THYROID HYPERTROPHIC EFFECT OF SEMOTIADIL FUMARATE, A NEW CALCIUM ANTAGONIST, IN RATS

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ABSTRACT — We studied the effects of semotiadil fumarate (SF), a new calcium antagonist with a unique benzothiazine structure, on the thyroid gland and liver in rats and compared them with those of another calcium antagonist, nicardipine (NCD), a well-known thyroidal hypertrophic agent and microsomal enzyme inducer, phenobarbital (PB), and goitrogen propylthiouracil (PTU). In oral 2-week treatment, SF caused increases in hepatic microsomal protein levels, uridine diphosphate glucuronosyltransferase (UDPGT) activity and an increase in serum thyroid stimulating hormone (TSH) level together with decreases in serum thyroid hormone levels. These results suggest that SF accelerates peripheral disposition of thyroid hormones and subsequently stimulates secretion of TSH from the pituitary gland as a compensatory response. PB and NCD had similar effects on the thyroid gland and the liver. PTU showed obvious thyroid hyperplasia and an increase in relative liver weight. Drastic increase in TSH level was observed in the PTUtreated group together with significant decreases in serum thyroid hormone levels and an increase in hepatic UDPGT activity. Histopathologically, PTU depleted the colloids in follicles, suggesting the inhibition of thyroid hormone synthesis. SF, PB and NCD showed thyroid hyperplasia, but the extent of the change was far more moderate than that induced by PTU. These results indicate that the effect of SF is similar to those of PB and thyroid hypertrophy seen in the oral 2-week treatment with SF, and may be caused by indirectly elevated TSH levels which resulted from the induction of hepatic UDPGT activity.

KEY WORDS: (+)-(R)-2-[5-methoxy-2-[3-[methyl-2-[(3,4-methylenedioxy)phenoxy]ethyl]amino]propoxy]phenyl]-4-methyl]-2H-1,4-benzothiazin-3(4H)-one hydrogen fumarate, Semotiadil fumarate, Calcium antagonist, Rat, Thyroid, Uridine diphosphate glucuronosyltransferase

INTRODUCTION

Thyroid stimulating hormone (TSH) is secreted from the pituitary gland and stimulates the thyroid gland to accelerate the secretion of thyroid hormones such as triiodothyronine (T3) and thyroxine (T4). Decreases in circulating thyroid hormone levels enhance the pituitary gland to secrete TSH. Thus, the pituitary-thyroid axis function is under the control of the feedback system (Neil et al., 1995).

Certain drugs are known to directly or indirectly affect this pituitary-thyroid homeostasis, leading to thyroid hypertrophic or hyperplastic changes. Indeed, drug-induced enlargement of the thyroid gland is frequently seen in rats (McClain, 1989). We have found that semotiadil fumarate (SF), a new calcium antagonist, caused enlargement of the thyroid gland in rats, but not in dogs, in 13-week oral toxicity studies. In a preliminary study, SF induced hepatic microsomal enzymes, such as cytochrome P-450 contents and aminopyrine demethylase activity, in both rats and dogs by 2-week treatment and 13-week treatment, respectively, suggesting elevations in metabolism and clearance of thyroid hormones. Therefore, we compared the functional and morphological effects of SF on the thyroid gland in rats with compounds having direct and
indirect hypertrophic effects on the thyroid gland, *i.e.*, propylthiouracil (PTU) and phenobarbital (PB), to evaluate the mechanism of the effect. PTU blocks the synthesis of thyroid hormones through the direct suppression of iodide organification catalyzed by thyroid peroxidase (Taurog, 1976), resulting most likely in hypothyroidism in various species (Capen *et al.*, 1983). PB has indirect goitrogenic effects in rats (Oppenheimer *et al.*, 1968, Japundzic, 1969) by stimulation of peripheral thyroid hormone disposition (Cavaliere and Pitt-Rivers, 1981). In addition to these reference compounds, since SF has a unique benzothiazine structure, we studied the effects of nicardipine (NCD), a calcium antagonist with a dihydropyridine structure which been reported to cause thyroidal changes in rats, to clarify whether the effects are common to calcium antagonists.

**MATERIALS AND METHODS**

**Animals**

Six-week-old female Wistar rats purchased from Japan SLC. Inc. (Hamamatsu, Japan) were used. The animals were housed 2 or 3 per cage in wire-mesh cages kept in an air-conditioned room with a 12-hr light-dark cycle at a temperature of 23 ± 2°C, a relative humidity of 55 ± 15% and a ventilation rate of 15 times per hr. Rats were allowed free access to a commercial diet (F-2, Funabashi Farm Inc., Funabashi, Japan) and chlorinated tap water.

**Treatment**

SF (Fujita *et al.*, 1990) synthesized at Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan), PTU and PB purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan), and NCD purchased from Sigma Chemical Co. (St. Louis, USA) were respectively suspended in 1.0% methylcellulose (MC, Nacarai Tesque Inc., Kyoto, Japan) solution using a teflon homogenizer. Animals receiving 1.0% MC solution alone served as controls. Each solution was administered orally once a day by a constant volume of 10 mL/kg body weight.

**Experimental Protocol**

Groups of six or seven female rats were treated with 40 and 160 mg/kg of SF, 50 mg/kg or 100 mg/kg of PTU, 100 mg/kg of PB and 160 mg/kg of NCD for 2 weeks. The dose levels of SF were set depending on those in the previous 13-week toxicity study. 40 mg/kg and 160 mg/kg of SF caused an increase in relative liver weights in that study (Table 1). 160 mg/kg of SF caused a significant increase in relative thyroid weight in males. On the other hand, 40 mg/kg of SF caused a tendency to increase relative thyroid weight in females. Furthermore, 160 mg/kg of SF and more than 40 mg/kg of SF induced hepatic cytochrome P-450 contents and aminopyrine demethylase activity in both males and females. These changes in hepatic microsomal enzymes were a little clearer in females rather than males. Therefore, female rats were used in the present study. Dose levels of PTU and PB were considered to cause changes in the thyroid and the liver, respectively. The dose level of NCD, one of the calcium antagonists, was determined to be the same as the high dose of SF to compare their effects. Six hr (only for the 160 mg/kg group of SF) and 24 hr following the last administration, blood samples for assays of hormones were taken from each rat by bleeding from the carotid artery under ether anesthesia, and the serum was separated and stored at −80°C until assay time. Rats were then killed and the thyroid and liver were taken from all rats for histopathological examination. In addition, a part of the liver was removed to measure hepatic microsomal enzyme activity.

**Determination of thyroid stimulating hormone and thyroid hormone levels in serum**

The TSH level was obtained by the radio immunoassay technique established by Dr. Kazuyoshi Taya (Laboratory of Veterinary Physiology, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, Japan). T3 and T4 levels were measured by enzyme immunoassays, using the Enzymun-Test T3 and T4 kits (Boehringer Mannheim Yamanouchi Co., Ltd., Tokyo, Japan), respectively, while free triiodothyronine (FT3) and free thyroxine (FT4) levels were assayed by radio immunoassays using the Amerlex M-FT3 and M-FT4 kits (Amersham International plc., Tokyo, Japan), respectively.

![Chemical structure of semotiadil fumarate.](image)
<table>
<thead>
<tr>
<th>Sex:</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg/day):</td>
<td>0</td>
<td>2.5</td>
</tr>
<tr>
<td>Variable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>367.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>351.0</td>
</tr>
<tr>
<td></td>
<td>± 6.4</td>
<td>± 7.5</td>
</tr>
<tr>
<td></td>
<td>10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10</td>
</tr>
<tr>
<td>Thyroid weight (mg/100g b.w.)</td>
<td>6.2</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>± 0.2</td>
<td>± 0.3</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Liver weight (g/100g b.w.)</td>
<td>3.16</td>
<td>3.09</td>
</tr>
<tr>
<td></td>
<td>± 0.05</td>
<td>± 0.03</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Cytochrome P-450 (nmol/mg p)</td>
<td>1.16</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>± 0.10</td>
<td>± 0.14</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Aminopyrine demethylase (nmol/mg p/30 min)</td>
<td>100.7</td>
<td>107.1</td>
</tr>
<tr>
<td></td>
<td>± 5.5</td>
<td>± 12.9</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Histopathology</td>
<td>Increase of colloid in the thyroid</td>
<td>1/10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>mean ± S.D.
<sup>b</sup>No. of animals.
<sup>c</sup>No. of animals showing the finding / total No.

h.w.: body weight.
mg p: mg protein.

Statistical significance: *p<0.05, **p<0.01 vs. control by Dunnett's test.
Measurement of hepatic microsomal enzyme (UDPGT) activity

Approximately 4 g of the liver was taken from each animal 24 hr after the last dosing, and was weighed exactly. The microsomal fraction of the liver was separated by the method of Kamataki et al. (1974), and its protein concentration was determined by the method of Miller (1959). UDPGT activity in the microsomal fraction from each animal was measured by a modification of the technique of Comer et al. (1985). The reactions were started by adding 0.05 mL of 60 mM uridine 5'-diphosphoglucuronic acid (UDPGA) in 0.4 M Tris buffer (pH 7.4) to 0.15 mL of a mixture containing 0.02 mL of microsomal suspension (2 to 3 mg / 0.02 mL), 4 mM p-nitrophenol as a substrate, 0.1% Triton X-100, 10 mM MgCl₂ and 0.4 M Tris buffer (pH 7.4). After incubation at 37°C for 30 min, 3.8 mL of ice-cold 0.1 N NaOH was added to stop the reaction. The assay mixture without UDPGA was treated as a blank. The absorbance of the mixtures was measured at 400 nm using a spectrophotometer (UV-730, Shimadzu Co., Kyoto, Japan), and the enzyme activity was expressed in terms of the content (moles) of p-nitrophenol glucuronide produced in 1 min.

Histopathological Examinations

The thyroid and liver were removed and fixed in 10% buffered formalin. After fixation, the tissues were processed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) or periodic acid schiff stain (PAS) for light microscopic examination.

The thyroid sections stained with H&E were also analyzed morphometrically according to the method of Denef et al. (1981). Five follicles of the thyroid of each animal were selected randomly, and the follicular area, the lumenal area, the colloid area, the epithelial height of follicles, the ratio of colloid area and the ratio of epithelial area were measured using a monochromatic TV camera (CTC 2600, Ikegami Tsushinki Co. Ltd., Tokyo, Japan) and an image analyzer (Luzex 5000 X, Nireco Co., Tokyo, Japan). For each animal, the mean of five follicles was used.

Statistical analysis

Quantitative data were expressed as mean±standard deviation (S.D.). The heterogeneity of variance was analyzed by Bartlett’s test (Bartlett, 1937) followed by Dunnett’s test (Dunnett, 1955) for homogeneous variance data (Nagata and Yoshida, 1997a), and by the Dunnett type rank test for heterogeneous variance data (Nagata and Yoshida, 1997b). A p-value less than 0.05 was considered statistically significant.

RESULTS

Effects of SF, PTU, PB and NCD on the thyroid and serum thyroid hormones

After 2 weeks of treatment, the relative thyroid weight of the PTU-treated group was about 7 times that of the control group. However, SF, PB and NCD had no significant effect on the relative thyroid weight (Fig. 2). Fig. 3 showed that PTU significantly decreased the serum levels of the thyroid hormones T₃, T₄, FT₃ and FT₄. The effects of PB and NCD on thyroid hormones were moderate in comparison with those of PTU. PB tended to lower FT₃ levels. NCD significantly decreased only FT₄. No obvious change of thyroid hormones was seen 24 hr after the last SF treatment, while FT₃ and FT₄ were significantly decreased 6 hr after the last treatment with 160 mg/kg of SF. As Fig. 4 indicates, PTU increased the serum TSH level up to approximately 14 times that of control. In addition, 160 mg/kg of SF and NCD increased the level significantly to 186% and 242% of the control, respectively. PB tended to increase the TSH level to 168% of the control.

Effects of SF, PTU, PB and NCD on the liver and hepatic microsomal enzymes

All drugs tested increased the liver weight relative to the body weight after 2 weeks of treatment (Table 2). The protein concentration and UDPGT activity in the

![Fig. 2. Effects of semotiadil fumarate (SF), propylthiouracil (PTU), phenobarbital (PB) and nicardipine (NCD) on the thyroid weight relative to the body weight in female rats. The thyroid weight was measured on day 15 after 14 consecutive daily administrations of each compound. Vertical bars show the S.D. from 6-7 rats. Statistical significance: ** p<0.01 vs. control by Dunnett’s test.](image-url)
Semotiadil fumarate and thyroid hypertrophy.

Fig. 3. Effects of semotiadil fumarate (SF), propylthiouracil (PTU), phenobarbital (PB) and nicardipine (NCD) on serum triiodothyronine (T$_3$), free triiodothyronine (FT$_3$), thyroxine (T$_4$) and free thyroxine (FT$_4$) level in female rats. Serum samples were taken 6 hr (160 mg/kg of SF alone) and 24 hr after 14 consecutive daily administrations of each compound. Vertical bars show the S.D. from 6-7 rats. Statistical significance: * p<0.05, ** p<0.01 vs. control by Dunnett’s test.

Fig. 4. Effects of semotiadil fumarate (SF), propylthiouracil (PTU), phenobarbital (PB) and nicardipine (NCD) on thyroid stimulating hormone (TSH) level in female rats. Serum samples were taken 6 hr (160 mg/kg of SF alone) and 24 hr after 14 consecutive daily administrations of each compound. Vertical bars show the S.D. from 6-7 rats. Statistical significance: ** P<0.01 vs. control by Dunnett’s test.
hepatic microsomes increased in rats receiving 40 mg/kg or more of SF and significantly (P<0.05) at a dose of 160 mg/kg. PB (100 mg/kg) and NCD (160 mg/kg) significantly increased the protein concentration and UDPGT activity in the hepatic microsomes, causing them to rise higher than the levels obtained with 160 mg/kg of SF. In contrast, 100 mg/kg of PTU increased the UDPGT activity, but not the microsomal protein concentration.

Histopathological examination

Histopathological changes in the thyroids and livers from rats receiving SF (160 mg/kg), PTU (50 mg/kg), PB (100 mg/kg) and NCD (160 mg/kg) for 2 weeks were as follows.

1. Thyroid

PTU induced diffuse follicular cell hyperplasia. Hyperplastic glands contained irregularly shaped follicles with narrowed lumens and scanty amounts of colloid. The follicular epithelium appeared more cuboidal than normal (Photo 1), and piled (Photo 2). Hyperplasia induced by SF, PB or NCD was far more moderate than that induced by PTU. The glands consisted of irregularly shaped follicles with granular colloid (Photo 3).

2. Liver

The liver from a rat treated with PB showed centrilobular hypertrophy of hepatocytes with glassy cytoplasm, indicating the induction of hepatic microsomal enzymes. In the present study, SF, PTU and NCD did not cause obvious hypertrophy of hepatocytes.

Table 3 shows the results of morphometric analysis of the thyroid. SF increased the epithelial height and the ratio of epithelial area significantly. Similar changes were observed in the PB- and NCD- treated groups. Furthermore, NCD decreased the luminal area significantly. PTU caused significant increases in the follicular area, epithelial height and ratio of the epithelial area as well as significant decreases in the luminal area, colloid area and ratio of the colloid area.

DUSCCUSSION

In the 2-week treatment, SF, PB and NCD significantly increased relative liver weights without alteration of relative thyroid weights. PTU showed significant increases in both the relative thyroid and liver weights. With respect to thyroid function, SF showed significant decreases in thyroid hormones FT₃ and FT₄, after 6 hr of the last administration, while these parameters recovered to normal levels after 24 hr. NCD significantly decreased FT₄ and PB tended to decrease in FT₃ at 24 hr. Although PB caused no significant changes in thyroid hormone levels, it tended to lower levels. PTU showed significant decreases in T₃, FT₃, T₄ and FT₄ concentrations. In the other study, the serum levels of FT₃ and FT₄ tended to be lower than control at 3, 10 and 24 hr after the repeated 2-week treatment of SF, PB and NCD, but it was noticed that the concentrations rose and fell. SF decreased both FT₃ and FT₄ at 3 hr after administration, but those increased to the same level as the control at 10 hr after administration. At 24 hr after administration, FT₃ and FT₄ decreased significantly again. PB and NCD decreased FT3 and FT₄ at 3 and 10 hr after administration. These levels increased at 24 hr after administration. In contrast, PTU decreased

<table>
<thead>
<tr>
<th>Compound⁹</th>
<th>Dose (mg/kg/day)</th>
<th>Relative liver weightb</th>
<th>Microsomal proteinb</th>
<th>UDPGT activityb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(g/100 g b.w.)</td>
<td>(mg/g liver)</td>
<td>(n moles/mg protein/min)</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>3.40 ± 0.13</td>
<td>9.70 ± 1.32</td>
<td>62.6 ± 4.2</td>
</tr>
<tr>
<td>SF</td>
<td>40</td>
<td>3.86 ± 0.19**</td>
<td>11.16 ± 1.67</td>
<td>81.2 ± 10.0</td>
</tr>
<tr>
<td>SF</td>
<td>160</td>
<td>4.61 ± 0.32**</td>
<td>12.53 ± 1.34*</td>
<td>105.0 ± 17.0*</td>
</tr>
<tr>
<td>PTU</td>
<td>100</td>
<td>4.29 ± 0.22**</td>
<td>10.25 ± 1.60</td>
<td>123.7 ± 11.9**</td>
</tr>
<tr>
<td>PB</td>
<td>100</td>
<td>5.09 ± 0.23**</td>
<td>16.01 ± 2.23**</td>
<td>193.2 ± 29.6**</td>
</tr>
<tr>
<td>NCD</td>
<td>160</td>
<td>6.46 ± 0.52**</td>
<td>30.08 ± 3.00**</td>
<td>149.4 ± 80.9**</td>
</tr>
</tbody>
</table>

⁹Each compound was administered orally for 2 weeks.

²Twenty-four hr after the last administration.

b.w.: body weight.

Statistical significance: *p<0.05, **p<0.01 vs. control by Dunnett’s test.
Semotiadil fumarate and thyroid hypertrophy.

**Photo 1.** Thyroid from a female control rat. No marked change is seen. H&E staining ×320.

**Photo 2.** Thyroid from a female rat treated orally with 50 mg/kg of PTU for 2 weeks. Diffuse hyperplasia of follicular epithelium and scanty colloid are seen. H&E staining ×320.
Photo 3. Thyroid from a female rat treated orally with 160 mg/kg of semotiadil fumarate for 2 weeks. Small follicles and granular colloid are visible. H&E staining ×320.

Table 3. Morphometric analysis of the effects of semotiadil fumarate (SF), propylthiouracil (PTU), phenobarbital (PB) and nicardipine (NCD) on the thyroid in female rats.

<table>
<thead>
<tr>
<th>Compound(^a)</th>
<th>Control</th>
<th>SF 160</th>
<th>PTU 50</th>
<th>PB 100</th>
<th>NCD 160</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg/day):</td>
<td>0</td>
<td>160</td>
<td>50</td>
<td>100</td>
<td>160</td>
</tr>
<tr>
<td>Variable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular area ((\mu)m(^2))</td>
<td>247.5(^b)</td>
<td>269.1</td>
<td>453.3**</td>
<td>227.4</td>
<td>203.4</td>
</tr>
<tr>
<td></td>
<td>± 85.8</td>
<td>± 58.0</td>
<td>± 73.8</td>
<td>± 44.1</td>
<td>± 72.5</td>
</tr>
<tr>
<td>Luminal area ((\mu)m(^2))</td>
<td>116.4</td>
<td>84.4</td>
<td>28.8**</td>
<td>75.5</td>
<td>60.0**</td>
</tr>
<tr>
<td></td>
<td>± 55.2</td>
<td>± 32.6</td>
<td>± 18.5</td>
<td>± 22.5</td>
<td>± 27.8</td>
</tr>
<tr>
<td>Colloid area ((\mu)m(^2))</td>
<td>115.7</td>
<td>78.8</td>
<td>0.0**</td>
<td>68.6</td>
<td>56.1</td>
</tr>
<tr>
<td></td>
<td>± 54.4</td>
<td>± 31.4</td>
<td>± 0.0</td>
<td>± 19.7</td>
<td>± 37.2</td>
</tr>
<tr>
<td>Epithelial height of follicles ((\mu)m)</td>
<td>2.81</td>
<td>3.99**</td>
<td>6.33**</td>
<td>3.59**</td>
<td>3.77**</td>
</tr>
<tr>
<td></td>
<td>± 0.59</td>
<td>± 0.39</td>
<td>± 0.44</td>
<td>± 0.26</td>
<td>± 0.46</td>
</tr>
<tr>
<td>Ratio of colloid area (%)(^c)</td>
<td>99.5</td>
<td>93.9</td>
<td>0.0**</td>
<td>91.2</td>
<td>92.7</td>
</tr>
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<td></td>
<td>± 0.9</td>
<td>± 7.1</td>
<td>± 0.0</td>
<td>± 3.4</td>
<td>± 5.8</td>
</tr>
<tr>
<td>Ratio of epithelial area (%)(^d)</td>
<td>54.8</td>
<td>69.5**</td>
<td>93.7**</td>
<td>67.3*</td>
<td>72.1**</td>
</tr>
<tr>
<td></td>
<td>± 8.5</td>
<td>± 8.6</td>
<td>± 3.2</td>
<td>± 5.9</td>
<td>± 10.9</td>
</tr>
</tbody>
</table>

\(^a\)Each compound was administered orally for 2 weeks.
\(^b\)Mean ± S.D. from 5 follicles of the thyroid.
\(^c\)Colloid area/luminal area × 100.
\(^d\)Follicular area/luminal area/follicular area × 100.

Statistical significance: *p<0.05, **p<0.01 vs. control by Dunnett’s test.
Semotiadil fumarate and thyroid hypertrophy.

thyroid hormone levels at all time points and kept steady low levels (data was not shown). Thus, the effects of PTU on the serum thyroid hormone levels are more potent than those of the other three compounds. Although the difference in potency of the effects can be ascribed to doses used or the duration of effect, it may also suggest a possibility that PTU acts through a different mechanism from that of SF, PB and NCD.

PTU increased the serum TSH level by approximately 14-fold with a 7-fold elevation of the relative thyroid weight. SF, PB, and NCD increased TSH levels by approximately 2-fold without elevations of relative thyroid weights. SF, PB and NCD induced microsomal protein content and enhanced UDPGT activity. In contrast, PTU potentiated UDPGT activity without an elevation of protein content. Thus, while all four agents increased UDPGT activity, only PTU showed a drastic increase in TSH level, suggesting that PTU increased TSH level through a mechanism different from that of the other three compounds.

PB induces microsomal enzymes (Conney, 1967) which accelerate the peripheral disposition of thyroid hormones and subsequently increase the secretion of TSH from the pituitary gland (McClain et al., 1989). PTU inhibits the synthesis of thyroid hormones and lowers the circulating thyroid hormone levels, leading to the secretion of TSH as a feedback response (Engler et al., 1982). In our histopathological examination, PTU induced more striking thyroid hyperplasia than SF, PB and NCD. In the morphometric analysis, PTU markedly increased the follicular area, epithelial height, and ratio of the epithelial area, indicating its potent hyperplastic effect (Thiele and Funke, 1983), different from the other three compounds. In addition, PTU caused severe decreases in both the colloid area and ratio of the colloid area, indices of colloid production and resorption (Thiele and Funke, 1983), suggesting that PTU inhibited thyroid hormone synthesis. On the other hand, SF, PB and NCD showed a slight decrease in the colloid area, indicating that follicular epithelium of these rats treated with SF, PB and NCD maintained the ability to synthesize the thyroid hormones and that these compounds enhanced thyroid hormone secretion. Thus, our study confirmed the results of the previous studies described above. SF and NCD had an effect similar to that of PB. In addition, PTU induced severe thyroid hyperplasia, while SF, PB and NCD showed slight hypertrophic effects. Therefore, the magnitude of thyroid hyperplastic changes most likely reflects differences in mechanisms affecting thyroid function as well as possibly dose levels.

In our study, PTU increased UDPGT activity. The metabolism of thyroid hormones occurs through a variety of pathways, the most important ones being deiodination and conjugation with glucuronate and sulfate (Curran and DeGroot, 1991). p-Nitrophenol, used as a substrate in this study, was reported to be a good marker for induction of UDPGT activity toward Ts (Semler et al., 1989). This hepatic conjugation by UDPGT is considered to be the rate-limiting reaction for the biliary clearance of the circulating thyroid hormones (Bastomsky, 1973). Therefore, our results may indicate that PTU enhanced biliary excretion of thyroid hormones at high doses, besides being an inhibitor of thyroid hormone synthesis.

We found that the effects of SF and NCD were similar to that of PB. Agents enhancing hepatic UDPGT activity are widely accepted to induce thyroid hypertrophy. PB (McClain et al., 1989, Atterwill et al., 1993), polychlorinated biphenyls (Bastomsky, 1974), and an imidazole derivative SC-37211 (Comer et al., 1985) have been demonstrated to enhance hepatic UDPGT activity and induce thyroid hypertrophy. Therefore, hypertrophic effects of SF and NCD on the thyroid gland in rats are thought to be a compensatory response to an increase in metabolism and clearance of thyroid hormones, resulting in the increased TSH secretion via the negative feedback system. Currently, it is not known if these hypertrophic effects of SF and NCD are associated with calcium entry blocking.

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