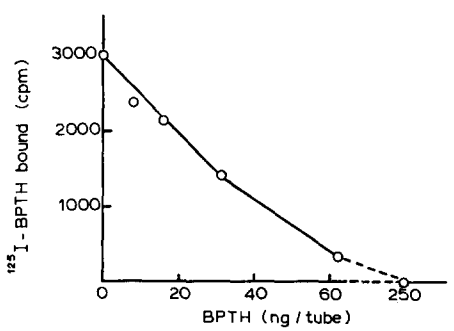

Fig. 2. Displacement by unlabelled bovine parathyrin of ^{125}I-labelled bovine parathyrin bound to porcine renal cortex plasma membranes. Membranes (100 μg) in 200 μl incubation buffer were incubated at 22 °C for 30 min with ^{125}I-labelled bovine parathyrin (9000 cpm) and increasing amounts of unlabelled bovine parathyrin. Binding data has been corrected for unspecific binding as described in Materials and Methods.

7.5 with a decrease in binding at higher pH followed by a further increase in binding with bicarbonate buffer at pH 9.5. NaOH (0.1 M) reduced binding to undetectable levels. The effect of pH was not due to alteration of subsequent antibody binding caused by residual incubation buffer since washing the membranes with NIRG buffer gave identical results.

Kinetics of binding. Since an apparent biphasic binding of ^{125}I-labelled bovine parathyrin was observed whereas no such effect was found in the labelled antibody system [12] the binding of bovine parathyrin to porcine renal membranes was re-examined over the first 30 min of incubation. At both 0 °C and 22 °C an initial rapid uptake could be demonstrated whereas at 37 °C the phenomenon was not detectable (Fig. 3). However, there is a discrepancy in the time of peak response since maximum early phase binding of ^{125}I-labelled bovine parathyrin was after 2 min and the trough at 4 min, whereas with bovine parathyrin the initial peak binding was after 5 min.

Binding of parathyrin to chick renal plasma membranes. The binding of bovine parathyrin (MRC or Wilson) or human parathyrin to porcine or bovine membranes does not demonstrate saturation of binding sites over the concentration range 80 pg–10 ng [12]. Chick membranes are extremely sensitive to parathyrin [23] and would therefore seem to be more suitable for studying hormone-receptor interaction in the absence of degradation. Bovine parathyrin and human parathyrin both demonstrated higher binding (per 100 μg protein) to chick, than to either porcine or bovine, plasma membranes and saturation was observed at 2.5 ng parathyrin/100 μg protein (Fig. 4). Since only low levels of human hormone were available the maximum concentration tested was 1 ng/tube.

Treatment of bovine parathyrin (1.25 or 2.5 ng) with hydrogen peroxide (as described in Methods) reduced subsequent detectable binding to control values, whereas when catalase was added to the reaction mixture prior to hydrogen peroxide there was no such reduction (Fig. 4). When mitochondria (porcine renal) were sub-

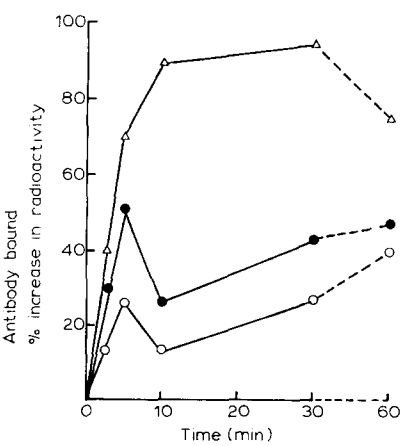

Fig. 3. Effect of incubation time and temperature on binding of bovine parathyrin to porcine renal membranes as demonstrated by labelled antibody binding. Bovine parathyrin (Wilson, 10 ng) was incubated for varying time periods and temperatures with porcine renal membranes followed by a 2 h incubation with labelled antibody (AS 262). Temperature of incubation with hormone: ○, 0 °C; ●, 22 °C; △, 37 °C. Further details as in Materials and Methods.

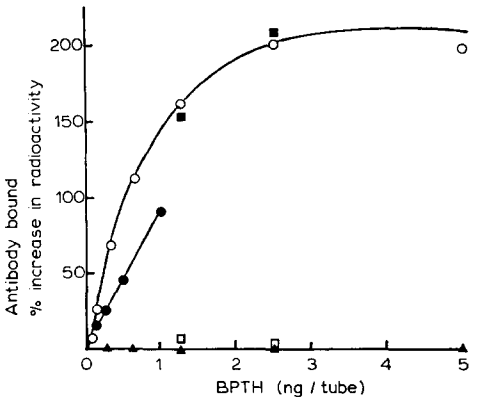

Fig. 4. Binding of parathyrin to chick renal plasma membranes as demonstrated by labelled antibody binding. Membranes (100 μg protein) were incubated with bovine parathyrin (MRC ○) or human parathyrin (O'Riordan ●) for 2 h at 22 °C followed by incubation with labelled antibody (Bouillon) for 1 h at 22 °C. Total cpm added 11 580. In some experiments bovine parathyrin was treated with hydrogen peroxide either before (□) or after (■) the addition of catalase and incubated with membranes as above (see Materials and Methods). The effect of substitution of renal mitochondria (100 μg protein) for plasma membranes is also shown (▲).

stituted for plasma membranes in the reaction mixture at an equivalent protein concentration bovine parathyrin binding, at all dose levels, was abolished (Fig. 4).

The presence of plasma or serum in the incubation medium reduced the binding of all points by 8–10 % but did not alter the minimum level of hormone producing an increase in bound antibody (80 pg). The effect of the presence of labelled antibody on the binding of hormone onto membranes was investigated by measuring the uptake by the usual sequential addition of hormone (2 h) and antibody (1 h), and comparing it with a system in which all reagents were added together and incubated for 3 h. It is evident that the presence of antibody (Fig. 5) inhibits the uptake of hormone although with very little loss in sensitivity.

Effect of enzyme treatment of membranes on binding of bovine parathyrin. Membranes were treated with phospholipase A or D, or Trypsin at various concentrations (Table II) and subsequently assayed for their ability to bind bovine parathyrin. Both phospholipase A and D caused dramatic decreases in binding. In the case of phospholipase A 1 μg/ml produced a significant decrease in binding and a maximum effect occurred at 50 μg/ml. There was no further decrease at higher concentrations. Concentrations of phospholipase D necessary to produce significant effects were much higher (50 μg/ml) although a more pronounced inhibition occurred at 100 μg/ml. Trypsin treatment (20 μg/ml) reduced subsequent bovine parathyrin binding to 50 % control values and 100 μg/ml almost completely abolished binding.

Control tubes were only slightly affected by the prior incubation at 30 °C or 37 °C with a maximum drop in binding of 10 % compared to a tube incubated at 22 °C.

Effect of peptide hormones on bovine parathyrin. Porcine renal membranes were incubated with a number of peptide hormones prior to the addition of bovine parathyrin and measurement of binding with antibody Bouillon A VII. Prior incubation with 1-34 Synthetic bovine parathyrin (1 μg) significantly reduced subsequent binding

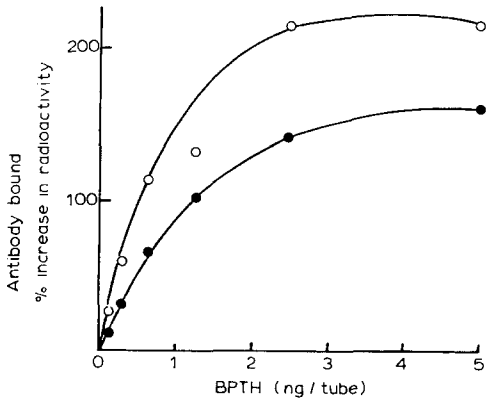

Fig. 5. Effect of antibody on binding of bovine parathyrin to chick renal membranes. Membranes were incubated with bovine parathyrin (MRC) for 2 h followed by 1 h with antibody (Bouillon) (○) or in a system containing all reagents together (●). Further details as in Materials and Methods.

of bovine parathyrin (Fig. 6). However, binding of 5 ng bovine parathyrin was only reduced by 50 % and the binding of 100 ng was not significantly inhibited. Addition of labelled anti-human 1–34 parathyrin antibody to membranes previously incubated with either human or bovine synthetic 1–34 fragment did not demonstrate any significant increase in binding over control membranes.

TABLE II

EFFECT OF ENZYME TREATMENT OF MEMBRANES ON SUBSEQUENT BOVINE PARATHYRIN BINDING

Phospholipase treatment: porcine membranes (2 mg/ml) were incubated at 37 °C in Krebs-Ringer-bicarbonate buffer + 1 % albumin with phospholipase A or phospholipase D in the concentrations shown above. With phospholipase A $CaCl_2$ was added to a final concentration of 10 mM. Membranes were than centrifuged, washed in incubation buffer and assayed for bovine parathyrin binding. Trypsin treatment: porcine membranes (2 mg/ml) were incubated with trypsin in 1 mM $KHCO_3$ pH 7.8 and after 30 min 30 °C, soybean trypsin inhibitor was added at ten times the concentration of enzyme. The membranes were then sedimented, washed and resuspended in incubation buffer. As controls membranes were treated identically as above but in the absence of phospholipases or by adding trypsin inhibitor before the 30 min incubation. Bovine parathyrin: MRC 71/324. Antibody: Bouillon A VII.

Enzyme treatment	Antibody bound (% Increase in radioactivity)
Control for phospholipases	190
Phospholipase A (1 μg/ml)	147
Phospholipase A (5 μg/ml)	75
Phospholipase A (10 μg/ml)	64
Phospholipase A (50 μg/ml)	60
Phospholipase D (50 μg/ml)	120
Phospholipase D (100 μg/ml)	40
Trypsin (20 μg/ml)	90
Trypsin (100 μg/ml)	3
Control for Trypsin	180

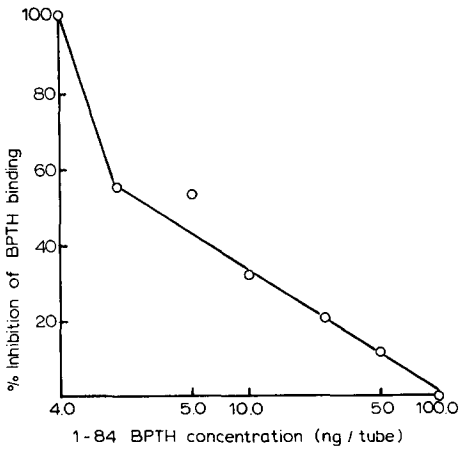

Fig. 6. Effect of prior incubation of membranes with 1-34 bovine parathyrin on subsequent binding of bovine parathyrin. Bovine Membranes (100 μg protein) were preincubated (1 h) with 1-34 bovine parathyrin (1 μg). Various concentrations of bovine parathyrin (MRC) were then added to the system and binding subsequently detected with labelled antibody (Bouillon). Data is expressed as the percentage inhibition of bovine parathyrin binding compared to control tubes pre-incubated in the absence of 1-34 bovine parathyrin.

Preincubation of membranes with growth hormone at concentrations of 20 and 100 ng significantly increased subsequent parathyrin binding by 79 ± 5 % and by 63 ± 10 % respectively. Calcitonin also increased subsequent binding by 13 ± 2 % (20 ng) and by 32 ± 4 % (100 ng). Gastrin, insulin and glucagon (up to 1 μg/tube) did not exert any displacement or enhancement of bovine parathyrin binding.

DISCUSSION

Investigation of the parathyrin receptor in kidney has been hampered by the lack of techniques required to produce biologically active labelled hormone. The classical chloramine-T method of iodination [19] generally produces biologically inactive hormone [8] although claims to the contrary have been made [9]. Biologically active labelled hormone may, however, be produced by the lactoperoxidase method of incorporating ^{125}I [8, 24], by electrolytic iodination [10], or by preparing a tritiated acetamidino derivative [11, 25, 26]. A further method of studying receptor bound hormone is by an indirect approach utilizing labelled antibodies [12].

The addition of excess antibody can completely dissociate binding of hormone from receptors [27] and a ternary cell-insulin-antibody complex could not be observed by Cuatrecasas [28]. However, the present studies were carried out with high specific activity ^{125}I-labelled antibodies. Recently insulin binding to liver receptors has been detected with ferritin labelled antibodies [29] confirming that receptor-hormone-antibody complexes can exist.

The time course of parathyrin binding to renal membranes is of a complex nature as indicated by both methods of measurement. A similar biphasic uptake has been observed for insulin binding to liver membranes [30] and adipocytes [31] and it has been suggested that two receptor sites, acting co-operatively, may be present [30,

32]. Other workers [27, 28] have not observed such uptake. It is possible that differences in the concentration of endogenously bound hormone could alter subsequent binding characteristics.

Displacement of bound ^{125}I-labelled bovine parathyrin occurred with concentrations of bovine parathyrin as low as 8 ng. Since a maximum of 60 % of bound hormone could be displaced it is probable that even lactoperoxidase iodination causes significant structural alteration, although only slight reduction in the activity of such material in the chick hypercalcaemia and adenylate cyclase assays has been reported [24].

The characteristics of the parathyrin receptor, determined by labelled antibody detection, demonstrated many similarities to the receptors for other polypeptide hormones [4]. A pH optimum of around 7.4 is consistent with a physiological role of binding, but the increase at higher pH may be an artefact. However, high pH binding has been observed in a number of hormone-receptor binding studies [33–35].

Studies of bovine parathyrin binding to porcine and bovine renal membranes failed to demonstrate saturation of binding sites and this was probably at least partially due to enzymic degradation of hormone [12]. The increased responsiveness of chick renal adenylate cyclase to parathyrin [23] is consistent with the present results. Saturation of binding occurred at 2.5 pmol/mg membrane protein and there was undetectable binding after prior oxidation of the bovine hormone with H_2O_2 in agreement with Malbon and Zull [11]. The absence of detectable binding with renal mitochondria is not in agreement with the localization studies of Nordquist and Palmieri [36] and would tend to indicate that the material observed by them was probably a degradation product.

The loss of parathyrin binding caused by prior treatment of membranes with phospholipase A or D suggests that phospholipids are crucial for the integrity of the parathyrin receptor. Whether this is due to a change in the receptor itself or due to changes in the membrane environment of the receptor clearly cannot be answered. A number of other hormone-receptor systems are also sensitive to phospholipase treatment [37–40]. Trypsin treatment also caused almost complete abolition of hormone binding at similar concentrations to those causing disruption of other polypeptide hormone receptors [41–44]. A recent study of the renal adenylate cyclase system demonstrated the possibility to selectively proteolyse the parathyrin receptor with 200 μg/ml trypsin while leaving the calcitonin receptor intact [45].

Inhibition of hormone binding by analogues or biologically active fragments is an important criterion for establishing specificity. The 1–34 synthetic fragment of parathyrin caused a reduction in binding of the intact hormone (Fig. 6). However, relatively high levels were required to produce an effect. Since adenylate cyclase stimulation by bovine parathyrin was approximately three times greater than by bovine 1–34 parathyrin (on a molar basis) it appears possible that the affinity of the receptor for intact hormone is much higher than for the fragment.

The stimulation of parathyrin binding by growth hormone may be of physiological significance, although it seems more likely that it is a reflection of the ability of growth hormone to interact with a number of membrane systems producing a conformational change in the membrane protein [46–48]. The slight effect of calcitonin is unlikely to be of physiological significance.

These studies favour the hypothesis that "intact" parathyrin binds to the

receptor since both N-terminal (i.e. biologically active) and C-terminal (immunologically active) regions of the molecule were simultaneously detected in the system. Two anomalies in the present results need further intensive study: the high levels of 1–34 synthetic fragment required to produce inhibition of binding and the inability to measure bound 1–34 fragment with an antibody directed against this fragment. Both results may be explained by an active enzyme system which rapidly metabolizes the 1–34 hormone, the C-terminal region thus serving to protect the intact hormone. Alternatively the former observation may be due to a much higher affinity of the receptor for intact hormone and the latter observation to an inability of the antibody to bind to 1–34 hormone when it is receptor-bound. These questions, among others, are at present under investigation.

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