Restoration of Prolactin Synthesis and Release by the Administration of Monoaminergic Blocking Agents to Pituitary Tumor-bearing Rats

Robert M. MacLeod and Joyce E. Lehmeyer

Department of Internal Medicine, University of Virginia School of Medicine, Charlottesville, Virginia 22901

SUMMARY

Pituitary glands from normal and pituitary tumor-bearing rats were incubated in vitro in the presence of leucine-4,5-3H, and the incorporation of radioactivity into prolactin present in the pituitary gland and incubation medium was measured. A significant decrease in prolactin production was observed in the glands from tumor-bearing rats. Incubation of these glands or normal pituitary glands with 4-(2-aminoethyl)pyrocatechol (dopamine) caused a significant decrease in the amount of radioactive prolactin secreted into the incubation medium. The administration of monoaminergic blocking agents, i.e., perphenazine, haloperidol, or pimozide, significantly overcame the dopamine-mediated inhibition of prolactin secretion. Injection of tumor-bearing rats with these agents completely restored to normal levels the incorporation of radioactive leucine into prolactin. These data suggest that the elevated serum prolactin concentration resulting from pituitary tumor secretion decreases the host's pituitary gland production of prolactin by acting through a catecholamine-dependent mechanism.

INTRODUCTION

The implantation of the prolactin-secreting pituitary tumors into the scapular region of rats greatly elevates serum prolactin levels (2, 5). An accepted concept for the control of normal pituitary gland function suggests that releasing and inhibiting factors in the hypothalamus regulate the secretion of adenohypophyseal hormones. The hypothalamus is under neural control which is apparently modified through a feedback mechanism sensitive to the concentration of specific serum hormones. In situ, release of prolactin from the pituitary gland into the circulatory system is suppressed by inhibitory factors in the hypothalamus. These pituitary tumors function independently of hypothalamic control. However, the tumor hormones are capable of exerting a significant effect on the pituitary gland of the host animal (14). The presence of these tumors suppresses the gland's content of prolactin and inhibits the release of the hormone from the gland.

Recently, we presented evidence that monoaminergic blocking agents exert a powerful action on the secretion of prolactin (9). In this study, we used some of these blocking agents in an attempt to elucidate the nature of the mechanism by which the pituitary tumors exert their suppressive effect on the pituitary glands of the host animals.

MATERIALS AND METHODS

Mature male and female Sprague-Dawley rats were obtained from Flow Research Animals, Dublin, Va. The rats were routinely housed 4 to 5/cage and allowed water and Purina laboratory chow ad libitum.

Mature female Wistar/Furth rats (obtained from ARS/Sprague-Dawley, Madison, Wis.) were inoculated with pituitary tumor MtTW15 as previously described (10). Pituitary tumor 7315a was transplanted into mature female Buffalo rats (obtained from Simonsen Laboratories, Gilroy, Calif.).

Following decapitation of the animals, the anterior pituitary glands were excised, hemisected, and incubated with 10 μCi of leucine-4,5-3H in tissue culture Medium 199 as previously described (11). At the termination of incubation, the pituitary glands were homogenized in 1 ml 0.05 m phosphate, pH 7.2, with a glass homogenizer fitted with a Teflon pestle, and the contents were frozen and thawed 3 times. Duplicate 25- or 50-μl aliquots of the homogenates or incubation medium were subjected to polyacrylamide gel electrophoresis according to the Reisfeld et al. (17) modification of the method of Jones et al. (8). The protein bands on the gels were stained with Amido black and identified by comparison of their electrophoretic mobility with those produced by purified rat prolactin or growth hormone. The authenticity of the prolactin and growth hormone bands on polyacrylamide gels has been documented by many investigators. The protein bands were dissolved in 30% H2O2 at 90°, and the radioactivity was determined by liquid scintillation techniques.

In some cases, the prolactin in the incubation media and in the sera of the animals was measured by double-antibody radioimmunoassay, with the reagents and protocol supplied by the Hormone Distribution Program, National Institute of Arthritis and Metabolic Diseases. Sheep anti-rabbit γ-globulin was obtained from Dr. Ann Johanson of the University of Virginia School of Medicine.

1 This investigation was supported by USPHS Grant CA-07535 from the National Cancer Institute.

Received September 4, 1973; accepted November 7, 1973.
Perphenazine was a gift from the Schering Corp., Union, N. J. Haloperidol (Haldol) was obtained from McNeil Laboratories, Inc., Fort Washington, Pa. Pimozide was a gift from Janssen Pharmaceuticals, Beerse, Belgium. All drugs were injected s.c.

Each group was composed of 3 or 4 flasks containing 3 or 4 hemipituitary glands. Student's t test was used for the statistical evaluation of the data, and the results are expressed as the mean ± S.E.

RESULTS

The ability of catecholamine to inhibit the in vitro release of prolactin has been previously reported (16). In the present study, the presence of $5 \times 10^{-7} \text{M}$ dopamine caused a 95% ($p < 0.01$) decrease in the amount of radioactive prolactin released into the incubation medium. In other experiments, the in vivo importance of the brain catecholamines was suggested by the finding that the injection of monoaminergic blocking drugs perphenazine and haloperidol caused a dramatic increase in serum prolactin.

The effects of these drugs on in vitro prolactin synthesis by glands of male and female rats are summarized in Chart 1. Normally, the glands of male rats incorporate little radioactive leucine into prolactin (10). When male rats were given injections daily for 4 days of 1 mg perphenazine or 0.1 mg haloperidol, in vitro synthesis and release of labeled prolactin increased 3- to 4-fold ($p < 0.01$). The glands of female rats synthesize and release large amounts of prolactin. Consequently, the effects of these drugs on in vitro synthesis by these glands were not as dramatic as that by glands of male rats. Nevertheless, both drugs significantly increased prolactin synthesis ($p < 0.02$).

The ability of these drugs to block the in vitro effects of dopamine is shown in Table 1. Once again, $5 \times 10^{-7} \text{M}$ dopamine inhibited release of newly synthesized prolactin into the incubation medium and allowed the labeled hormone to accumulate within the tissue ($p < 0.01$). Subsequently, total synthesis was reduced. Treatment of female rats with 4 daily injections of 1 mg perphenazine significantly increased ($p < 0.01$) the amount of newly synthesized prolactin found within the glands, although it did not increase total prolactin synthesis. When dopamine was added to the incubation medium of glands of perphenazine-treated animals, it was found that the inhibitory effect of the catecholamine on prolactin release was largely blocked. Similar results were observed with glands of animals given 4 daily injections of 0.1 mg haloperidol. Haloperidol treatment significantly increased the amount of newly synthesized prolactin found within the tissue, although it did not increase total prolactin synthesis. When dopamine was added to the incubation medium of glands of haloperidol-treated animals, it was found that the inhibitory effect of the catecholamine on prolactin release was completely without effect on the secretion of prolactin ($p < 0.01$).

Implantation of pituitary tumors decreases the prolactin content of the pituitary gland of the host animal and dramatically suppresses the in vitro synthesis of prolactin by the gland. Normal female rat pituitary glands synthesize large amounts of prolactin, most of which is released into the incubation medium (Chart 2). As observed before, the addition of $5 \times 10^{-7} \text{M}$ dopamine to the medium suppresses the release of the newly synthesized hormone ($p < 0.01$). We previously reported that when the glands of rats bearing the prolactin-secreting pituitary tumor MtTW15 were incubated in the presence of leucine-$^3\text{H}$, very little of the labeled amino acid was incorporated. The data presented here show that these glands synthesized and released only 14% as much prolactin as did control glands. The addition of $5 \times 10^{-7} \text{M}$ dopamine to the medium further suppressed the release of labeled prolactin and caused a slight accumulation of the hormone within the tissue. As with the glands of MtTW15-bearing rats, the glands of rats with the prolactin-secreting tumor 7315a synthesized and released considerably less prolactin ($p < 0.01$) than did controls. This diminished release of newly synthesized prolactin was even further suppressed by the addition of dopamine to the incubation medium.

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1 The trivial name used is: dopamine, 4-(2-aminoethyl)pyrocatechol.
Drugs and Prolactin Secretion

Table 1
Antagonistic effects of perphenazine and haloperidol on the dopamine-mediated inhibition of the in vitro synthesis and release of prolactin

Four hemipituitary glands per flask; 3 flasks per group.

<table>
<thead>
<tr>
<th></th>
<th>Incorporation into prolactin (cpm/mg tissue)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Glands</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Dopamine, $5 \times 10^{-7} \text{M}$</td>
<td>1509 $\pm$ 252$^a$</td>
</tr>
<tr>
<td>Perphenazine (chronic)$^c$</td>
<td>1451 $\pm$ 350$^b$</td>
</tr>
<tr>
<td>Perphenazine (chronic)$^c$ and dopamine</td>
<td>1481 $\pm$ 327</td>
</tr>
<tr>
<td>Haloperidol (chronic)$^d$</td>
<td>1034 $\pm$ 18$^d$</td>
</tr>
<tr>
<td>Haloperidol (chronic)$^d$ and dopamine</td>
<td>975 $\pm$ 44</td>
</tr>
<tr>
<td>Perphenazine (acute)$^c$ and dopamine</td>
<td>555 $\pm$ 43</td>
</tr>
<tr>
<td>Perphenazine (acute)$^c$ and dopamine</td>
<td>597 $\pm$ 21</td>
</tr>
</tbody>
</table>

* Mean ± S.E.
$p < 0.01.$
$^a$ Perphenazine, 1.0 mg, was injected s.c. daily for 4 days.
$^b$ Haloperidol, 0.1 mg, was injected s.c. daily for 4 days.
$^c$ Perphenazine, 1.0 mg, was injected s.c. 30 min before decapitation.

Animals bearing these tumors were given 4 daily injections of 0.1 mg haloperidol. Chart 3, left, shows that the drug stimulated in vitro prolactin synthesis by glands of normal animals ($p < 0.01$). The glands of MtTW15-bearing rats showed a greatly impaired ability to synthesize prolactin. When these animals were treated with haloperidol, however, the glands were able to synthesize 55% more prolactin than normal controls ($p < 0.01$). The effect of this drug treatment on circulating prolactin levels is shown in the right side of the chart. Normal females had prolactin levels of 49.3 ± 16.8 ng/ml. Haloperidol increased this level to 1100 ± 140 ng/ml. The circulating levels of the untreated tumor animals were 1355 ± 241 ng/ml, while those of the rats given injections were increased to 2625 ± 573 ng/ml.

The effect of another pharmacological agent, pimozide, on the synthesis and release of prolactin was studied. The data in Table 2 demonstrate that injection of this drug significantly increased the in vitro synthesis of prolactin by normal glands and completely restored the suppressed production of prolactin by pituitary glands from rats bearing pituitary tumor MtTW15.

Since we had previously observed that perphenazine can increase serum prolactin as soon as 10 min after injection (9), we investigated the ability of a single, relatively short-term injection of perphenazine or haloperidol to restore prolactin production in the glands of tumor-bearing rats. MtTW15-bearing rats received a single injection of 0.5 mg haloperidol 8 hr before sacrifice. As noted before, the glands of MtTW15-bearing rats synthesize and secrete very small amounts of radioactive prolactin (Chart 4, left). Treatment of these animals 8 hr earlier with haloperidol significantly increased in vitro incorporation of leucine-3H into prolactin ($p < 0.01$), although it did not restore it to control levels. A radioimmunoassay carried out on the incubation media revealed that MtTW15 glands released significantly less radioimmunoassayable prolactin than normal glands (Chart
Chart 3. Restoration of in vitro prolactin production by pituitary glands from tumor-bearing rats. Four daily s.c. injections of 0.1 mg haloperidol were administered to normal and tumor-bearing rats. The serum prolactin levels were measured by radioimmunoassay and, subsequently, the pituitary glands were incubated in vitro and prolactin synthesis and secretion was studied.

Table 2

<table>
<thead>
<tr>
<th>Incorporation into prolactin (cpm/mg tissue)</th>
<th>Glands</th>
<th>Incubation medium</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>638 ± 47*</td>
<td>2047 ± 191</td>
<td>2685 ± 144</td>
</tr>
<tr>
<td>Control + pimozide</td>
<td>2516 ± 250*</td>
<td>2153 ± 158</td>
<td>4669 ± 404*</td>
</tr>
<tr>
<td>MtTW15</td>
<td>167 ± 148</td>
<td>162 ± 19</td>
<td>329 ± 129</td>
</tr>
<tr>
<td>MtTW15 + pimozide</td>
<td>1430 ± 93*</td>
<td>2359 ± 119*</td>
<td>3789 ± 212*</td>
</tr>
</tbody>
</table>

* Mean ± S.E.
* p < 0.01.

Interestingly, the glands of haloperidol-treated tumor animals released even less prolactin, as detected by radioimmunoassay, in spite of the fact that in vitro synthesis and release of labeled prolactin was increased in these glands. A similar experiment was carried out with 7315a-bearing rats which were given 2 mg perphenazine 8 hr before sacrifice (Chart 5). Again, the drug overcame the tumor-induced suppression of prolactin synthesis, although it did not restore synthesis to normal levels within this 8-hr period. The radioimmunoassay of the incubation media gave a pattern that was very similar to that of the previous experiment. In spite of the fact that perphenazine stimulates new synthesis of prolactin in vitro, the glands of treated tumor-bearing animals released less radioimmunoassayable prolactin into the incubation medium than did the glands of nontreated tumor-bearing animals. This observation is probably related to the action of perphenazine and haloperidol to cause the secretion of prolactin in vivo.

DISCUSSION

The mechanism through which serum prolactin modifies the synthesis and release of the hormone by the pituitary gland and thus regulates its own concentration is an important experimental and clinical concept to comprehend. Previous studies showed that implantation of hormone-secreting pituitary tumors into rats produced atrophy of the pituitary gland and decreased its hormonal content (5, 12–14). Subsequent studies showed that the in vitro incorporation of radioactive amino acids into prolactin was greatly decreased in these glands (11). Although the high serum prolactin concentration is presumably responsible for the suppression of pituitary gland function, the mechanism whereby the serum prolactin acts is unknown.

Catecholamines demonstratedly decrease in vitro prolactin release when incubated with pituitary glands (4, 15). Reduction of hypothalamic catecholamines or injection of agents that inhibit their synthesis results in an increased production of prolactin. The present findings indicate that the injection into rats of the monoaminergic adrenergic blocking agents, perphenazine, haloperidol, and pimozide, causes an increased synthesis of prolactin and a pituitary gland which, when incubated with dopamine, is completely refractory to the inhibitory effects of the catecholamine. The biochemical specificity of these pharmaceutical agents described by Anden et al. (1) suggests that they primarily block dopamine receptors. Their injection into rats causes a blockade of the catecholamine receptors and increases brain catecholamine turnover. Large increases in serum pro-
Drugs and Prolactin Secretion

**Chart 4.** Rapid restoration of prolactin synthesis following the injection of haloperidol into tumor-bearing rats. Eight hr before sacrifice, 0.5 mg haloperidol was injected and the pituitary glands, were incubated with radioactive leucine, as described in the text. The amount of radioimmunoassayable prolactin released into the incubation medium was also determined.

**Chart 5.** Rapid restoration of prolactin synthesis following the injection of perphenazine into tumor-bearing rats. The experimental design was as described in Chart 4, except that 2 mg of perphenazine was injected.

Prolactin follows their injection into normal male and female or ovariectomized rats (3, 6, 16).

It seemed possible that a causal relationship might exist between the increased serum prolactin concentration in rats bearing pituitary tumors and the dopamine-mediated decrease in pituitary prolactin production. The administration of haloperidol, perphenazine, or pimozide to tumor-bearing rats was found to restore to normal the in vitro incorporation of leucine-\(^3\)H into prolactin. This fact suggested that the tumor hormone suppressed pituitary gland prolactin production through a catecholamine-mediated mechanism. This hypothesis is consistent with the observation of Hokfelt and Fuxe (7) who found marked activation of the tuberoinfundibular dopamine neurons following the injection of prolactin.

However, these data, do not establish whether the serum prolactin of tumor-bearing rats stimulates the production of increased amounts of hypothalamic catecholamines or whether it causes the pituitary gland to have a greater sensitivity to the circulating catecholamine. Studies are currently being conducted to determine whether hypothalamic catecholamine turnover is altered in tumor-bearing rats.

**ACKNOWLEDGMENTS**

We would like to thank Ronald C. Pace for his excellent technical assistance.

**REFERENCES**

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