Congenital hypothyroidism in two cats due to defective organification: data suggesting loosely anchored thyroperoxidase

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Abstract. Two cats with congenital hypothyroidism are described. In vivo discharge of accumulated labelled iodide by perchlorate administration revealed defective organification of iodide, which was complete in one cat and partial in the other. In the cat with the partial organification defect, thyroid tissue was obtained for biochemical studies. No membrane-bound peroxidase activity could be demonstrated. The activity was found in the 100,000 x g supernatant. It is suggested that the loose enzyme anchoring caused decreased availability of peroxidase and as a consequence reduced capacity for organic binding of trapped iodide.

Several congenital disorders in thyroid hormone synthesis have been described. Identification of the molecular nature of the defects has been greatly facilitated for those defects for which a model system has become available, e.g. hereditary congenital goitre in Dutch goats (1-4) and Afrikander cattle (5,6). Thus far only defects in thyroglobulin synthesis have been found in these animal diseases. Another defective step in iodothyronine synthesis may be the binding of iodine to the tyrosyl residues of thyroglobulin, a process requiring H₂O₂ generation and thyroperoxidase (7). In animals these so-called organification defects have been demonstrated by in vivo discharge tests in two dogs (8,9) and in one cat (10).

Here we describe two hypothyroid cats in which the organification defect was demonstrated in vivo. In one of these cats thyroid tissue was obtained and a partial peroxidase defect was found. Evidence is presented that the enzyme was loosely anchored in the membrane.

Animals and Methods

Case reports

Kitten 1. In July 1985, an 8-week-old male European short-haired kitten, body mass 0.6 kg, was presented for retarded growth, slow mental activity and disproportionate features. According to the owner the animal was half the size of its littermates. Physical examination revealed a strikingly large and round head with small ears set far apart, a short and broad neck, and short limbs. The kitten had a slightly distended abdomen and tended to fall asleep during the examination. The plasma thyroxine concentration (11) of 4 nmol/l fell clearly below the reference range (9-46 nmol/l) for adult cats (12).

The prescribed thyroxine replacement therapy was discontinued by the owner several times and stopped definitely when the animal was two years old. Six months later the cat was hospitalized for further studies, at which time the plasma thyroxine concentration was 15 nmol/l. Nine months after this, at the time of hemithyroidectomy, both thyroids were found to be goitrous and extended from the larynx to the thoracic inlet. The plasma thyroxine concentration was 19 nmol/l.

Kitten 2. In July 1986, an 8-week-old male European short-haired kitten, body mass 0.5 kg, was presented with one of its littermates (Fig. 1). The animal was striking in its juvenile appearance, characterized by a round head, tiny ears and blue irises. In the littermates the colour of the irises had already changed to the yellow of adulthood.
Other features were similar to those of kitten 1. Radiographic examination revealed retarded skeletal development. The plasma thyroxine concentration was 1 nmol/l. On replacement therapy with 10 μg thyroxine once daily the kitten developed to a normal-sized, somewhat lethargic cat. Further studies were performed at the age of about two years. At this time the replacement therapy had been discontinued by the owner for several months, resulting in a plasma thyroxine concentration of <2 nmol/l. In this animal the thyroid glands could not be palpated.

Reference materials
Thyroid glands of 6 hyperthyroid cats were obtained by surgical hemithyroidectomy similar to the procedure in kitten 1. Thyroid tissues of 3 healthy adult cats were obtained immediately following euthanasia by iv injection of pentobarbitone. All 9 cats were house cats and had been fed commercial cat foods.

Methods
Following iv injections of 1.8 MBq Na$^{231}$I the thyroidal radiiodine uptake (RIU) was measured at 1, 4, 24, 48, and 72 h as previously described (13). For the perchlorate discharge test the RIU measurements were carried out every 15 min for a period of 4 h. At 1 h after injection of 0.9 MBq Na$^{231}$I, 20 mg aqueous potassium perchlorate was injected iv.

Immediately following excision the thyroid tissue was dissected free from fat. About 1/3 was placed in liquid nitrogen and stored at −70°C and the remaining 2/3 was fixed in 10% formalin for histological examination.

The frozen tissues were homogenized in a Sorvall Omnimixer in 0.1 mol/l TRIS-HCl at pH 8.3, containing 0.1 mol/l KI and 0.06 mmol/l PMSF (phenyl methane sulphonl fluoride). After centrifugation at 1000 g a microsomal fraction was prepared by centrifugation (100 000 x g at 4°C). The supernatants were used for measurements of thyroglobulin and thyroperoxidase activity. The pellets containing the microsomes were resuspended in a TRIS-HCl buffer (pH 8.3), containing 0.5% sodium deoxycholate to solubilize the lipophilic membranes. For further solubilization the membranes were treated with trypsin (14).

Peroxidase activity was measured in the supernatants and the solubilized membranes by the oxidation of ABTS (2,2-amino-di-[3-ethyl-benzthiazoline-(6)-sulphonic acid], diammonium salt) from Sigma, St. Louis, MO, using a H$_2$O$_2$ concentration of 25 μmol/l (13). The experiments were carried out on a Shimadzu UV-200 spectrophotometer at 420 nm.

The 100 000 x g supernatants were precipitated with 50% ammonium sulphate. The precipitates were solubilized in and dialysed against PBS. Electrophoresis of these preparations was performed on a polyacrylamide gel gradient (4-32%) in a Pharmacia system. The positions of the bands were compared with those of high molecular weight standards (Pharmacia, Uppsala).

The presence of thyroxine in the thyroglobulin of these preparations was demonstrated in an ELISA. Nunc 96-well plates were coated with thyroglobulin preparations from cat 1, the euthyroid control cats and the hyperthyroid cats in amounts ranging from 0 to 200 ng protein per well and allowed to dry overnight at 37°C. The plates were incubated with monoclonal antibody B2 and subsequently with the conjugate rabbit anti-mouse Ig conjugated to alkaline phosphatase. Bound phosphatase activity was revealed with p-nitrophenyl phosphate (16).

The iodine content was measured as described previously (1). Essentially, the 152I content was determined by measurement of the slope of the log extinction at 420 nm as a function of time, in a spectrophotometer (Cobas Bio Diagnostica, Hoffman La Roche, Basle, Switzerland), after digestion of the iodine-containing compounds with HClO$_3$. The iodine content was related to the protein content as measured by absorption at 280 nm, using the extinction coefficient for thyroglobulin (17).

![Fig. 1.](image) Two 8-week-old littermate kittens. As compared with the healthy littermate (left) the hypothyroid kitten 2 (right) has a juvenile appearance that is manifested by the round head and small ears.
Results

In vivo studies
In both cats the injected radioiodide accumulated very rapidly in the thyroid glands, as compared with the low initial uptakes observed in healthy cats (Fig. 2). In cat 1 the radioiodide uptake values reached a very high maximum (87%) at 24 h after injection, followed by a slight decrease. Cat 2 had a rapid initial uptake that remained at about the same level (17%) until 24 h, and then decreased slightly.

After four measurements at 15-min intervals the iv injection of perchlorate caused a rapid decline in thyroidal radioactivity (Fig. 3). At 30 min after the perchlorate injection the uptake decreased by 51% in cat 1 and by 63% in cat 2. In both cats thyroidal radioactivity remained at the same level for the following 2.5 h, although the levels were different (in cat 1 approximately 20% and in cat 2 approximately 8%).

In vitro studies
In the thyroid tissue of cat 1 no thyroid peroxidase activity could be detected in the microsomal fraction before or after trypsin treatment. However, in the 100 000 xg supernatant there was detectable activity. In the tissues collected from hyperthyroid cats there was thyroid peroxidase activity in the

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Fig. 2. Thyroidal radioiodine uptake (RIU) curves (expressed as percentage of the injected dose) in hypothyroid cats 1 (dashed line) and 2 (solid line) as compared with the range observed in 10 healthy house cats (shaded area) (13).

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Fig. 3. Measurements of thyroidal radioiodine uptake (RIU) at 15-min intervals (solid lines) in cat 1 (upper panel) and cat 2 (lower panel). In both cats radioiodine uptake studies were repeated three days later (interrupted lines), but with iv administration of 20 mg potassium perchlorate (arrow), 1 h after the administration of radioiodide.

Table 1. Thyroperoxidase (TPO) activity measured with ABTS as substrate in trypsin-treated microsomes and supernatants (100 000 x g) of thyroid tissue of a congenitally hypothyroid cat (kitten 1) and 6 hyperthyroid cats. n.d. = not detectable.

<table>
<thead>
<tr>
<th>TPO activity (μmol ABTS • 1 min⁻¹ • (mg tissue)⁻¹)</th>
<th>Microsomes</th>
<th>Supernatant</th>
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<tbody>
<tr>
<td>Hypothyroid</td>
<td>n.d.</td>
<td>19</td>
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<tr>
<td>Hyperthyroid</td>
<td>20</td>
<td>n.d.</td>
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microsomal fraction (Table 1). In the thyroid tissue (100 000 x g sediment and supernatant) of the healthy cats no thyroid peroxidase activity could be detected.

The polyacrylamide gel electrophoresis of proteins precipitated by ammonium sulphate (50% saturation) revealed that substantial amounts of thyroglobulin (Tg) (molecular weight 660 000) was present in the thyroid tissue of the hypothyroid cat (Fig. 4). The iodine content (w/w) of the ammonium sulphate-precipitated proteins was 0.19% in the hypothyroid cat, 0.16% in the euthyroid cats, and 0.22% in the hyperthyroid cats.

In the tissue of all the cats the ELISA demonstrated the presence of thyroglobulin. Experiments with monoclonal antibody B2 revealed the presence of thyroxine in all thyroglobulin preparations, including that of the hypothyroid cat (Fig. 5).

Histology

Microscopic examination of formalin-fixed, HE-stained sections of the goitrous tissue of cat 1 revealed rather large follicles and cuboidal to flat epithelium. The histological features did not differ from those observed in healthy control cats.

Discussion

In the organic binding of iodine in the thyroid gland at least three steps are involved: generation of H₂O₂, oxidation of iodide, and binding of the oxidized iodide to tyrosyl residues of thyroglobulin. This machinery of protein iodination is geared to bind efficiently into covalent linkage any iodide trapped by the thyroid gland (7).

The radioiodide uptake studies in both hypothyroid cats demonstrated that iodide transport into the thyroid was increased. However, competitive inhibition of the iodide transport system by perchlorate caused a rapid decrease in thyroidal radioactivity in both cats. This indicated the presence of unbound iodide that was free to diffuse from the thyroid cells into the circulation, and thereby proved that organification was defective.

Fig. 4.
Electrophoresis of different thyroglobulin preparations on a 4/80 PAA gradient gel. Lanes 1 and 5: HMW markers. Lanes 2 to 4: normal cats (14, 7 and 3.5 µg protein, respectively). Lanes 6 to 8: hypothyroid cat 1 (70, 35 and 17.5 µg protein). Lanes 9 to 11: hyperthyroid cats (50, 25 and 12.5 µg protein).

Fig. 5.
ELISA with a specific monoclonal antibody against thyroglobulin. Plates were incubated with the thyroglobulin preparations from the hypothyroid cat (—O—), the normal cats (—△—), and the hyperthyroid cats (—□—), in amounts ranging from 0 to 200 ng protein per well and tested with the monoclonal antibody B2 (16).
Three observations indicated that the defect in cat 2 was complete. First, the circulating thyroxine concentration was always very low in the periods without thyroxine supplementation. Secondly, the initial thyroidal radioiodide uptake was high but quickly reached a plateau. Thirdly, perchlorate administration caused a decrease in radioactivity in the neck to a level compatible with circulating radioactivity only, i.e. similar to the early uptake levels in the healthy cats.

In cat 1 the low-normal circulating thyroxine concentration indicated a partial defect. This was substantiated by the perchlorate test, in which the resulting level of radioactivity in the neck was above that due to circulating radioactivity alone. At the time of hemithyroidectomy the animal appeared euthyroid. Histological examination of the thyroid did not reveal features consistent with hyperactivity and the plasma thyroxine concentration was normal. Apparently the thyroid hyperplasia and the high iodine content of the food (see below) allowed the animal to compensate for the partial defect.

No peroxidase activity could be demonstrated in the thyroid tissues of the healthy cats. This might be explained by low thyroidal uptake of iodide owing to high levels of iodine in commercial cat foods, as has been shown in commercial dog foods (18). In support of this explanation, thyroidal radioiodine uptake values in healthy cats on commercial cat foods were low (13). In hyperthyroid cats injected radioiodide accumulated rapidly in the thyroid gland (13), as in the hypothyroid cats described here. Therefore comparison of the thyroperoxidase activity of the congenitally hypothyroid cats with that of the hyperthyroid cats is more relevant.

In cat 1 microsomally bound peroxidase activity could not be detected, but peroxidase activity was found in the 100 000 xg supernatant. We have not observed this previously in any species nor in the hyperthyroid cats used as reference material. It may suggest that thyroperoxidase is loosely anchored. This can lead to decreased availability of the peroxidase. The capacity of the enzyme to iodinate thyroglobulin seemed only slightly reduced, since, with the high iodine content of the food, the thyroglobulin contained $T_3$ in amounts comparable to those in normal thyroglobulin. The reduced capacity to catalyse the iodination of tyrosine residues in thyroglobulin prohibited the instantaneous organization of all trapped iodide.

According to the review of Dumont et al. (7), several different types of thyroperoxidase defects can be distinguished, including 1. absent or abnormal thyroid peroxidase, 2. peroxidase apoenzyme-prosthetic group defect, 3. abnormal subcellular localization of the enzyme, and 4. abnormal interaction with the protein substrate or an abnormal substrate. In this classification, the defect in cat 1 would appear to be type 3. There have been two case reports of this type of defect in humans (19,20). However, in both women the enzyme was present in the particulate fractions and alterations in the structure of the enzyme were suggested. In cat 1, however, peroxidase activity was found only in the supernatant, without any prior treatment with digestive enzymes or detergents. Hence the defect in this cat does not appear to have been described previously and comprises a loosely-anchored thyroperoxidase that is easily released either following isolation in vitro or already in vivo.

References


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