Thyroid Hyperactivity Induced by Methimazole, Spironolactone and Phenobarbital in Marmosets (Callithrix jacchus): Histopathology, Plasma Thyroid Hormone Levels and Hepatic T₄ Metabolism

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ABSTRACT. To determine drug-induced hyperfunction of marmoset thyroids due to inhibition of synthesis or enhancement of metabolic elimination of thyroid hormones, males were orally administered 10 and 30 mg/kg/day methimazole (MMI), 30 and 100 mg/kg/day spironolactone (SPL), or 50 mg/kg/day phenobarbital (PB) for 4 weeks. MMI caused marked hypertrophy of follicular epithelial cells in accordance with a significant decrease in the plasma thyroxin (T₄) level. Hypertrophied epithelial cells were filled with dilated rough endoplasmic reticulum and reabsorbed intracellular colloids, and the luminal surface was covered with abundant microvilli. The colloid included vacuoles positive to anti T₄ immuno-staining. SPL and PB also caused similar histomorphological changes, although they were less severe than those due to MMI and were not clearly associated with decrease in the plasma T₄ levels. Hepatic T₄ UDPGT activities tended to increase due to SPL and PB treatment, however, which were not so significant as increases in microsomal cytochrome P-450 contents. Some animals treated with SPL and PB showed marked increases in thyroid weights due to inactive dilated follicles. In conclusion, hyperactivity of thyroid follicles was induced in marmosets not only due to inhibition of T₄ synthesis produced by MMI but also because of enhancement of hepatic T₄ elimination produced by SPL and PB. However, hypertrophic effects of SPL and PB were less severe than MMI, because plasma T₄ levels were maintained at almost pretreatment or control levels after SPL or PB treatment.—KEY WORDS: marmoset, methimazole, phenobarbital, spironolactone, thyroid hyperactivity.
Table 1. Group constitution of the study

<table>
<thead>
<tr>
<th>Test substance</th>
<th>Dose (mg/kg/day)</th>
<th>Number and sex of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Vehicle</td>
<td>6 males</td>
</tr>
<tr>
<td>SPL</td>
<td>30 and 100</td>
<td>3 males in each dose level</td>
</tr>
<tr>
<td>PB</td>
<td>50</td>
<td>3 males</td>
</tr>
<tr>
<td>MMI</td>
<td>10 and 30</td>
<td>3 males in each dose level</td>
</tr>
</tbody>
</table>

a) Test substance were suspended in 0.5% carboxymethylcellulose-Na (Wako Pure Chemical, Osaka, Japan) solution, and the dosing volume of each group was 5 ml/kg.
b) SPL was synthesized at Mitsubishi Chemical (Tokyo, Japan).
c) PB was purchased from Wako Pure Chemical (Osaka, Japan).
d) MMI was purchased from Aldrich (Milwaukee, WI).

Available ad libitum from water bottles. After approximately 2 months of acclimatization, twenty-one animals were divided into 6 groups as shown in Table 1. The groups were treated orally once daily for 28 consecutive days. Control animals were treated with vehicle. At the commencement of the treatment, body weights ranged from 271 to 401 g and ages ranged from 17 to 42 months. High doses of MMI and SPL, and 50 mg/kg/day PB, which were decided on the basis of the preliminary study, were maximum tolerated doses without death or severe body weight reduction for marmosets in repeated administrations. The animals were treated with appropriate care and respect, according to the Guidelines for Animal Experimentation of our Laboratory, Mitsubishi Chemical Safety Institute Ltd.

Liver biopsy: To obtain pretreatment values of hepatic microsomal enzyme activity, a liver biopsy was performed on all animals 5 or 6 weeks before the commencement of the drug treatment under sodium pentobarbital (30 mg/kg, ip) anesthesia. After disinfecting the skin with povidone-iodine and 70% ethanol, an abdominal wall incision was made over the mid-line, and the wedge of the right lateral lobe was excised. A collagen sheet (AVITEN, Zeria Shinyaku, Tokyo, Japan) was used for hemostasis of the excised site in the liver. The suture site was disinfected with povidone-iodine and antibiotics.

Analysis of plasma triiodothyronine (T3) and thyroxin (T4): Prior to, and two and four weeks after commencement of treatment, blood samples were obtained from the femoral vein. The plasma was then separated by centrifugation. Total and free T3, and total and free T4 concentrations were determined by radioimmunoassay using commercially available kits (Amerlex M T3, free T3, T4, and free T4, respectively; Amersham, International plc, UK). Plasma samples were stored at −80°C until analysis.

Analysis of cytochrome P-450 content: Homogenates of the liver samples obtained at biopsy and autopsy were prepared with 0.25 M sucrose, 10 mM HEPES, and 1 mM dithiothreitol solution (pH 7.0) using Potter-type glass and a teflon homogenizer. Then, microsomes were obtained by centrifugations (10,000 × g for 20 min and 100,000 × g for 60 min at 4°C) and suspended in phosphate buffer (pH 7.4) containing 3 mM EDTA and 1 mM dithiothreitol.

Cytochrome P450 contents in hepatic microsomes were determined from the reduced carbon monoxide difference spectrum using a spectrophotometer based on the extinction coefficient of 91 mM−1 cm−1 [20]. The protein concentration was determined using the method of Lowry et al. [14] using bovine serum albumin as a standard. Microsome samples were stored at −80°C until assayed.

Analysis of thyroxin UDP-glucuronosyltransferase (T4 UDPGT): T4 UDPGT activities in hepatic microsomes were determined according to the method of Beetstra et al. [2]. The microsome sample (1 mg protein/ml) was incubated with 1 µM T4 (Sigma, St. Louis, MO, U.S.A.) and 0.1 µCi 125I-T4 (New England Nuclear, Boston, MA, U.S.A.) in 0.1 M Tris-HCl buffer (pH 7.4) containing 5 mM MgCl2 and 0.05% Brij 56 (Sigma, St. Louis, MO, U.S.A.). The reaction was initiated by adding UDP glucuronic acid (Nacalai Tesque, Kyoto, Japan) at a final concentration of 5 mM. It was reacted for 120 min at 37°C, and was terminated by adding ice-cold methanol. After centrifugation (10,000 × g for 2 min), the supernatant was filtered through a 0.45 µm LCR membrane (Ultrafree C3-LH, Millipore, Tokyo, Japan), and was used for HPLC analysis [7]. An Inertsil ODS-2 column (4.6 × 150 mm; GL Science, Tokyo, Japan) and 57:43 (v/v) methanol/20 mM potassium phosphate buffer containing 1% (v/v) triethylamine adjusted to pH 7.0 with orthophosphoric acid was used for separation (flow rate: 1.0 ml/min). The peaks were detected using a radioisotope detector and the activity was calculated using the % of radioactivity of formed T4-glucuronide.

Pathological examination: On the day after the final dosage, all animals were sacrificed by exsanguination of the abdominal aorta under sodium pentobarbital anesthesia and underwent necropsy. The liver and thyroid were fixed in 10% neutral buffered formalin. Light microscopic examination was performed on the sections of both organs routinely stained with hematoxylin and eosin. Additionally, T4 immuno-staining using monoclonal antibody (rabbit anti-human thyroxin, DAKO Japan, Kyoto, Japan) and PCNA immuno-staining using monoclonal anti-PCNA antibody (PC10, DAKO Japan, Kyoto, Japan) were performed on the thyroid sections. Portions of the thyroid and liver were fixed in 0.1 M phosphate-buffered 3% glutaraldehyde solution and then in 0.1 M phosphate-buffered 1% osmium tetroxide. Electron microscopic examination was performed on the thin sections stained with uranyl acetate and lead citrate.

Statistical analysis: Data are expressed as mean ± standard deviation. Statistical analyses were performed using paired t test and/or Student’s t-test. The significance level was set at P<0.05 and 0.01.

RESULTS

Body weight change, Liver and Thyroid weights: Table 2 shows the body, liver and thyroid weights. The final body weights were not affected by any treatment. PB caused
**Table 2.** Final body weight, and liver and thyroids weights in marmosets treated with SPL, PB and MMI

<table>
<thead>
<tr>
<th>Test substance (mg/kg/day)</th>
<th>Dose</th>
<th>n</th>
<th>Final body weight</th>
<th>Liver weight</th>
<th>Thyroid weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>g (% to initial value)</td>
<td>g (× 10^−3 % to FBW)</td>
<td>mg (× 10^−3 % to FBW)</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>6</td>
<td>322 ± 33 (101 ± 4)</td>
<td>11.3 ± 1.1 (3.53 ± 0.31)</td>
<td>59.8 ± 6.0 (18.9 ± 3.7)</td>
</tr>
<tr>
<td>SPL</td>
<td>30</td>
<td>3</td>
<td>308 ± 45 (97 ± 4)</td>
<td>13.1 ± 4.0 (4.21 ± 0.78)</td>
<td>68.8 ± 6.2 (22.6 ± 0.4)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>3</td>
<td>315 ± 37 (97 ± 2)</td>
<td>12.6 ± 1.0 (4.01 ± 0.24)*</td>
<td>87.0 ± 23.6 (27.6 ± 7.0)</td>
</tr>
<tr>
<td>PB</td>
<td>50</td>
<td>3</td>
<td>325 ± 37 (102 ± 1)</td>
<td>18.1 ± 1.8** (5.57 ± 0.08)**</td>
<td>91.9 ± 37.1 (29.3 ± 15.2)</td>
</tr>
<tr>
<td>MMI</td>
<td>10</td>
<td>3</td>
<td>315 ± 26 (103 ± 1)</td>
<td>12.3 ± 1.1 (3.91 ± 0.05)*</td>
<td>53.3 ± 10.5 (17.0 ± 3.5)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3</td>
<td>323 ± 20 (104 ± 2)</td>
<td>13.3 ± 2.1 (4.11 ± 0.43)</td>
<td>65.6 ± 13.0 (20.6 ± 5.3)</td>
</tr>
</tbody>
</table>

Marmosets were treated orally for 28 consecutive days. Data are shown in mean ± S.D. for 3 or 6 animals per group.

* P<0.05, **P<0.001 against control (Student’s t-test).

significant increases of the absolute and the relative (against body weight) liver weights. The thyroid weights were markedly increased at 100 mg/kg/day SPL and 50 mg/kg/day PB due to the dilated and colloid-rich follicles observed in one animal in each group, although the changes were not statistically significant due to the large variation. MMI did not affect the thyroid weight at any dose, although the substance caused significant hypertrophy of follicular cells.

**Plasma T₃ and T₄ concentration:** Figures 1 and 2 show plasma total and free T₄ levels. Plasma T₄ level in marmosets receiving 100 mg/kg/day SPL showed only a slight reduction compared with their pretreatment and control value after 4 weeks of treatment. PB did not affect the plasma total and free T₄ levels. However, MMI markedly decreased plasma total and free T₄ levels at all doses in a dose-dependent manner. Treatment related changes in plasma total and free T₃ concentrations were not obvious in any treatment group due to large variations.

**Hepatic cytochrome P450 content:** Table 3 shows hepatic microsomal cytochrome P450 contents. Cytochrome P450 contents in SPL-treated marmosets tended to be increased or were significantly increased at all doses compared with their individual pretreatment values and with control value on a milligram microsomal protein, a gram liver and a total liver basis. In marmosets treated with PB, about 5 or 10-fold increases in cytochrome P450 contents were observed on a microsomal protein and a gram liver basis, respectively, compared with their pretreatment value and the content was about 14 times higher than the control value on a total liver basis. However, cytochrome P450 contents in MMI-treated marmosets tended to decrease at all doses.

**Hepatic T₄ UDPGT activity:** Table 4 shows the hepatic T₄ UDPGT activities. T₄ UDPGT activity tended to be increased on a gram liver or a total liver basis by the treatment with SPL at all doses and PB compared with their pretreatment or control values. However, the changes were not statistically significant except in 100 mg/kg/day SPL, and were less obvious as compared with increases in cytochrome P450 contents. No change in T₄ UDPGT activity was observed in marmosets treated with MMI.

**Histopathological findings:** Histopathological findings of the thyroids and liver were summarized in Table 5. In the thyroids, 30 and 100 mg/kg/day SPL, 50 mg/kg/day PB
and 10 mg/kg/day MMI induced mild hypertrophy of follicular cells, and the most prominent hypertrophy was induced by 30 mg/kg/day MMI. The follicles were lined by cuboidal to columnar epithelial cells and contained faint eosinophilic and scanty colloid (Fig. 3). Colloid vacuoles called reabsorption lacunae, which showed positive staining for anti-T4 antibody, appeared in all treatments (Fig. 4). However, treatment-related change was not observed in the PCNA-index in any treatment groups. Dilated follicles were also present in one animal treated with 100 mg/kg/day SPL and 50 mg/kg/day PB in each group. These follicles were lined with flattened epithelial cells, and the thyroids

Table 3. Hepatic P-450 contents in marmosets treated with SPL, PB and MMI

<table>
<thead>
<tr>
<th>Test substances (mg/kg/day)</th>
<th>Dose</th>
<th>n</th>
<th>nmol/mg protein</th>
<th>nmol/g liver</th>
<th>nmol/liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pretreatment</td>
<td>28 days after</td>
<td>Pretreatment</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>6</td>
<td>0.38 ± 0.11</td>
<td>0.39 ± 0.11</td>
<td>11.6 ± 5.5</td>
</tr>
<tr>
<td>SPL</td>
<td>30</td>
<td>3</td>
<td>0.47 ± 0.05</td>
<td>0.80 ± 0.07**</td>
<td>12.0 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>3</td>
<td>0.41 ± 0.13</td>
<td>0.65 ± 0.14**</td>
<td>11.5 ± 3.9</td>
</tr>
<tr>
<td>PB</td>
<td>50</td>
<td>3</td>
<td>0.47 ± 0.11</td>
<td>2.22 ± 0.18***</td>
<td>9.4 ± 2.9</td>
</tr>
<tr>
<td>MMI</td>
<td>10</td>
<td>3</td>
<td>0.48 ± 0.05</td>
<td>0.46 ± 0.07</td>
<td>13.2 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3</td>
<td>0.51 ± 0.12</td>
<td>0.30 ± 0.11***</td>
<td>11.9 ± 3.0</td>
</tr>
</tbody>
</table>

Marmosets were treated orally for 28 consecutive days. Data are shown in mean ± S.D. for 3 or 6 animals per group.
* P<0.05, **P<0.01 against control (Student’s t-test).
# P<0.05, ##P<0.01 against pretreatment value (paired t-test).

Table 4. Hepatic T4 UDPGT activities in marmosets treated with SPL, PB and MMI

<table>
<thead>
<tr>
<th>Test substances (mg/kg/day)</th>
<th>Dose</th>
<th>n</th>
<th>pmol/mg protein/min.</th>
<th>pmol/g liver/min.</th>
<th>pmol/liver/min.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pretreatment 28 days after</td>
<td>Pretreatment 28 days after</td>
<td>28 days after</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>6</td>
<td>0.64 ± 0.14</td>
<td>0.59 ± 0.07</td>
<td>19.1 ± 4.7</td>
</tr>
<tr>
<td>SPL</td>
<td>30</td>
<td>3</td>
<td>0.55 ± 0.21</td>
<td>0.59 ± 0.12</td>
<td>14.4 ± 6.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>3</td>
<td>0.69 ± 0.10</td>
<td>0.71 ± 0.08</td>
<td>19.6 ± 2.0</td>
</tr>
<tr>
<td>PB</td>
<td>50</td>
<td>3</td>
<td>0.96 ± 0.17</td>
<td>0.75 ± 0.13*</td>
<td>18.7 ± 2.0</td>
</tr>
<tr>
<td>MMI</td>
<td>10</td>
<td>3</td>
<td>0.66 ± 0.04</td>
<td>0.75 ± 0.14*</td>
<td>18.4 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3</td>
<td>0.72 ± 0.11</td>
<td>0.50 ± 0.03</td>
<td>16.7 ± 0.5</td>
</tr>
</tbody>
</table>

Marmosets were treated orally for 28 consecutive days. Data are shown in mean ± S.D. for 3 or 6 animals per group.
* P<0.05 against control (Student’s t-test).

Fig. 2. Plasma free T4 concentration in the marmosets treated orally with saline (control, □), Spironolactone (SPL) 30 ( △ ) and 100 ( □ ) mg/kg/day, Phenobarbital (PB) 50 ( ▲ ) mg/kg/day and Methimazole (MMI) 10 ( ◊ ) and 30 ( □ ) mg/kg/day for 28 consecutive days.
containing dilated follicles showed a marked increase in weight.

Ultrastructurally, the following findings were obtained from animals in all treatment groups except animals showing dilated follicles and flattened epithelial cells in SPL (100 mg/kg/day) and PB-treatment groups. Hypertrophied epithelial cells were filled with microvesicularly or cisternally dilated rough endoplasmic reticulum, which were occasionally intermingled with reabsorbed intracellular colloids. The luminal surface was covered with abundant microvilli. Ultrastructural changes caused by MMI was also most severe in three drugs (Fig. 5).

In the liver, centrilobular hepatocellular hypertrophy was observed in all animals treated with PB. Hepatocytes from the central area to midzone were swollen with eosinophilia. Hypertrophy of hepatocytes was attributed to the proliferation of the smooth endoplasmic reticulum observed using electron microscopy.

DISCUSSION

Marmosets (Callithrix jacchus) belong to the suborder Platyrrhini and inhabit the Amazon basin. This animal came to be used in toxicological studies because of ease of handling, their small size and good reproductive ability [12]. There are very few basic toxicological profiles for marmosets, thus it is important to investigate its basic toxicological profile. There are marked species differences in thyroid physiology between rats and humans, therefore, toxicological data on the thyroid obtained from primate studies might be important. However, great care must be taken when interpreting the toxicological effects on marmoset thyroid, because marmosets, like rats, lack TBG, a main carrier protein of thyroxin.

MMI, an anti-thyroid drug, impairs the synthesis of thyroid hormones by inhibiting the peroxidase [25]. In rats, a 4-fold increase in MMI treatment (90 ppm in diet, approx. 9 mg/kg/day) accompanied with diffuse hypertrophy and hyperplasia of follicular epithelial cells [26].

In the present study, MMI (10 and 30 mg/kg/day) induced marked hypertrophy of follicular epithelial cells in marmosets also. The appearance of reabsorption lacunae in colloids stained with anti-T4 antibody and ultrastructural findings, such as dilated rough endoplasmic reticulum, increase of reabsorbed colloid and luminal surface covered with abundant microvilli, evidenced that the follicles were activated.

Hypertrophic change was well correlated with the reduction in plasma T4 levels. Since hepatic microsomal P450 content or T4 UDPGT activity was not affected by MMI treatment, hyperactivities of thyroid follicles might be caused secondarily by the lowered circulating thyroid hormones due to inhibition of hormone-synthesis. However, increase in thyroid weight or hyperplastic change of follicular epithelial cells observed in rats was not seen in marmosets even at the maximum tolerated dose. This may indicate that the feedback mechanism of the hypothalamus-pituitary-thyroids axis in marmosets was less sensitive to alterations in the blood T4 level than that in rats. However, it is possible that hyperplastic changes will be induced under chronic reduction of the blood T4 level, because PCB, a potent inducer on thyroid function and morphology, induced hyperplasia of follicular epithelial cells in accordance with the decrease in serum T4 and the increase in serum TSH in marmosets as well as in rats [27].

SPL, an aldosterone antagonist, is a hepatic microsomal enzyme inducer. In rats, a 13-week administration of 200 mg/kg/day SPL caused hypertrophy of follicular cells secondary to the enhancement of T4 elimination in the liver [22]. PB was also a potent hepatic microsomal enzyme inducer in humans as well as in rats [5, 9]. In rats, PB increased the thyroid weight with induction of hypertrophy or hyperplasia of follicular cells [11, 16], where the mechanism is also secondary to the enhancement of hepatic T4 elimination [17]. However, in rhesus monkeys, no changes were observed in the thyroid gland after administration of 250 mg/kg/day SPL for 52 weeks [15]. Moreover, in humans, no decrease in circulating thyroid hormone levels has been observed under conditions where hepatic microsomal enzymes are induced [19], and hypertrophic or hyperplastic change in the thyroid caused by SPL and PB has not been reported in humans.

<table>
<thead>
<tr>
<th>Organs</th>
<th>Test substances:</th>
<th>SPL Dose (mg/kg/day):</th>
<th>PB</th>
<th>MMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30 100 50 10 30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertrophy of follicular epithelium</td>
<td>1+</td>
<td>3 2 2 – –</td>
<td></td>
<td></td>
</tr>
<tr>
<td>with vacuoles in the follicle</td>
<td>2+</td>
<td>– – – – 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dilatation of follicles</td>
<td>–</td>
<td>1 1 – –</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centrilobular hypertrophy of hepatocytes</td>
<td>–</td>
<td>– 3 – –</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Marmosets were treated orally for 28 consecutive days. Data are shown in the number of animals with lesions for 3 animals per group. a) 1+: slight; 2+: moderate.
Fig. 3. Photomicrographs of hematoxylin-eosin stained sections of thyroid gland in control (a), treated with 100 mg/kg/day SPL (b), 50 mg/kg/day PB (c) and 30 mg/kg/day MMI (d). Follicular epithelia in treated animals were taller than in control animals and absorption lacunae were prominent. (× 480)

Fig. 4. Photomicrographs of T₄-immunostained sections of thyroid gland in control (a) and treated with 30 mg/kg/day MMI (b). Colloid vacuoles showed positive staining for anti-T₄ antibody in MMI-treated animals. (× 400)
EFFECTS OF GOITROGEN IN MARMOSETS

The present study demonstrated SPL and PB, as well as MMI, also induced hypertrophy of follicular cells in marmosets, and in addition, other histologic and electron microscopic changes in the thyroids were similar to that of MMI-treated marmosets. SPL and PB treatment caused a significant increase in hepatic microsomal cytochrome P450 content, and T₄ UDPGT activity was also slightly increased in a dose-dependent manner. Therefore, hyperactive changes of follicular cells induced by SPL and PB in marmosets are probably secondary to hormone imbalance due to the enhancement of metabolic clearance of T₄ in the liver. Seo et al. [23] reported that marmosets were lacking TBG and showed high concentrations of circulating free T₄. Thus, hyperactivity of thyroid follicles secondary to the enhancement of T₄ metabolism may be due to the high concentration of the free form in marmosets, which is similar to rats. However, induction of hepatic T₄ UDPGT was not so drastic as to cause reductions in plasma T₄ levels, which were maintained at almost pretreatment or control levels after SPL or PB treatment. As a result, histologic changes in the thyroids might be less severe than that observed in MMI-treated marmosets. Moreover, the thyroids in some animals treated with high dose of SPL and PB showed marked increases in weights and dilated follicles with flattened lining epithelial cells. These findings suggest that follicles were in an inactive stage rather than active. These findings also indicate that hyperactivity of thyroid follicles, induced by SPL and PB treatment, may be temporary.

To confirm that the present follicular cell hypertrophy induced by SPL, PB, and MMI was secondary to hormone imbalance, it was necessary to determine the plasma TSH concentration. However, we were unable to measure the TSH concentration with the currently available kit for humans and rat TSH, due to the lack of cross-reactivity.

In conclusion, our findings indicate that not only MMI, thyroxin synthesis inhibitor, but also SPL and PB, hepatic microsomal enzyme inducers, induce hyperactivity of thyroid follicles in the marmoset as well as in the rat. Hypertrophic effects of SPL and PB, which were not associated with reductions of blood thyroid hormone levels, were less severe than that of MMI which was associated with significant reductions in the hormone levels.

Fig. 5. Hypertrophied follicular cells in rats treated with 100 mg/kg/day SPL (a) and 30 mg/kg/day MMI (b). Cytoplasmic expansion is characterized by dilated profiles of rough endoplasmic reticulum and increase in number of long microvilli. Bar=1 μm.
ACKNOWLEDGMENTS. Authors thank Dr. Minoru Tsuchitani for valuable discussions and Mr. Takeshi Kawasuso for good technical assistance.

REFERENCES


