Potentiation by Indomethacin of TRH-Induced TSH Secretion in the Rat

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Synopsis

We have studied the effect of two inhibitors of prostaglandin synthesis on the basal and TRH-stimulated plasma TSH levels in the rat. Animals were injected sc daily with indomethacin (3 mg/0.5 ml) or aspirin (16-30 mg/0.5 ml) for 3 days. The plasma T₄ and T₃ were consistently lower in the indomethacin or aspirin groups than in the controls, while the basal TSH levels did not change. Indomethacin treatment significantly potentiated the TSH response to synthetic TRH (200 ng, iv) in intact and thyroidectomized rats. The pituitary TSH content was markedly increased by indomethacin, while hypothalamic TRH content did not change. In contrast, aspirin inhibited the TSH response to TRH in intact rats, when pituitary TSH content decreased significantly. No potentiation by aspirin of TRH-stimulated TSH response in thyroidectomized rats was observed.

The increased sensitivity of plasma TSH response to exogenous TRH in the indomethacin group is presumably due to higher pituitary TSH content than in the controls. The action of indomethacin appears to be mediated, at least in part, at the pituitary level. In addition, there is a dissociation between the action of indomethacin and the action of aspirin in the TSH response to TRH.

There has been so far no consensus regarding the effect of prostaglandin (GS) on pituitary TSH secretion. Some investigators reported a stimulatory effect of PGE₁ (Dupon and Chavancy, 1972) and PGE₂ (Vale et al., 1972), but others failed to find the effect of PGs on TSH secretion from the pituitary in vitro (Tal et al., 1974). Brown and Hedge (1974) found that PGs had no effect on the basal TSH and PGE₁ actually inhibited the response of the pituitary TSH to the subsequently administered TRH when given iv to the rat. On the other hand, PGs potentiated the response of pituitary TSH to TRH only if the PGs were injected directly into the pituitary (Brown and Hedge, 1974). Thompson and Hedge (1976) reported that indomethacin or aspirin, inhibitors of PG synthesis, significantly inhibited the TSH response to TRH in the rat. In contrast, Ramey et al. (1976) observed that indomethacin had no effect on serum TSH response to TRH in man.

Because of these conflicting results, we considered that more detailed information on TSH secretion by indomethacin and aspirin would be of value. The present study indicates that indomethacin, but not aspirin, causes a significant augmentation of plasma TSH response to TRH in the rat.

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Materials and Methods

Male Wistar rats weighing 180–200 g were used. Each group of rats consisted of 5–6 animals. They were fed Oriental laboratory chow and tap water ad libitum. Animals were housed in group cages at a constant temperature and with a lighting schedule in which lights were turned on at 0600 hr and turned off at 2000 hr. Indomethacin (Sumitomo Kagaku Pharmaceutical Co., Osaka) was used at a daily dose of 3 mg/0.5 ml/injection for 3 days. Aspirin (Junsei Pharmaceutical Co., Tokyo) was used in a daily dose of 16–30 mg/0.5 ml/injection for 3 days. These drugs were suspended in 15% gelatin for sc injection. The indomethacin or aspirin treatment employed by us was considered to decrease pituitary and hypothalamic or plasma PGF levels, as reported by Orczyk and Behrman (1972). The total dose of these drugs administered for 3 days was approximately similar to those used within 24 hr by Thompson and Hedge (1976).

In experiment 1, 24 animals were divided into 2 groups, one of which (12 animals) was injected with indomethacin for 3 days. The other group of 12 rats were treated with 15% gelatin instead of indomethacin gelatin suspension. Twenty four hr after the last injection of indomethacin or gelatin alone, 6 rats from each group were injected with 200 ng synthetic TRH (Tanabe Pharmaceutical Co., Osaka) iv via tail vein under ether anesthesia. One ml of heparinized blood was taken through the jugular vein just before and 20 min after the injection of TRH. Six rats from each group were killed by decapitation without TRH injection. The anterior pituitary was separated from the neurohypophysis and weighed on Librare electrobalance. It was then homogenized in 0.5 ml of phosphate buffered saline solution (0.01 M phosphate and 0.15 M NaCl, pH 7.6, PBS) containing 1% bovine serum albumin (BSA), centrifuged and the supernatant was diluted 1:25 for storage (Wilber and Utiger, 1967). In experiment 2, thyroidectomy was carried out by conventional methods under ether anesthesia. Seven days after thyroidectomy, 10 rats were injected with indomethacin daily for 3 days. Another 10 rats were treated with gelatin alone. Three days later, autopsy was performed as in experiment 1. The hypothalamic slices were rapidly dissected in ice-cold 90% methanol, homogenized and extracted with methanol twice, and the extract was evaporated to dryness with a vacuum pump. The dried extract was dissolved in PBS containing 1% BSA for subsequent assay (Reichlin et al., 1970). In experiment 3, 24 rats were divided into 2 groups and the animals were injected with aspirin or gelatin alone daily for 3 days. The other experimental conditions were the same as in the experiment 1. Furthermore, 24 thyroidectomized rats were divided into 2 groups and the animals were injected with aspirin or gelatin for 3 days.

Plasma samples were kept at −20°C until assay. TSH was assayed using radioimmunoassay (RIA) materials provided by the NIAMDD Rat Pituitary Hormone Distribution Program and the data were expressed as mU/100 ml of plasma and mU/pituitary. Plasma T₄ was measured by T₄ RIA kit (Dainabot Radioisotope Lab., Tokyo). Plasma T₃ was measured by T₃ RIA kit (Daichi Radioisotope Lab., Tokyo). The normal ranges for plasma T₄, T₃ and TSH in normal rats were 2.6–6.5 µg/100 ml, 50–90 ng/100 ml and 6–20 mU/100 ml, respectively. The minimal detectable levels of T₄ and T₃ in our assay were 0.4 µg/100 ml and 25 ng/100 ml, respectively. TRH was labeled with ¹²⁵I, using the Greenwood-Hunter technique (1972). Synthetic TRH served as the reference preparations. All samples for each experiment were analyzed in the same assay for each hormone. Inter- and intra-assay coefficients of variation were less than 10% in all assays. Statistical evaluation of the data was made with Student’s t test.

Results

1. Effect of indomethacin treatment on plasma TSH response to TRH in intact rats.

The plasma TSH response following TRH stimulation is shown in Fig. 1. The basal TSH level between the indomethacin and gelatin control group did not differ significantly (p>0.05). In contrast, a significant augmentation of TSH response to TRH was observed in the indomethacin-treated group (p<0.01). As shown in Table 1, both plasma T₄ and T₃ concentrations were below the detectable limitation in the indomethacin group. In addition, higher pituitary TSH content was detected in the indomethacin group than in the gelatin group (p<0.05).

2. Effect of indomethacin treatment on plasma TSH response to TRH in thyroidectomized rat.

Thyroidectomized rats were treated with indomethacin or gelatin alone for 3 days. Both plasma T₄ and T₃ levels were un-
detectable in our assay (Table 2). The basal TSH levels in the two groups increased following thyroidectomy, but the rise was significantly lower in the indomethacin group than in the gelatin control group (p<0.01). The plasma TSH response to TRH is shown in Fig. 2. The higher plasma TSH response to TRH was observed in the indomethacin group than in the control group (p<0.01). The pituitary TSH content was significantly increased by indomethacin treatment (p<0.02), while hypothalamic TRH content in the two groups was not different (p>0.1).

3. Effect of aspirin treatment on plasma TSH response to TRH in intact and thyroidectomized rats.

Intact rats were treated with aspirin or gelatin alone for 3 days. As shown in Table 3, both plasma T₄ and T₃ levels were significantly lower in the aspirin group than those in the gelatin control group (T₄: p<0.05, T₃: p<0.02). The basal TSH level was slightly lower in the aspirin group than in the control group, although this difference was statistically not significant

Table 1. Effect of chronic administration of indomethacin on plasma T₄, T₃, TSH and pituitary TSH content in intact rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body wt (g)</th>
<th>Plasma T₄ (µg/100 ml)</th>
<th>Plasma T₃ (ng/100 ml)</th>
<th>Plasma TSH (mU/100 ml)</th>
<th>Pituitary TSH (mU/gland)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>176±9</td>
<td>3.4±0.1</td>
<td>80.0±5.7</td>
<td>9.1±1.0</td>
<td>59.7±7.2</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>163±7</td>
<td>n.d.</td>
<td>n.d.</td>
<td>7.5±0.7</td>
<td>149.4±44.6*</td>
</tr>
</tbody>
</table>

All results expressed as mean±SEM of 6 rats per group. "n.d." indicates that the hormone concentration in all samples was below the limit of detectability. Statistical comparison between the two groups was made by Student's t test; * p<0.05. Values without an asterisk were not significantly different (p>0.05).

Table 2. Effect of indomethacin treatment on hypothalamic-pituitary-thyroid axis in thyroidectomized rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body wt (g)</th>
<th>Plasma T₄ (µg/100 ml)</th>
<th>Plasma T₃ (ng/100 ml)</th>
<th>Plasma TSH (mU/100 ml)</th>
<th>Pituitary TSH (mU/gland)</th>
<th>Hypothalamic TRH (ng/hypothalamus)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>185±11</td>
<td>n.d.</td>
<td>n.d.</td>
<td>39.1±2.5</td>
<td>39.2±4.8</td>
<td>7.6±0.3</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>176±10</td>
<td>n.d.</td>
<td>n.d.</td>
<td>17.1±3.2*</td>
<td>115.1±21.3**</td>
<td>8.3±0.5</td>
</tr>
</tbody>
</table>

The mean±SEM of 5 rats per group is shown. "n.d." is shown as in Table 1. * p<0.01, ** p<0.02.
Fig. 2. Effect of indomethacin treatment on plasma TSH response to TRH in thyroidectomized rats. The mean±SEM of 5 rats per group is shown for each point. The differences between the TSH levels of the control and indomethacin groups were statistically significant at time 0 and 20 min (*p<0.01).

Fig. 3. Effect of aspirin treatment on plasma TSH response to TRH in intact and thyroidectomized rats. The mean±SEM of 6 rats per group is shown. Difference between the control and aspirin groups in intact rats was statistically significant at 20 min after TRH injection (*p<0.01).

Table 3. Effect of chronic administration of aspirin on plasma T₄, T₃, TSH and pituitary TSH content in intact and thyroidectomized rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body wt (g)</th>
<th>Plasma T₄ (μg/100 ml)</th>
<th>Plasma T₃ (ng/100 ml)</th>
<th>Plasma TSH (mU/100 ml)</th>
<th>Pituitary TSH (mU/gland)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Intact</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>195±7</td>
<td>2.9±0.1</td>
<td>61.2±3.2</td>
<td>14.0±1.7</td>
<td>62.3±4.3</td>
</tr>
<tr>
<td>Aspirin</td>
<td>193±5</td>
<td>1.4±0.4*</td>
<td>44.0±3.0**</td>
<td>8.8±0.7</td>
<td>46.9±4.1*</td>
</tr>
<tr>
<td>II. Thyroidectomized</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>190±5</td>
<td>n.d.</td>
<td>n.d.</td>
<td>53.5±4.2</td>
<td>62.4±2.2</td>
</tr>
<tr>
<td>Aspirin</td>
<td>185±5</td>
<td>n.d.</td>
<td>n.d.</td>
<td>58.0±4.0</td>
<td>48.1±4.6</td>
</tr>
</tbody>
</table>

Aspirin was injected sc daily to intact (16 mg/0.5 ml gelatin solution) and thyroidectomized (30 mg/0.5 ml gelatin solution) rats for 3 days. The mean±SEM of 6 rats per group is shown.

"n.d." is shown in Table 1. *p<0.05, **p<0.02.
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(p > 0.05). In contrast to indomethacin, aspirin inhibited the TSH response to TRH (p < 0.01, Fig. 3). The pituitary TSH content was significantly lower in the aspirin group than in the control group (p < 0.05).

Thyroidectomized rats were also treated with aspirin or gelatin for 3 days. No difference in the basal TSH, TSH response to TRH and pituitary TSH content between the two groups was observed (Table 3, Fig. 3).

**Discussion**

No increase in the plasma TSH level in indomethacin-treated animals was observed, even when plasma $T_4$ and $T_3$ concentrations decreased to the undetectable level (Table 1). Thompson *et al.* (1977) reported that a dramatic decrease in circulating $T_4$ and $T_3$ in the indomethacin-treated group was due at least in part to a marked reduction in thyroid responsiveness to TSH. On the other hand, Tsukui *et al.* (1972) reported that indomethacin displaced $T_4$ from plasma binding proteins in vivo and in vitro. It is thus possible that the effect of indomethacin on the plasma TSH level may correlate with an increase in free thyroid hormone concentration followed by decreasing total $T_4$ level, since several drugs which inhibit $T_4$ binding to $T_4$-binding proteins were reported to suppress TSH secretion (Yamamoto *et al.*, 1972; Good *et al.*, 1965). However, Thompson *et al.* (1977) demonstrated that indomethacin increased the percent of free $T_4$ in the serum, but because of the extremely low level of $T_4$, the concentration of free $T_4$ was significantly less than in the controls. Similar changes might be seen in our study, although we could not determine free thyroid hormone concentrations in the plasma, because total $T_4$ and $T_3$ levels in the indomethacin-treated group were below limits of detectability. Therefore, it is likely that indomethacin blocked the rise in plasma TSH following a marked decrease in $T_4$ and $T_3$ by some other mechanism than the effect on thyroid hormone binding on plasma proteins.

To explore further a possible central effect of indomethacin on TSH secretion, thyroidectomized rats were used (Table 2), since no significant alteration by the drug in the total and free $T_4$ and $T_3$ levels could be attained owing to the very low total plasma thyroid hormone concentrations (Yamamoto *et al.*, 1972). Nevertheless, indomethacin treatment suppressed an elevation in the basal plasma TSH level following thyroidectomy (p < 0.01), although this TSH level in indomethacin-treated group was still higher than in intact rats (Table 1). Thus, it is suggested that indomethacin treatment inhibits the compensatory TSH rise by acting directly at the hypothalamic-pituitary level.

Thompson and Hedge (1976) reported that indomethacin or aspirin decreased the pituitary TSH response to TRH in thyroidectomized rats with $T_4$ replacement. In contrast, we have found that indomethacin treatment potentiated the pituitary response of TSH to TRH in both intact and thyroidectomized rats without $T_4$ replacement (Fig. 1 and 2). Harada *et al.* (1975) postulated that increased sensitivity of pituitary TSH response to TRH was roughly correlated with pituitary TSH content. It is of interest that there was a significant increase in the pituitary TSH content in the indomethacin-treated animals (Table 1 and 2). Therefore, it is possible that the indomethacin prevents an expected increase in the plasma TSH level after thyroidectomy followed by an accumulation of TSH in the pituitary, which in turn results in the potentiation of TSH secretion to TRH. Since the rats were given $T_4$ replacement after thyroidectomy in the work of Thompson and Hedge (1976), there might be no sti-
mulating effect on pituitary TSH response to TRH due to reduced T4 and T3 levels. Under such conditions, one would neither expect a great accumulation of TSH in the pituitary with indomethacin treatment, nor the potentiation of TSH response to TRH.

Ramey et al. (1976) reported that aspirin blocked the responsiveness to TRH in man by a mechanism other than the inhibition of PG synthesis, while indomethacin had no effect on it. It is of interest to note that aspirin consistently inhibits the TSH response to TRH, in contrast to indomethacin (Thompson and Hedge, 1976; Ramey et al., 1976), although no inhibitory effect of aspirin on TSH response to TRH was observed in thyroidectomized rats (Fig. 3). In this connection, it seems likely that there is a dissociation between the action of indomethacin and that of aspirin in the TSH response to TRH.

The result of the present study is the first demonstration that indomethacin treatment potentiated the pituitary TSH response to TRH in intact and thyroidectomized rats. The finding that indomethacin caused an increase in pituitary TSH content, without affecting hypothalamic TRH content (Table 2), suggests that the action of indomethacin is mediated, at least in part, at the pituitary level.

References