The Tissue Localization of Tritiated Parathyroid Hormone in Thyroparathyroidectomized Rats*

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SUMMARY

Tritiated acacetamidino-parathyroid hormone localizes rapidly and specifically in the kidneys of thyroparathyroidectomized rats within 30 min after subcutaneous injection. There is some indication of saturation of the kidney by the hormone very early after injection with doses of greater than 40 µg per rat. The hormone is metabolized rapidly to tri-chloroacetic acid-soluble components with a resultant rapid loss of radioactivity from the kidney. Localization in bone is not demonstrable when data are calculated on a tissue weight basis. When calculated as disintegrations per min per g of cells, the bone cells are more radioactive than any other. However, direct evidence for binding to bone cells is not available. It was established that the radioactivity in bone is not due to vascular parathyroid hormone.

Localization of 3H-labeled parathyroid hormone in kidney is less when the parathyroid glands are not removed, suggesting competition by endogenous parathyroid hormone. Neither 3H-insulin or methyl 3H-acetimidate localize in the kidney in the same manner as does parathyroid hormone, but oxidized, inactive, parathyroid hormone binds to kidney in a manner identical with native hormone.

An extensive body of information suggests that the parathyroid hormone exerts its physiological effects by direct action on the kidney and on the bone (1-3). Some evidence also suggests that there may be an action of this hormone on the small intestine (4). However, it has not been possible to investigate direct association of this hormone with its target tissues since techniques for such experiments have not been available. Perhaps the most direct evidence on this point comes from the work of Orimo and Fugita (5) who showed that kidney extracts from normal dogs contain some parathyroid hormone activity which was not present in extracts from parathyroidectomized dogs. In addition, Martin, Melick, and deLuise (6) and deLuise, Martin, and Melick (7) performed tissue distribution studies with iodinated parathyroid hormone which showed a high incorporation of this hormone into the kidney. Further, they demonstrated a rapid metabolism of 125I-labeled parathyroid hormone by kidney microsomes. However, judgment of the significance of their data must await demonstration of the biological activity of such iodinated parathyroid hormone preparations since they appear to be inactive in the classical bioassays.

Our successful preparation of potent tritiated parathyroid hormone of high specific radioactivity makes possible direct investigation of the hormone-tissues interactions (8). This paper reports the tissue localization and distribution of tritiated parathyroid hormone given to thyroparathyroidectomized rats, the time and dose dependency of this distribution, and experiments attempting to delineate the specificity of localization and distribution of acetamidino-parathyroid hormone.

GENERAL METHODS AND MATERIALS

3H-Labeled Parathyroid Hormone—Tritiated acetamidino hormone was prepared as described previously (8). The specific radioactivity of the hormone preparations ranged from 1 to 1.8 × 10^6 dpm per µg.

Animals—Male Sprague-Dawley rats, 150 g, were placed on a low calcium diet for 4 days. The animals were then subjected to surgical thyroparathyroidectomy and subcutaneous injections of labeled hormone or other test solutions were administered within 30 min after surgery. After killing and bleeding the animals, the tissues were removed and dried to constant weight for combustion and counting.

Counting—Tissue samples of 1 g or less were mechanically compressed into pellets. The pelleted tissues were combusted in a Packard Tri-Carb model 300 Trinitium Oxidizer, the tritiated water being collected in counting vials with 15 ml of a mixture consisting of 5 g of 2,5-diphenyloxazole (PPO), 250 mg of 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP), 110 g of naphthalene, 135 ml of absolute methanol, 135 ml of toluene, and 730 ml of dioxane. Counting efficiency for tritium was 10 to 20%.

Oxidation of Parathyroid Hormone—Oxidized parathyroid hormone and 3H-labeled parathyroid hormone were prepared by the addition of 50 µg of labeled or of native parathyroid hormone to a solution of 0.15 M H2O and 5 × 10^-4 M acetic acid in a total volume of 1 ml. The solution was incubated for one hour at 37°C and the reaction then stopped by slow addition of 5 µg of catalase (Sigma). Bioassay showed the oxidized hormone to be inactive in calcium mobilizing activity.

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1 L. M. Sherwood, personal communication.
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FIG. 1. Time dependence for incorporation of radioactivity in the tissues of thyroparathyroidectomized rats after injection of \(^{3}H\)-acetamidino-parathyroid hormone. Data are presented as specific radioactivity normalized to the total injected dose.

TABLE I
Calculation of maximum amount of \(^{3}H\)-labeled parathyroid hormone present in vascular system in femur, from distribution of \(^{3}H\)albumin in blood and femur

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Blood</th>
<th>Femur</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin, dpm/g (\times 10^4)</td>
<td>145:138</td>
<td>1.4:1.4</td>
</tr>
<tr>
<td>Vascular tissue volume (ml/g tissue)</td>
<td>1</td>
<td>0.01:0.001</td>
</tr>
<tr>
<td>(^{3}H)-labeled parathyroid hormone, dpm/g (\times 10^4)</td>
<td>25:24</td>
<td>8.5:8.3</td>
</tr>
<tr>
<td>Maximum parathyroid hormone dpm in vascular system (\times 10^4)</td>
<td>25:24</td>
<td>0.25:0.24</td>
</tr>
<tr>
<td>Percentage of parathyroid hormone dpm in bone vascular system</td>
<td>3.3:2.9</td>
<td></td>
</tr>
</tbody>
</table>

\(^{3}H\)Acetamidino-parathyroid hormone \((10^{7} \text{dpm})\) was injected into two 120-g rats. After 30 min the animals were killed and the blood collected in a heparinized syringe. Disintegrations per min (dpm) per ml of plasma were then determined and whole blood disintegrations per min per ml calculated. Parathyroid hormone data are from earlier experiments on two separate animals.

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Although Fig. 1 shows only kidney, skeletal muscle, bone, lung, and liver, the level of counts in spleen, heart, brain, small and large intestines, and skin were also determined. The data for the liver and lung are representative of these tissues.

Two other significant points were indicated by these data. First, there are very few counts in the bone, although, because of the low cellular content of bone, this result is not too surprising. However, in the bone it was possible that the radioactivity was localized in one or more of three general compartments, the cells, the bone matrix, or the vascular space. Direct demonstration of cellular localization has not yet been possible, but the following experiment was conducted to estimate what portion of the bone radioactivity might be present in the vascular system of this tissue. Labeled serum albumin was prepared by reaction with methyl acetimidate; the labeled albumin was then injected into

TABLE II
Percentage of total disintegrations per min of trichloroacetic acid extractable from kidney and from muscle at 30 min and 6 hours after injection of \(^{3}H\)-labeled parathyroid hormone

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Percentage of disintegrations per min of trichloroacetic acid soluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>64 ± 9</td>
</tr>
<tr>
<td>Muscle</td>
<td>99 ± 1</td>
</tr>
<tr>
<td>Kidney</td>
<td>80 ± 2</td>
</tr>
<tr>
<td>Kidney</td>
<td>92 ± 2</td>
</tr>
</tbody>
</table>

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rungs from two rats were combined and a 5-μl sample was counted for 3H in Bray’s counting solution.

<table>
<thead>
<tr>
<th>Dose (μg)</th>
<th>3H count (% of injected dose per ml urine)</th>
</tr>
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<tbody>
<tr>
<td>40</td>
<td>3.73 (8.4), 1.40 (6.5), 0.47 (14.2)</td>
</tr>
<tr>
<td>15</td>
<td>1.40 (5.7), 1.40 (6.9), 0.47 (8.6)</td>
</tr>
<tr>
<td>5</td>
<td>0.47 (12.8), 0.47 (7.2), 0.093 (5.7)</td>
</tr>
<tr>
<td>1</td>
<td>0.093 (14.0), 0.093 (14.0), 0.093 (14.0)</td>
</tr>
</tbody>
</table>

These data suggest two things. (a) The counts accumulated in muscle after 6 hours are not native parathyroid hormone, but some trichloroacetic acid-soluble breakdown product of the labeled hormone; and (b) the kidney is very active in metabolizing parathyroid hormone since even at 30 min more than 70% of the high amount of radioactivity in this tissue is trichloroacetic acid-soluble. Further investigations on the metabolism of parathyroid hormone by kidney now in progress suggest that, in fact, there are three main products of in vivo parathyroid hormone metabolism in the kidney, two of which are of relatively low molecular weight (<1000) and a third of higher molecular weight.

In regard to the apparent specific localization in the kidney, we investigated whether this might simply reflect concentration of tritium in the urine due to an active excretion by the kidney. However, as shown in Table III, the time course and extent of excretion of counts in the urine does not correlate in any manner with the data of Fig. 1. Excretion appears to reach an early steady state and then remains relatively constant for several hours.

The dependency of the tissue binding of 3H-labeled parathyroid hormone on the administered dose was investigated quite extensively in the hope of showing saturation of the kidney by the hormone, as further indication of specificity of binding. Fig. 2 indicates that at 30 min the specific activity of the kidney is not a purely linear function of dose and supports the suggestion that some saturation occurs. On the other hand, the other tissues did not show this relationship, total tissue radioactivity being a linear function of administered dose. At 2 hours, however, the kidney cannot be saturated with the concentrations used in this experiment (Fig. 3). This fact probably is a reflection of rapid metabolism of hormone suggested by the data in Fig. 1, leading to considerably reduced levels of active parathyroid hormone for binding to the receptor site after 2 hours.

Further evidence for specificity was sought by comparison of the radioactivity present in kidney after 30 min in normal rats compared to thyroparathyroidectomized animals. It was hoped that endogenous hormone might compete with tritiated hormone...
and thereby reduce the binding of the labeled parathyroid hormone. Table IV indicates that there is indeed a significant reduction in the specific activity of the kidney at 30 min when thyroparathyroidectomy is omitted. Again this effect occurs only in the kidney and thus is further evidence for the specific nature of the kidney-parathyroid hormone interaction. Although there was also a small decrease in the average specific activity of the bone, this difference is not statistically significant.

On the other hand, attempts to show competition of exogenous parathyroid hormone with 3H-labeled parathyroid hormone injected into the animals at the same time were not successful and we were unable to show any reduction in specific activity of the kidney by the simultaneous injection of an excess of cold parathyroid hormone. However, in this particular experiment the amount of radioactivity in the kidney at 30 min was considerably less than that which we observed for all other such experiments. Therefore this result is still in question and is the subject of further investigation at this time.

As further test of specificity, the distribution of methyl [3H]-acetamide, [3H]-acetamido-insulin, and oxidized [3H]-labeled parathyroid hormone were compared to that of the [3H]-labeled hormone. These data are in Table V and VI. Both insulin and the imidate ester show some localization in the kidney at 30 min. However, the specific activity attained is not of the same order of magnitude as that for parathyroid hormone. In the case of insulin it should also be noted that the 20 μg dose administered is a huge excess in terms of the amounts of insulin normally administered for physiological experiments. In addition, earlier studies with 311-labeled insulin had also revealed some localization in the kidney (9).

Perhaps the most unexpected result is the apparently identical localization of oxidized compared to native parathyroid hormone (Table V). There is apparently no significant reduction in binding when the hormone-methionine residues are oxidized although the hormone is inactivated by such treatment. Thus, normal methionine residues are not required for the initial binding of parathyroid hormone to its target organ.

**Discussion**

All of our experimentation with the tritiated acetamidino-parathyroid hormone is predicated on the belief that this material is a valid tracer for parathyroid hormone. Although the specific localization in kidney, and the apparent full biological activity argue that this is the case, the final establishment of this point can only come when labeled native parathyroid hormone is available. Thus, the following discussion is presented with the understanding that although we are not tracing native hormone, the data on acetamidino-parathyroid hormone are highly relevant to the function and properties of the native hormone.

These data, then, appear to present direct evidence that the kidney is a receptor tissue for parathyroid hormone. Although this result was predicted from physiological data, this is the first demonstration of direct binding of active preparations of this hormone by kidney. Specific association with other potential target tissues such as bone or intestine is more difficult to show. Although there is radioactivity present in bone, the levels are low and suggest no greater specificity than for any other organ. However, if the bone data are converted to a cellular basis, the localization is quite impressive. For example, if the total radioactivity in bone shown in Fig. 1 is converted to a cellular basis, the specific activity is 50% dose per g of bone cells. However, in the absence of direct demonstration of parathyroid hormone...
binding by bone cells, evaluation of this point is not possible. Although our data suggest that the parathyroid hormone counts in bone cannot be primarily present in the vascular system, we have not excluded the possibility that hormone is bound to other acellular components.

It was hoped that direct data concerning this question might be gathered by autoradiographic techniques but our data show that the level of radioactivity is not adequate for this type of study. However, since the physiological data suggest a direct action on bone, it may be important to compare this tissue with the kidney. In this regard, the most interesting observation is that the bound radioactivity remains relatively constant in bone while peaking and then dropping dramatically in kidney. If “in fact” bone cells do specifically bind parathyroid hormone, the metabolic fate of the hormone must be quite different between the two tissues. These points are presently under investigation in our laboratory.2

In regard to metabolism of the labeled parathyroid hormone, the data suggest that the hormone is rapidly broken down into trichloroacetic acid-soluble fragments, at least one of which then accumulates in muscle. The nature of this product (or products) cannot yet be stated, and is presently being investigated. In general, our results confirm the earlier observations of Martin et al. (6) on the metabolism of parathyroid hormone by kidney. However, their data were obtained with 125I-labeled parathyroid hormone which is biologically inactive. It thus appears that their studies can now be accepted as probably characteristic of the general pattern of behavior for fully active parathyroid hormone. This point illustrates particularly well the utility of the acetamidino-labeled parathyroid hormone for such studies. While iodinated parathyroid hormone is metabolized to identifiable products, these products must contain tyrosine in order to be detected. On the other hand we are able to detect any fragments containing lysine. Since the parathyroid hormone molecule contains only one tyrosine and nine lysines, the advantages of the tritiated acetamidino-parathyroid hormone are obvious.

We are able to detect and should be able to identify the fragments produced by metabolism of parathyroid hormone thereby elucidating questions regarding control of hormonal action and determining whether the kidney converts native parathyroid hormone to an active fragment (or fragments).

Investigation into the question of specificity of the hormone binding to kidney has raised several interesting points. First, the rather striking increment in binding found in thyroparathyroidectomized rats compared to animals with intact glands argues strongly for a specific interaction. One difficulty with this interpretation, however, is that the apparent levels of 125I-labeled parathyroid hormone in blood at 30 min are quite high (~100 ng per ml). Thus, since the steady state level of endogenous parathyroid hormone is 1 ng per ml (11) it would not be expected to be as effective a competitor as shown in Table IV. On the other hand, from the data in Table II it is clear that large amounts of parathyroid hormone in tissue are not native parathyroid hormone but degradation products of the hormone. Therefore it is possible that the levels of native active hormone in the blood do not greatly exceed physiological amounts at any time after the injection, and the high radioactivity of the blood reflects the presence of metabolites of native hormone. In addition, many of the binding sites for parathyroid hormone in kidney may not be immediately available for binding injected hormone simply because of the prior binding of endogenous parathyroid hormone.

The inability of excess native parathyroid hormone injected simultaneously with 125I-labeled parathyroid hormone to inhibit binding of the tritiated hormone is also confusing. However, it may be that the tritiated hormone is more tightly bound to tissue receptor than is native parathyroid hormone, or that absorption from the injection site is more rapid or efficient in the case of the acetamidino derivative. In addition, since competition theoretically can best be shown at saturation levels of hormone, the difficulty of achieving saturation (e.g. Fig. 2) and the fact that in these negative experiments we observed a lower accumulation of radioactivity in the control kidneys may simply indicate that saturation was not achieved and sites were readily available for binding both native and labeled parathyroid hormone. However, unless the latter arguments, it is not clear why the very low levels of endogenous parathyroid hormone should effectively compete with injected hormone (Table IV). Elucidation of these questions awaits further experiments on the mechanism of transport of parathyroid hormone in the blood, the rate of absorption from its site of injection, the relative affinities of acetamidino and native parathyroid hormone for the kidney receptor, and the effects, if any, of parathyroidectomy on the number and kinds of sites available in the kidney for binding parathyroid hormone. Most of these questions are amenable to direct investigation and are the subject of present research in this laboratory.

A highly interesting observation is the fact that oxidized (inactive) parathyroid hormone localizes in the kidney in the same manner as does the native hormone. Thus, it is less surprising that the data of deLuise et al. (7) showing specific localization of 125I-labeled parathyroid hormone is in agreement with our data although the oxidated hormone is inactive. This observation also is in concert with the report that oxidation of methionine does not inhibit binding of parathyroid hormone to its antibody (10). Apparently the initial binding of parathyroid hormone by kidney in vivo, is similar at least in this regard to binding of parathyroid hormone to antibody. Also, it seems clear that the methionine residues are probably not important in the initial receptor mechanism in the target organ. This is in direct contrast to binding of tritiated parathyroid hormone by isolated plasma membrane in vitro, where a reduced methionine is essential.2

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The Tissue Localization of Tritiated Parathyroid Hormone in Thyroparathyroidectomized Rats
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