Proliferative Pituitary Lesions in Rats Treated with Salmon or Porcine Calcitonin

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ABSTRACT
Calcitonin, the serum calcium-lowering hormone, has been used in the treatment of hypercalcemia of malignancy and postmenopausal osteoporosis in humans for several years without any adverse effects. Recent studies in rats have indicated that calcitonin may be associated with morphologic effects on the pituitary. A large study was performed on 2 strains of rats, Sprague-Dawley (SD) and Fischer-344 (F-344), with 2 types of calcitonin, salmon-derived (sCT) and porcine-derived (pCT) calcitonin, to evaluate possible effects on the pituitary. Sixteen groups of 42 male and 42 female SD or F-344 rats were given 0 (vehicle control), 1.25, 5.0, or 80.0 IU/kg/day of sCT or pCT, once daily, subcutaneously, for 1 yr.

An increased incidence of adenomas of the adenohypophysis was observed in male SD rats at all dose levels of sCT, female SD rats given 80 IU/kg/day of sCT, male SD rats at the high dose level of pCT, and male F-344 rats at the high dose level of sCT. Also, an increased incidence of total proliferative lesions, due mostly to an increased incidence of focal hyperplasia of the pars distalis, occurred in female F-344 rats given the high dose of sCT. These pituitary proliferations were histologically similar to those that occur spontaneously, and the incidences observed were comparable to those that could occur in rats on 2-yr or lifetime studies, indicating that the injection of calcitonin had decreased the latency period.

Keywords: Adenohypophysis; adenocarcinoma; adenoma; hormone; hypercalcemia; osteoporosis; SD rats; F-344 rats

INTRODUCTION
It has long been recognized that the endocrine system of rats, and particularly the pituitary gland, has a propensity for the development of spontaneous tumors. Typically, pituitary adenomas occur in the adenohypophysis of aged rats where they probably originate from hyperplastic foci (9, 15, 16). In general, there is a female predominance; however, both sexes are affected. Because of expansive growth and compression of the overlying brain, pituitary adenomas are frequently lethal. Malignancy, which is usually recognized by local invasion, is rare. Because of the high spontaneous incidence of pituitary adenomas, it is often difficult to determine whether drugs or other xenobiotics are responsible for an increase in pituitary adenomas. Nevertheless, an increased incidence of these tumors has been reported with a wide variety of agents including drugs (pirazolate, ribavirin, leuprolide) (6), caffeine (18), estrogens (4, 8, 13), and ionizing radiation (5, 13, 15).

The serum calcium-lowering hormone, calcitonin, has been used for more than 15 yr in the treatment of hypercalcemia (e.g., of malignancy) and for other human diseases, including postmenopausal osteoporosis. Despite this long history, there are no reports of adverse findings related to the pituitary in humans. However, in a study conducted by Boorman et al (3), pituitary tumors occurred in rats that received transplants of homologous calcitonin-secreting thyroid medullary carcinomas. Also, Lee et al (10) observed that thyroid C-cell proliferative lesions in aged Long-Evans rats were associated with proliferative lesions in the adenohypophysis. This is noteworthy in that rats differ from what is observed in multiple endocrine neoplasia syndrome in humans, where there is no correlation between the occurrence of thyroid medullary carcinoma and pituitary adenoma (10). Therefore, it is very likely that these pituitary effects in rats treated with calcitonin are species-specific.
In the present study, we report an increased incidence of proliferative lesions of the pituitary in Sprague-Dawley (SD) and Fischer-344 (F-344) rats that is associated with the daily subcutaneous injection of salmon-derived (sCT) or porcine-derived (pCT) calcitonin at dosages as high as 80 IU/kg/day for 1 yr.

**Materials and Methods**

**Test Compounds.** The pharmacology and structure of pCT and sCT have been described (1). In this study, 1 ml of either pCT or sCT containing 1.25, 5.0, or 80.0 IU in their respective vehicles was used.

**Animals and Treatment Groups.** A total of 336 male and 336 female SD [Crl:CDR(SD)BR] and 336 male and 336 female F-344 [CDF(F-344)/CrlBR] weanling rats were used in the study. The study consisted of 2 segments (No. 82878 and No. 82879), using both strains of rats in each segment, which ran concurrently in adjoining animal rooms. In each segment, the rats were randomized into 8 treatment groups of each strain and sex. The groups consisted of 2 vehicle control groups, 3 groups that received sCT at dosages of 1.25, 5.0, or 80.0 IU/kg/day, and 3 groups that received pCT at dosages of 1.25, 5.0, or 80.0 IU/kg/day. The rats were injected with the vehicle or test substances once daily for 52 wk. In the first segment there were 20 animals per sex per group of each strain and, in the second, there were 22 animals per sex per group of each strain.

**Pathological Examination.** Necropsy examinations were performed on all rats, including those that died (unscheduled deaths) and those that survived to the 1 yr terminal necropsy. At necropsy, the pituitaries were fixed in situ in Bouin's solution except for some early deaths in which the pituitaries were fixed in 10% neutral-buffered formalin. A representative sample of the pituitary from each rat was processed, embedded in paraffin, and sectioned at 5 μm. In the first segment of the study, 2 hematoxylin-and-eosin-stained sections, approximately 30 sections apart from each pituitary, were prepared and examined. In the second segment, a single representative hematoxylin-and-eosin-stained section from each pituitary was prepared and examined. Since there was essentially no difference in the incidence of proliferative lesions between the first and second sections of pituitary in the first segment of the study, only 1 section was prepared and examined from rats of the second segment of the study.

**Results**

Significant numbers of unscheduled deaths only occurred in the male SD rats administered 80 IU/kg/day sCT, and most of the deaths (32/42) occurred after week 45 of the study. All of the rats had pituitary adenomas, and although histopathologic examination was not performed on any other tissue, it was reasonable to conclude that the tumors were contributory to the death of these rats.

Gross examination of the pituitary revealed enlargement and/or discoloration in rats of all groups; however, the incidences of these gross changes increased in the SD and F-344 males receiving 80 IU/kg/day sCT.

The incidence of microscopic proliferative lesions in the pituitary glands of male and female rats of the SD and F-344 strains given sCT or pCT is summarized in Tables I and II. An increased incidence of adenomas of the adenohypophysis occurred in male SD rats at all dosages of sCT, female SD rats given the high dose of sCT, male F-344 rats given the high dose of sCT, and male SD rats given the high dose of pCT. Female F-344 rats given the high dose of sCT had an increased incidence of total proliferative lesions, which was predominantly due to an increased incidence of focal hyperplasia of the pars distalis compared to the respective control group. A single adenocarcinoma was diagnosed in 1 male SD rat given the mid-dose of sCT.

The histomorphologic characteristics of these proliferative lesions in the adenohypophysis were consistent with those that occur spontaneously, and the criteria for their diagnosis was that which is commonly used for the pituitary in laboratory rats (11, 15). Lesions diagnosed as focal hyperplasia of the pars distalis were variably sized areas of an increased cellularity with either a uniform population of cells or a mixture of cell types that blended imperceptibly with the adjacent parenchyma (Figs. 1 and 2). These foci did not cause compression or distortion of the surrounding tissue. Affected pituitaries had either single or multiple foci. Lesions classified as adenomas of the adenohypophysis were usually larger areas or nodules with either a pleomorphic or monomorphic population of cells with evidence of growth by expansion and resulting compression of adjacent tissue. In some instances, the tumors involved the entire lobe and had resulted in compression and distortion of the pars intermedia and pars nervosa (Figs. 3 and 4). The cells within the adenomas often were variable in their morphologic characteristics. In several of the tumors, the cells were large with an abundant eosinophilic cytoplasm and large basophilic and pleomorphic nuclei. In other tumors, a variety of cell types was involved. Other changes within the tumors included cystic degeneration, congestion, hemorrhage, and/or necrosis. Although many of the tumors had varying degrees of pleomorphism and mitotic activity,
TABLE I.—Incidence of proliferative pituitary lesions of the adenohypophysis in SD and F-344 male rats injected with salmon or porcine calcitonin.

<table>
<thead>
<tr>
<th>Dose level (IU/kg/day):</th>
<th>SD</th>
<th>Salmon</th>
<th>0</th>
<th>1.25</th>
<th>5.0</th>
<th>80.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose group (n = 42):</td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Adenoma, pars distalis</td>
<td></td>
<td></td>
<td>42</td>
<td>42</td>
<td>41</td>
<td>42</td>
</tr>
<tr>
<td>Adenocarcinoma, pars distalis</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hyperplasia, pars distalis, focal</td>
<td></td>
<td></td>
<td>15</td>
<td>16</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>Adenoma, adenohypophysis</td>
<td></td>
<td></td>
<td>14</td>
<td>19</td>
<td>22</td>
<td>25†</td>
</tr>
<tr>
<td>Number examined</td>
<td>42</td>
<td>42</td>
<td></td>
<td>42</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>Number of rats with hyperplasia only and neoplasia</td>
<td>20</td>
<td>33*</td>
<td>35*</td>
<td>39**</td>
<td>38†</td>
<td>38†</td>
</tr>
</tbody>
</table>

*; Fisher's exact test, one tail, p ≤ 0.05.
†; Fisher's exact test, one tail, p ≤ 0.05.
**; Fisher's exact test, one tail, p ≤ 0.05.
††; Fisher's exact test, one tail, p ≤ 0.05.

TABLE II.—Incidence of proliferative pituitary lesions of the adenohypophysis in SD and F-344 female rats injected with salmon or porcine calcitonin.

<table>
<thead>
<tr>
<th>Dose level (IU/kg/day):</th>
<th>SD</th>
<th>Salmon</th>
<th>0</th>
<th>1.25</th>
<th>5.0</th>
<th>80.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose group (n = 42):</td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Adenoma, pars distalis</td>
<td></td>
<td></td>
<td>42</td>
<td>42</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>Adenocarcinoma, pars distalis</td>
<td></td>
<td></td>
<td>14</td>
<td>14</td>
<td>15</td>
<td>28*</td>
</tr>
<tr>
<td>Hyperplasia, pars distalis, diffuse</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Number examined</td>
<td>42</td>
<td>42</td>
<td></td>
<td>42</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>Number of rats with hyperplasia only and neoplasia</td>
<td>27</td>
<td>29</td>
<td>25</td>
<td>38*</td>
<td>30</td>
<td>32</td>
</tr>
</tbody>
</table>

*; Fisher's exact test, one tail, p ≤ 0.05.
†; Fisher's exact test, one tail, p ≤ 0.05.
**; Fisher's exact test, one tail, p ≤ 0.05.
††; Fisher's exact test, one tail, p ≤ 0.05.
pituitary tumors of this type in the absence of invasion or metastasis are considered to be benign in rats (11). The single lesion, diagnosed as adenocarcinoma, had invaded the overlying brain.

The other microscopic changes observed in the pituitary of rats of the various groups of both strains, including the controls, were of the type that are commonly encountered as incidental findings in the pituitary of aged laboratory rats, and their type or incidence was not influenced by injection with sCT or pCT. Such incidental findings included cysts, congestion, dilatation of the hypophysial cavity, and dilatation of small vascular sinuses.

**DISCUSSION**

An increased incidence of adenomas of the adenohypophysis occurred in male SD rats at all 3 dosages of sCT and in female rats given the high dose of sCT and in male SD rats given the high dose of pCT. In the F-344 rats, an increased incidence of adenomas (male rats) or the combined incidence of focal hyperplasia and adenomas (female rats) occurred at the high dose of sCT. These incidences were statistically significantly higher than the controls and were considered to be treatment-related (Tables I and II).

Histologically, the adenomas in the rats of the vehicle control, as well as the sCT- and pCT-exposed rats, were comparable to those that occur spontaneously in rats. Only the incidence of these tumors was influenced by the compound administration and not the histomorphology. There was no evidence of an increased incidence of malignant transformation in the pituitary glands of any of the affected groups of rats given sCT or pCT of either strain. Only 1 adenocarcinoma was diagnosed in the entire study.

The relatively high spontaneous incidence of total proliferative pituitary lesions in control rats at 1 yr was unexpected. The incidences of total proliferative lesions in the adenohypophysis in the control rats were as follows: male SD rats 50/84 (60%), female SD rats 57/84 (68%), male F-344 rats 48/84 (57%), and female F-344 rats 25/84 (30%). In fact, the incidence of total proliferative lesions in several of the control groups, particularly of the SD strain, of this study was within the range of or even higher than the incidence of pituitary tumors reported in some long-term (>2-yr) studies reported in the literature (7, 15, 17). This is undoubtedly attributable in large part to the focus of the study on the pituitary,
EFFECTS OF CALCITONIN ON RAT PITUITARY

The relatively large number of animals in the study, and the detailed character of all facets of the study related to evaluation of the pituitary, i.e., fixation in situ, embedding, sectioning, and, in one segment of the study, examining at least two sections of each pituitary.

The histopathologic findings from this study are supportive of the concept that hyperplastic lesions of the pituitary are precursors to adenomas. Microscopically, there appears to be a continuum from the hyperplastic lesions to the small adenomas.

The results of this study also suggest that the proliferative lesions in the adenohypophysis of the pituitary may develop earlier than expected in laboratory rats. Therefore, compounds that have an effect on endocrine organs could cause an increased incidence of proliferative lesions in the pituitary when given to particular species, such as the rat, which have a propensity for spontaneously occurring proliferative lesions in the endocrine system.

The results from immunohistochemical assays and radioimmunoassays on material collected from this study have been reported elsewhere (7). These evaluations have demonstrated that the pituitary adenomas related to calcitonin treatment express free alpha-subunit that is common to the glycoprotein hormones, leutinizing hormone, follicle-stimulating hormone, and thyroid-stimulating hormone. Although lactotrophic (PRL) tumors are the most common type of pituitary tumor in rats, McComb et al (12) and Sandusky et al (14) have shown that approximately 10-20% of spontaneously occurring pituitary adenomas are nonfunctional. We have had the opportunity to study 5 of the nonfunctioning tumors reported by Sandusky et al (14) and have found immunohistochemical evidence of alpha-subunit in 3. It is also noteworthy that a few of the pituitary tumors in control rats of this study were shown to have alpha-subunit expression by immunohistochemical studies (7). As in humans, it appears that a major fraction of the pituitary tumors in rats that were previously classified as nonfunctioning tumors are expressing alpha-subunit (2).

Nevertheless, the proliferative reaction in the pituitary of rats in response to calcitonin appears to be species-specific. No adverse effects to the pituitary have been reported in humans, and neither focal hyperplasia nor adenomas have been observed in chronic studies in mice or in dogs (unpublished data).

We conclude that, although administration of calcitonin at doses 400 times the human clinical dose for 1 yr reduces the latency period for the production of nonfunctional proliferative lesions in the ade-

Fig. 3.—Adenoma, pars distalis. A large mass originating in the pars distalis with dilated blood-filled sinuses causing peripheral compression from a male SD rat given sCT vehicle. H&E. × 12.5.

Fig. 4.—Higher magnification of the pituitary adenoma, with a solid mass of proliferating cells with a discrete border causing peripheral compression. H&E. ×50.
nohypophysis of rats, it neither induces the hyperplastic/neoplastic process nor does it result in a substantial increase in the overall incidence of proliferative lesions. This suggests that long-term administration of calcitonin in rats perturbs the spontaneous pathophysiologic event(s) that leads to the development of nonfunctional ("null"") tumors in this species.

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REFERENCES


