Loss of Calcitonin Receptors: A Genetically Transmitted Defect in Rats with High Incidence of C-Cell Tumors


ABSTRACT

C-cell tumors (medullary thyroid carcinoma) occur in humans and several other mammalian species. This tumor develops spontaneously with a high incidence (50%) in old Wag/Rij (Wistar-derived strain) rats. We have recently shown that calcitonin binding sites, which are present in the Wistar rats, are lost from renal medulla of the Wag/Rij rats before they reach the age of 1 month. In the present work, we investigated the distribution of calcitonin binding sites in the kidneys of first and second generation hybrids of Wistar and Wag/Rij rats. The absence of calcitonin binding sites from the renal medullas of 25% of F2 hybrids indicates that the deficiency is inherited in a Mendelian fashion and opens the way to establishing inbred strains lacking renal medullary calcitonin binding sites.

INTRODUCTION

MTC1, a calcitonin-secreting tumor, occurs as either spontaneous or familial forms. The familial form of the disease is inherited with the development of multiple endocrine neoplasias (MEN 2a). Penetration of the gene is essentially complete by the age of 40 years. The gene of MTC has recently been assigned to chromosome 10 (4, 5).

Patients suffering from established disease have elevated basal levels of CT (6) and an exaggerated response to secretagogues such as calcium (7, 8) or pentagastrin (9). However, family members at risk from the inherited form of MTC frequently have normal basal CT levels, although the abnormal response to pentagastrin or calcium generally develops later, by the age of 30 in 95% of cases (10). At present, patients at risk of developing familial MTC can only be identified by the presence of elevated basal plasma CT and/or exaggerated CT response to secretagogues. These criteria clearly develop too late for early diagnosis and treatment. There is therefore a need for a reliable method for the early identification of these individuals.

One of the most promising approaches to this problem may be the study of a rat model, the Wag/Rij rat (11). Fifty percent of the rats of this Wistar-derived strain spontaneously develop thyroid medullary carcinoma with age. The tumors have biochemical and morphological characteristics similar to those of human MTC. Wag/Rij rats show the exaggerated response to calcium challenge, develop thyroid C-cell hyperplasias (12) and develop tumors by the age of 18–24 months.

We have recently shown (13) that the renal medullas of these Wag/Rij rats lack CT-binding sites by the time the rats are 3 weeks old. This loss of renal receptors precedes the development of the exaggerated response to calcium challenge.

MATERIALS AND METHODS

ABSTRACT

We have recently shown that calcitonin binding sites, which are present in the Wistar rats, are lost from renal medulla of the Wag/Rij rats before they reach the age of 1 month. In the present work, we investigated the distribution of calcitonin binding sites in the kidneys of first and second generation hybrids of Wistar and Wag/Rij rats. The absence of calcitonin binding sites from the renal medullas of 25% of F2 hybrids indicates that the deficiency is inherited in a Mendelian fashion and opens the way to establishing inbred strains lacking renal medullary calcitonin binding sites.

INTRODUCTION

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3 The abbreviations used are: MCT; medullary thyroid carcinoma; CT, calcitonin; sCT, salmon calcitonin.

The present study was carried out to establish the genetic origin of this defect by crossing Wag/Rij rats with Wistar rats and studying the distribution of CT-binding site in the kidneys of the F1 and F2 hybrids. The results clearly show that the absence of renal binding sites in the Wag/Rij rats is genetically transmitted.

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was raised in the sheep against the unconjugated intact synthetic human molecule.

The reproducibility of the calcium challenge was checked by subjecting 18 F2 hybrids, (nine males and nine females, 3 months old) to two calcium challenges at a 1-week interval. The blood samples obtained from these two challenges were assayed for calcitonin as indicated above.

Calcitonin Extraction. Individual thyroid glands were extracted with 4 M guanidium thiocyanate diluted 100x in radioimmunoassay buffer and aliquots were taken for CT determination.

Statistical Analysis. All data were analyzed by analysis of variance: the mean were compared using Student's t test (18). Confidence limits for the number of calcitonin binding sites in the medulla I in the F2 hybrids were computed for \( P < 0.05 \) and the animals classified according to these criteria into three types, Wistar, Wag/Rij, and intermediate.

### RESULTS

Weight. The male rats of all the strains studied were significantly \( (P < 0.001) \) heavier than the females. The Wistar rats of both sexes were significantly heavier \( (P < 0.001) \) than the Wag/Rij rats. The weights of the F1 and F2 hybrid males and females were intermediate between those of the parents.

Renal Calcitonin Binding Sites. Wistar and Wag/Rij rats pattern of \(^{125}\text{T}-\text{sCT}\) renal binding sites were identical to those previously described (13). The sCT binding sites in a typical Wistar kidney (Fig. 1A) are patchy in the peripheral cortical area. The outer medulla (OM) of these rats can be divided into three layers: medulla I and medulla III which are very rich in binding sites, and between them, medulla II, with few binding sites. Mean values ± SE are reported on the first line of Table 1. The Wag/Rij kidney (Fig. 1B) has few medullary binding sites (Table 1, fourth line), while the cortical sites do not seem to be affected. The patterns in males and females of both strains are similar, and therefore the results of both sexes were pooled.

The number of cortical binding sites in F1 and F2 hybrids were not significantly different from those of the parent strains and were the same in males and females. The number and distribution of medullary binding sites in the F1 and F2 hybrids showed no sex-dependent difference, the values for males and females were therefore pooled. The F1 kidneys showed the three regions seen in the parent strains (Fig. 1, C and D). The receptor concentrations of medulla I and III were similar and intermediate between the equivalent values of the parent kidneys, but closer to the Wistar values (Table 1, second line).

The mean numbers of calcitonin binding sites in the medullary areas of F2 kidneys were intermediate between those of the F1 and Wag/Rij (Table 1, third line). However, the distributions in individual animals varied from that of a typical Wistar rat (Fig. 1E) to a typical Wag/Rij pattern (Fig. 1F) with intermediate forms (Fig. 1, G and H).

Calcium Challenge. Plasma calcium and CT values, 4 min after the challenge are shown in Table 2. The greatest increase in plasma calcium was recorded for F1 and the lowest in Wag/Rij; Wistar and F2 animals had intermediate values.

The increase in plasma CT following calcium challenge was significantly greater in the Wag/Rij (2951 pg/ml ± 253) than the Wistar rats (1254 pg/ml ± 96). \( (P < 0.001) \).

The mean increase in plasma CT in F1 hybrids (6444 pg/ml ± 1103) was greater than that of the Wag/Rij rats, and significantly different from those of both parent strains \( (P < 0.001). \)

The mean increase in plasma CT of F2 hybrids (2375 pg/ml ± 271 pg/ml) was intermediate between the mean values of the parent strains. However, individual responses ranged from low (Wistar type) to greater than typical Wag/Rij values (Fig. 2).

### Table 1

<table>
<thead>
<tr>
<th>Series</th>
<th>Medulla 1</th>
<th>Medulla 2</th>
<th>Medulla 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar (5)*</td>
<td>68.5 ± 4.1</td>
<td>10.8 ± 0.7</td>
<td>49.6 ± 4.3</td>
</tr>
<tr>
<td>F1 (4)</td>
<td>43.0 ± 2.2</td>
<td>11.8 ± 0.8</td>
<td>32.0 ± 2.5</td>
</tr>
<tr>
<td>F2 (24)</td>
<td>32.3 ± 3.5</td>
<td>9.6 ± 0.8</td>
<td>22.2 ± 2.2</td>
</tr>
<tr>
<td>Wag/Rij (5)</td>
<td>8.6 ± 0.8</td>
<td>4.7 ± 0.3</td>
<td>12.5 ± 1.4</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, number of animals.

### Table 2

<table>
<thead>
<tr>
<th>Series</th>
<th>Medulla 1</th>
<th>Medulla 2</th>
<th>Medulla 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar vs. Wag/Rij</td>
<td>t1 = 6.5(^*)</td>
<td>t'1 = 3.0(^*)</td>
<td>t&quot;1 = 6.1(^*)</td>
</tr>
<tr>
<td>Wistar vs. F1</td>
<td>t2 = 2.6(^*)</td>
<td>t&quot;2 = 0.4</td>
<td>t&quot;2 = 1.0</td>
</tr>
<tr>
<td>Wistar vs. F2</td>
<td>t3 = 5.1(^*)</td>
<td>t&quot;3 = 0.8</td>
<td>t&quot;3 = 5.8(^*)</td>
</tr>
<tr>
<td>Wag/Rij vs. F1</td>
<td>t4 = 3.5(^*)</td>
<td>t&quot;4 = 3.3(^*)</td>
<td>t&quot;4 = 4.8(^*)</td>
</tr>
<tr>
<td>Wag/Rij vs. F2</td>
<td>t5 = 3.3(^*)</td>
<td>t&quot;5 = 3.0(^*)</td>
<td>t&quot;5 = 2.0(^*)</td>
</tr>
<tr>
<td>F1 vs. F2</td>
<td>t6 = 1.4</td>
<td>t&quot;6 = 1.2</td>
<td>t&quot;6 = 4.0(^*)</td>
</tr>
</tbody>
</table>

\(^*\) Significantly different, \( P < 0.05 \).

Plasma CT values for the 18 F2 hybrids challenged twice with calcium at a 1-week interval ranged from 1101 to 7105 pg/ml. The correlation between the results of the two assays was 0.952 \( (P < 0.001) \), and the slope of the regression curve...
DISCUSSION

Early identification of subjects at risk of developing familial MTC is extremely difficult as there is a complete absence of early clinical signs of calcitonin hypersecretion. The Wag/Rij rat may be a useful model of the disease as a high percentage (50%) develop C-cell tumor by the age of 18–24 months, respond to calcium challenge by the age of 3 months, and lack renal medullary CT receptors by the age of 1 month. However, the Wag/Rij rat cannot be a perfect model because almost all of these rats eventually develop tumors, while the percentage of human family members who develop familial MTC is much lower. The present study was designed to answer two questions: (a) Is the renal receptor defect acquired or genetically transmitted? (b) Is the enhanced secretion of CT after calcium challenge associated with the reduction in medullary CT receptors?

The results of this study of F1 and F2 hybrids of Wistar and Wag/Rij crosses confirmed our earlier results showing that Wag/Rij rats had an exaggerated response to calcium challenge and severely reduced renal medullary CT receptors. They also demonstrate that both these parameters varied between the parental norms in individual F1 hybrids.

The distribution of CT binding sites in the medulla of the F1 hybrids could indicate that the genetic defect responsible for the disappearance of these sites is dominant because concentration of these sites was lower in the kidneys of F1 hybrids than in those of the Wistar strain. The F1 hybrids also have higher peak calcitonin responses to a calcium challenge than did either the Wistar strain or the Wag/Rij strain. This could imply that the genes responsible for this enhanced response are also dominant and that the increased response is partly due to their higher expression in animals which are heavier and healthier than the original Wag/Rij strain, or to the more pronounced hypercalcemia in this group.

The distribution of calcitonin binding sites in the kidneys of the F2 hybrids is compatible with a classical Mendelian inheritance pattern: roughly one quarter of the animals had kidneys lacking calcitonin binding sites (typical Wag/Rij), one quarter had sites present at a density comparable to that of the Wistar strain, and the remaining animals had an intermediate type of distribution. Calcium challenge elicited increases in circulating calcitonin in F2 hybrids, which were either in the range of the normal Wistar rat, close to the normal Wag/Rij rat or intermediate between the two.

The loss of CT medullary binding sites is apparently not associated with a change in the CT secretion pattern, as the number of medullary CT binding sites is not correlated with either the thyroid gland CT content or the postchallenge increase in circulating CT.

The calcium challenge used in this study is a fairly good measure of C-cell secretory capacity as there was a positive significant correlation between the thyroidal CT content and

### Table 2. Increase of plasma calcium and circulating calcitonin levels 4 min after calcium challenge comparatively in the four series

<table>
<thead>
<tr>
<th>Series</th>
<th>Ca Increase (ΔCa)</th>
<th>CT Increase (ΔCT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar (44)</td>
<td>57.7 ± 1.0</td>
<td>1254 ± 96</td>
</tr>
<tr>
<td>F1 (18)</td>
<td>68.6 ± 3.0</td>
<td>6444 ± 1103</td>
</tr>
<tr>
<td>F2 (42)</td>
<td>59.7 ± 1.5</td>
<td>2375 ± 271</td>
</tr>
<tr>
<td>Wag/Rij (42)</td>
<td>47.0 ± 1.2</td>
<td>2951 ± 263</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Wistar vs. Wag/Rij</th>
<th>Wistar vs. F1</th>
<th>Wistar vs. F2</th>
<th>Wag/Rij vs. F1</th>
<th>Wag/Rij vs. F2</th>
<th>F1 vs. F2</th>
</tr>
</thead>
<tbody>
<tr>
<td>t1</td>
<td>5.6</td>
<td>t2 = 4.3</td>
<td>t3 = 1.0</td>
<td>t4 = 8.6</td>
<td>t5 = 6.5</td>
<td>t6 = 3.5</td>
</tr>
<tr>
<td>t1'</td>
<td>3.75</td>
<td>8.8</td>
<td>2.4</td>
<td>5.9</td>
<td>1.25</td>
<td>6.9</td>
</tr>
</tbody>
</table>

* Highly significant, \( P < 0.01 \).
* Significantly different, \( P < 0.05 \).

Almost unity (1.13 ± 0.009, \( P < 0.001 \)).
the increased secretion of calcitonin, and as the response of the animals to the test is reproducible. It is therefore likely that the genes involved in the exaggerated secretion of calcitonin and those responsible for the loss of the renal medullary CT binding sites are segregated independently. Furthermore, while the distribution of the renal medullary binding sites is compatible with a Mendelian type of inheritance, the enhanced secretion of CT in response to a calcium challenge is not. A long-term study of the development of tumours in F2 animals hybrids will be required to establish whether the loss of calcitonin binding sites in the renal medulla and/or the enhanced secretion of calcitonin in response to a calcium challenge are associated with the development of C-cell tumours.

F2 hybrids represent a better animal model for high risk individuals in the familial form of the disease than do the parental Wag/Rij rats, because not all animals show an exaggerated secretion of calcitonin in response to a calcium challenge. All rats of the Wag/Rij strain show abnormal increase in plasma calcitonin in response to a secretagogue, even though, according to Boorman (11), only 50% are liable to develop tumours.

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REFERENCES


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