EFFECT OF HYPERPROLACTINEMIA INDUCED BY NEUROLEPTIC AGENT, TIMIPERONE, ON PORPHYRIN CONTENT OF MOUSE HARDERIAN GLAND

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ABSTRACT — The histology and porphyrin content of the Harderian gland and the serum prolactin levels were examined in male B6C3F1 mice treated with neuroleptic butyrophenones (timiperone and haloperidol) and treated concurrently with timiperone and 2-bromo-α-ergocryptine (bromocriptine), a potent suppressor of prolactin.

Light-microscopically, both timiperone and haloperidol increased the number of accretions of porphyrin pigment within the Harderian gland lumina. Timiperone treatment of mice increased both the porphyrin content of the Harderian gland and the serum prolactin levels. Administration of bromocriptine to timiperone-treated mice distinctly prevented the rise in both tissue porphyrin and serum prolactin levels. In intact mice, bromocriptine also exerted inhibitory effects on both the Harderian gland porphyrin content and the serum prolactin level. Electron microscopic investigation revealed that the cytoplasm of type A cells in the Harderian glands of mice treated with timiperone contained trilaminar profiles similar to those seen in the intraluminal pigment masses.

These results indicate that timiperone accelerates porphyrin secretion from the type A cells of the mouse Harderian gland by increasing the serum prolactin levels.

KEY WORDS: Harderian gland, Porphyrin, Prolactin, Neuroleptics, Mouse

INTRODUCTION

The Harderian gland is a tubuloalveolar orbital gland present in many vertebrates such as mice, rats, and hamsters, but not in dogs, cats, and primates (Sakai, 1981). Its functions remain unclear, although it is usually regarded as a source of lubrication for the nictitating membrane (Kennedy, 1970). The organ has been proposed to be a site of immune response (Mueller et al., 1971; Albini et al., 1974), a link in a retino-pinealo-gonadal system (Wetterberg et al., 1970a; Reiter and Klein, 1971; Clabough and Norvell, 1973; Shirama, 1978), a source of pheromones (Thiessen et al., 1976; Payne, 1977) or a source of thermoregulatory lipids (Thiessen and Kittrell, 1980).

In rodents (rat, mouse and hamster), the Harderian gland contains brown porphyrin pigments which exhibit a characteristic red fluorescence in the ultraviolet light in the alveolar lumina (Grafflin, 1942). But pigments are visible as solid intraluminal accretions in histological preparations only when the amount of porphyrin is excessive. We observed microscopically the increased porphyrin pigments within the acinar lumina of the Harderian glands of male and female B6C3F1 mice treated with timiperone, a neuroleptic butyrophenone, in our preliminary study. Butyrophenones are known to induce hyperprolactinemia due to antidopaminergic effects (Dickerman et al., 1974; Horowski and Graf, 1979). Therefore we speculated that increased serum prolactin might stim-

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ulate the porphyrin secretion in the Harderian glands of mice. Gupta and Maiti (1981) reported that prolactin stimulated secretory activity in the Harderian glands of chicks, but did not refer to porphyrins. Buzzell et al. (1989) stated that bromocriptine, a dopaminergic agonist, prevented the castration-induced rise in porphyrin concentration in the Harderian glands of the male hamster, but it had no effect on the porphyrins in either intact males or intact females. In mice, it has been suggested that testosterone and progesterone were directly responsible for regulating the porphyrins of the Harderian gland (Shirama et al., 1981). As yet there is no information on the prolactin regulation of the Harderian glands of mice.

In the present study, we performed histological and biochemical examinations to determine whether timiperone increases the porphyrin content of the Harderian glands in mice. We studied further the influence of a combination of timiperone and bromocriptine, a potent suppressor of prolactin secretion, treatment to clarify the role of the serum prolactin on the Harderian gland porphyrins.

MATERIALS and METHODS

Animals

All animals used in this study were 6-week-old B6C3F1 male mice obtained from Charles River Japan Inc., Yokohama, Kanagawa. They were housed in polycarbonate cages in a room in which temperature (23 ± 2 °C), humidity (55 ± 10 %) and light (turned on at 8 AM and off at 8 PM) were controlled, and they were given a basal laboratory diet (CRF-1, Oriental Yeast Co. Ltd., Tokyo) and tap water ad libitum.

Chemicals

Timiperone (4'-fluoro-4-[4-(2-thioxo-1-benzimidazolyl) piperidino] butyrophenone) was obtained from Daiichi Pharmaceutical Co. Ltd., Tokyo, Japan, and haloperidol (4-[4-(p-chlorophenyl)-4-hydroxypiperidino]-4'-fluoro butyrophenone) from Teikoku Chemical Industries Co. Ltd., Osaka, Japan. Animals were given these chemicals mixed with basal diet (CRF-1) at a concentration of 250 ppm. Bromocriptine (CB-145, Sigma Chemical Company, St. Louis, MO, USA.) was used to reduce circulating prolactin levels. CB-154 suspended in olive oil was administered subcutaneously every day at 0.4 mg/0.1 ml/mouse. Control mice received 0.1 ml of olive oil s.c. per day.

Experimental design and treatment

Experiment 1

The effects of timiperone and haloperidol on the Harderian gland porphyrins were examined histologically. Male mice were divided into three experimental groups of ten mice each. Group 1 was given the basal diet and served as the intact control. Groups 2 and 3 were fed a diet containing timiperone and haloperidol, respectively, for 5 weeks. Five animals from each group were killed by exsanguination under ether anesthesia at week 1 and the remainder at week 5 after the commencement of the treatment. For light microscopic examination, the Harderian glands were removed at necropsy followed by fixation with Bouin's solution. Paraffin sections of these samples were made and stained with hematoxylin and eosin. The number of porphyrin accretions present in the Harderian glands was counted under a microscope, and expressed as number per mm² of the Harderian gland section.

Experiment 2

The porphyrin content of the Harderian gland was determined in mice treated with timiperone. Fifty male mice were divided into two groups of twenty-five animals each, as follows: 1) animals given basal diet for 5 weeks, 2) animals given diet containing timiperone for 3 weeks, followed by feeding on basal diet for 2 weeks. Five mice from each group were sacrificed weekly by exsanguination under ether anesthesia. Body weights were recorded, and both Harderian glands were removed, weighed, and stored at -20 °C until use for porphyrin analyses. The Harderian glands of mice treated with timiperone for 3 weeks and of intact mice at the same age were examined by electron microscopy. Samples were fixed for 2 hr in 3 % phosphate-buffered glutaraldehyde. After postfixation for 2 hr in 1 % phosphate-buffered osmium tetroxide, the tissues were dehydrated in graded alcohols and embedded in Quetol 812.
Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a Hitachi H-500 transmission electron microscope.

**Experiment 3**

The serum prolactin levels and the Harderian gland porphyrins were examined in mice subjected to combinations of the bromocriptine injection and the dietary administration of timiperone. Twenty male mice were divided into four groups of five animals each, as follows: 1) animals given basidial diet, 2) animals given daily injections of bromocriptine, 3) animals given basidial diet mixed with timiperone, 4) animals given bromocriptine during the timiperone treatment period. The experiment was terminated at week 2. The animals were decapitated and the blood samples for prolactin assay were collected. All blood samples were centrifuged at 3,000 rpm for 10 min and sera were stored at -80 °C until use for hormone assays. Both Harderian glands were removed immediately after blood sampling, weighed, and stored at -20 °C until use for porphyrin analyses.

**Measurement of the Harderian gland porphyrin content**

For assay the Harderian glands were thawed and homogenized in 10 ml of a mixture of ethyl acetate and glacial acetic acid (4 : 1). A portion of the homogenate was diluted 1 : 50 with 1.5 N hydrochloric acid and centrifuged at 3,000 rpm for 10 min. Porphyrin content was determined by fluorescence at 602 µm upon activation at 405 µm. Tetramethyl-coproporphyrin (Sigma) stock standard (1.0 µg/ml) was used as a reference substance. This assay followed the standard procedure used by Wetterberg et al. (1970b).

**Measurement of the serum concentration of prolactin (PRL)**

Highly purified mouse PRL and rabbit antimume PRL serum were donated by Dr. A. F.

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**Table 1.** Average body weights, food consumption and drug intake of male mice treated with neuroleptics for 5 weeks in experiment 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Food consumption (g/mouse/day)</th>
<th>Drug intake (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>initial 1 week 5 week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Control</td>
<td>24.6±0.8 26.1±1.1 29.9±1.2</td>
<td>6.1±1.1</td>
<td>-</td>
</tr>
<tr>
<td>2. TPN</td>
<td>24.6±0.9 24.5±1.4** 30.1±2.0</td>
<td>4.3±0.6</td>
<td>40.2±8.2</td>
</tr>
<tr>
<td>3. HPL</td>
<td>24.5±0.7 23.4±0.8** 27.6±1.3</td>
<td>4.3±0.9</td>
<td>42.9±8.4</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D.
Significantly different from control (**: p<0.01).
TPN: timiperone, 250 ppm in the diet.
HPL: haloperidol, 250 ppm in the diet.

**Table 2.** Numbers of porphyrin accretions in the Harderian glands of male mice treated with neuroleptics for 5 weeks in experiment 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>1 week</th>
<th>5 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of mice</td>
<td>No. of porphyrin accretions/mm²</td>
</tr>
<tr>
<td>1. Control</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>2. TPN</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>3. HPL</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D.
Significantly different from control (**: p<0.01).
TPN: timiperone, 250 ppm in the diet.
HPL: haloperidol, 250 ppm in the diet.
Parlow, Pituitary Hormones and Antisera Center, Harbor-UCLA Medical Center, Torrance, California, USA. We used a modification of the method of Sinha et al. (1972) for the radioimmunoassay of mouse PRL.

**Statistical analysis**

The significance of inter-group differences in the body weights, the food consumption, the organ weights, the histological data, the amounts of Harderian gland porphyrin, and the serum PRL levels were assessed using Student's t-test. A value for P of <0.05 was regarded as significant.

**RESULTS**

**Experiment 1**

The body weight, food consumption and drug intake are summarized in Table 1. The body weight for mice receiving timiperone or haloperidol was significantly less than control value at week 1, but recovered to levels comparable to the controls after 5 weeks of treatment. The Food consumption of the timiperone- and haloperidol-treated groups was decreased. The drug intake in the timiperone- and haloperidol-treated groups during the study was 40.2 and 42.9 mg/kg/day, respectively. Macroscopically, brown pigments were seen in the Harderian glands of mice treated with timiperone or haloperidol for 5 weeks. Microscopically, brown pigments were observed as granular or solid accretions in the alveolar lumina (Photo. 1) and the pigment accretions were identified as porphyrins because of their red fluorescence under ultraviolet light. The numbers of pigment accretions in the timiperone-treated group were almost the same as those in the haloperidol-treated group (Table 2). No pigments were demon-

![Photo 1](image-url)  
**Photo 1.** Light micrograph of the Harderian gland from a male mouse treated with timiperone for 5 weeks. Some of the alveoli contain intraluminal accretions of porphyrin pigment (asterisk). H.E. Staining, ×80.
Fig. 1. Changes in body and Harderian gland weights of male mice treated with timiperone for 3 weeks followed by a 2-week recovery period in experiment 2. Values are expressed as mean ± S.D. (n=5). Significantly different from control (*: p<0.05, **: p<0.01).

○: control, ●: timiperone, 250 ppm in the diet.
—: body weight, ---: Harderian gland weight.

Fig. 2. Changes in porphyrin content in the Harderian glands of male mice treated with timiperone for 3 weeks followed by a 2-week recovery period in experiment 2. Values are expressed as mean ± S.D. (n=5).

Significantly different from control (**: p<0.01).

○: control, ●: timiperone, 250 ppm in the diet.
stable in the Harderian glands of untreated control mice (Table 2).

**Experiment 2**

The Body weight and Harderian gland weight are shown in Fig. 1. The weekly sequent levels of the Harderian gland porphyrins are illustrated in Fig. 2. The body weight for mice receiving timiperone was significantly less than control value at weeks 1 and 2, but recovered to a level comparable to the control after 3 weeks of treatment. The treatment with timiperone also decreased the weight of Harderian glands. The porphyrin content was 3.39 μg /100mg gland tissue in the timiperone-treated mice, as against 0.39 μg /100mg in controls at week 1. At week 3, the porphyrin content (34.43 μg /100mg) in the timiperone-treated mice was more than about 55 times that in the controls. The increased porphyrin levels were decreased gradually after the withdrawal of timiperone treatment.

Electron microscopic examination showed that the intraluminal pigments were aggregates of straight and curved linear trilaminar profiles, as seen in Photo. 2. The intraluminal pigments with these profiles were intermingled with lipid droplets and cell debris. The glandular epithelium consisted of two types of secretory cells (A and B types) with abundant lipid vacuoles. In a few of the A-cells from timiperone-treated mice, the cytoplasm contained linear trilaminar profiles (Photo. 3) similar to those seen in the intraluminal pigment masses. Linear profiles were scattered in the cytoplasmic matrix between dilated endoplasmic reticulum. No linear trilaminar form was found in either the alveolar lumen or the acinar cell cytoplasm from control mice.

**Photo 2.** Electron micrograph of the Harderian gland from a male mouse treated with timiperone for 3 weeks, showing the intraluminal pigment mass. The substance consists of straight and curved trilaminar profiles (arrow) mixed with the cytoplasmic component of degenerated cells (star).
Photo 3. Electron micrograph of the Harderian gland from a male mouse treated with timiperone for 3 weeks, showing the cytoplasm of A-cell. There is a group of straight and curved linear profiles (arrow) in a cytoplasmic matrix (a). b shows details of the linear profiles; they are trilaminar with a lighter central core and electron-opaque outer rims.
Experiment 3
The effects of timiperone and/or bromocriptine on serum prolactin and Harderian gland porphyrins are shown in Fig. 3. Serum prolactin concentration in timiperone-treated mice was significantly increased, compared to untreated control levels. When timiperone and bromocriptine were given together, serum prolactin levels were significantly decreased compared with those in the timiperone-treated mice and tended to be lower than values in the untreated control mice. Timiperone treatment led to marked increases in Harderian gland porphyrin levels; this was prevented by bromocriptine. In mice

![Graph showing serum prolactin (PRL) levels and Harderian gland porphyrin content in male mice treated with timiperone (TPN) alone or in combination with bromocriptine (CB-154) in experiment 3. Values are expressed as mean ± S.D. (n=5). Significantly different from control group (*: p<0.05, **: p<0.01). Significantly different from TPN group (#: p<0.05, ##: p<0.01). CB-154: bromocriptine, 0.4mg/mouse/day, S.C. for 2 weeks. TPN: timiperone, 250 ppm in the diet for 2 weeks.]

Fig. 3.
receiving bromocriptine alone, prolactin levels tended to be low and porphyrins were significantly less than those in control animals.

DISCUSSION

Both timiperone and haloperidol increased the porphyrin content of the Harderian glands of mice, demonstrating that this effect is a characteristic common to butyrophenones.

It has been reported that temperature, lighting and sex hormones were the factors influencing porphyrin synthesis in the Harderian glands of mice (Shirama et al., 1981; Strum and Shear, 1982) rats (Ulrich et al., 1974; Shirama et al., 1987) and hamsters (Hoffman, 1971; Wetterberg et al., 1972; Payne et al., 1977; Spike et al., 1985). Buzzell et al. (1989) have proposed that prolactin stimulated the porphyrin synthesis in the hamster Harderian gland. On the other hand, Shirama et al. (1981) have suggested that, in mice, testosterone and progesterone were directly involved in regulating the porphyrins of the Harderian gland. To date, there has been no information regarding the effect of prolactin on the porphyrins of the gland in mice. Butyrophenones are known to exert an inhibitory effect on the hypothalamus and lead to increased blood prolactin levels (Dickerman et al., 1974; Horowski and Graf, 1979). Bromocriptine has the ability to suppress prolactin secretion of the pituitary and does not interfere substantially with the secretion of the other pituitary hormones (Yanai and Nagasawa, 1970 and 1972; Welsch and Gribler, 1973; Sinha et al., 1974).

In the present study, timiperone treatment increased both the porphyrin content of the Harderian gland and the serum prolactin level, the increase being prevented by bromocriptine. These results indicate that prolactin exerts a stimulatory effect on glandular porphyrins.

In mice (Shirama et al., 1981) and hamsters (Payne et al., 1977), castration reduces the blood testosterone levels and elevates the Harderian gland porphyrins. It has been reported that testosterone showed a marked inhibitory effect on glandular porphyrins in mice (Shirama et al., 1981) and hamsters (Hoffman, 1971; Payne et al., 1977). By the way, treatment of intact mice with bromocriptine can reduce peripheral testosterone levels (Bartke, 1976). Thus, bromocriptine may increase the Harderian gland porphyrins. In the present study, however, both Harderian gland porphyrin and serum prolactin concentrations were decreased below the control level in intact mice given bromocriptine. Therefore, the stimulatory effect of prolactin appears to be more responsible rather than the inhibitory effect of testosterone for regulating the porphyrins of the Harderian glands in mice. Prolactin may be involved in the rise in the Harderian gland porphyrins in castrated male mice. Further investigation is required to clarify this point.

In mice, two types of secretory cells (A type and B type) comprise the glandular epithelium. It is still unknown whether an intraluminal component which may consist of porphyrin compounds is produced by the type A or type B cells. Strum and Shear (1982) have demonstrated that lipid droplets of the type A cells in the glands of mice displayed a red fluorescence, but those in the type B cells did not. On this basis, they concluded that porphyrins were present in the lipid droplets of the type A cells. On the other hand, the ultrastructure of the porphyrin seems to be identical with lamellated fibrous membranes observed in the secretory vacuoles of the type B cells, suggesting that the porphyrins may also be secreted by the type B cells (Watanabe, 1980). Carriere (1985) found trilaminar profiles which resembled the structures identified as protoporphyrin crystals in mouse hepatocytes and dilated vesicles in the cytoplasm of A cells in rats. He concluded that a holocrine process might be considered as a mode of porphyrin release from the secretory A cells, since the acinar lumina contained pigments with abundant trilaminar profiles mixed with the cytoplasmic component of degenerated cells. The present results indicate that a holocrine process from the secretory A cells may also be involved in mice, and timiperone appears to stimulate this porphyrin secretion in the mouse Harderian gland.

The present results appear to suggest that timiperone probably accelerated the porphyrin secretion of the mouse Harderian gland through an increase in the blood prolactin level. And it is believed that prolactin plays an important role
in increasing the presence of porphyrins in the Harderian glands of mice.

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REFERENCES


lactin secretion in mice by a homologous radioimmunoassay. Endocrinology, 91, 1045-1053.


