EFFECTS OF PROLACTIN
ON PITUITARY-ADRENAL FUNCTION IN INTACT AND OVARIECTOMIZED RATS

By

Silvia B. Vasquez1) and Julian I. Kitay

ABSTRACT

The influence of prolactin treatment (100 μg/100 g body wt. sc daily for 7 days) on plasma corticosterone levels, adrenal steroid production in vitro and in vivo and pituitary-adrenal responses to stress were studied in intact and castrated female rats. Prolactin enhanced plasma corticosterone levels and corticosterone production in vitro and in vivo in intact rats after stress. Differences were abolished with ACTH treatment. In contrast, prolactin administration to ovariectomized rats inhibited plasma corticosterone response to stress. Combined treatment with ACTH reversed these findings. A greater in vitro production of corticosterone by adrenal slices and adrenal homogenates associated with an effective inhibition of adrenal 5α-reductase activity were also observed. Secretion of DHB in adrenal venous blood was decreased as well, without changes in corticosterone or THB secretion rates. However, combined treatment with prolactin and ACTH produced greater increments in secretion rates of corticosterone than those obtained with prolactin alone. The data suggest that prolactin treatment to ovariectomized rats has a dual effect: a) adrenal responsiveness to ACTH is enhanced by its effects on adrenal 5α-reductase activity, and b) pituitary-adrenal response to stress is dampered by prolactin treatment. The effects of prolactin on adrenal 5α-reductase activity and corticosterone production in vitro were paralleled in vivo only after the

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exogenous administration of ACTH. The presence of the gonads apparently prevented the inhibitory effect of prolactin on ACTH secretion and in turn seemed to act synergistically with prolactin to facilitate pituitary-adrenal response to stress.

Considerable evidence is available to indicate that pituitary-adrenal function is altered by prolactin. Changes in pituitary ACTH and adrenal content (Thatcher & Tucker 1968, 1970), hypothalamic CFR activity (Endröczi & Nyakas 1972), and response of the pituitary-adrenal axis to stress are among many of the variables measured during lactation in the rat. The most consistent findings involve a diminished response of plasma corticosterone concentration to different stressors (e.g., Thoman et al. 1970; Kamoun 1970a; Endröczi & Nyakas 1974). This has been substantiated by a concomitant decrease in ACTH release (Kamoun 1970b) and an increase in CRF activity at hypothalamic level (Endröczi & Nyakas 1972). Similar plasma corticosterone responses to stress have been observed with administration of high doses of prolactin to intact rats (Schleif et al. 1974). Other studies utilizing small doses of prolactin have shown a direct effect of this hormone on the adrenal secretion of steroids (Witorsch & Kitay 1972; Gustafsson & Stenberg 1975). Replacement with prolactin (or oestradiol) reverses the decline in corticosterone production associated with ovariectomy. This effect is attributable, at least in part, to changes in intra-adrenal metabolism of corticosterone (Kitay et al. 1970; Witorsch & Kitay 1972). Ovariectomy increases adrenal 5α-reductase activity, enhancing the conversion of corticosterone to 5α-dihydrocorticosterone (DHB) and to 3β, 5α-tetrahydrocorticoesterone (THB)\textsuperscript{1}. As a result, DHB and THB secretion increases and corticosterone output declines. Treatment with either prolactin (Witorsch & Kitay 1972) or oestradiol (Colby & Kitay 1972) restores reductase activity and corticosterone production to control levels.

The present studies were undertaken to substantiate in vivo the effects of prolactin on the adrenal function previously demonstrated in vitro, to obtain additional information concerning the effects of prolactin on the pituitary-adrenal axis and to evaluate the role of the ovaries as mediator of its action.

**MATERIAL AND METHODS**

Adult, virgin (151–175 g), albino female rats (Charles River CD strain from Wilmington, Massachusetts) were maintained under standardized conditions of light (0600–1800) and temperature (22.0°C ± 0.5°C) on a diet of Purina Laboratory Chow and water

\textsuperscript{1} The following trivial names or abbreviations are used in the text: corticosterone, 11β, 21-dihydroxy-4-pregnene-3, 20-dione (Cpd. B); DHB, 5α-dihydrocorticosterone; THB, 3β, 5α-tetrahydrocorticoesterone.
were 

Total 

lactin-treated 

Effects 

trations 

(2), 

Cortrosyn-Orgonan) 100 mU, was administered ip with 

decapitation 15 min later. Adrenal tissue and peripheral blood were then collected; plasma was separated and immediately frozen.

Adrenal slices and homogenates were prepared and incubated as previously re

ported (Kitay et al. 1970). Corticosterone production in the incubates was measured 

by acid fluorescence (Guillemin et al. 1959) and total steroid production was mea- 

sured by the blue tetrazolium reaction (Nowaczynski et al. 1955). Adrenal 5α-reductase 

activity was also assayed in homogenates (Kitay et al. 1970). Adrenal venous blood 

was collected for 15 min following induction of anaesthesia with ether (Kitay et al. 

1971). Half the animals in each treatment group were given ACTH, 100 mU in 0.1 

ml of saline iv, 5 min prior to blood collection. Peripheral blood was collected from 

the abdominal aorta at the end of each cannulation. Steroids were extracted and 

separated by paper chromatography as previously reported (Kitay et al. 1970). Plasma 

corticosterone, DHB and THB were measured by competitive binding methods (Colby 

& Kitay 1972; Murphy 1967). The Duncan’s multiple range test was used to analyze 

the data (Steel & Torrie 1960).

R E S U L T S

Effects of prolactin on plasma corticosterone concentrations

Prolactin administered sc daily for 7 days to intact rats produced no changes 

in plasma corticosterone concentrations either at rest or after ACTH adminis-

tration (Table 1); however, the response to ether stress was augmented. Pro-

lactin-treated ovariectomized rats showed a diminished response to ether stress. 

In contrast, combined treatment with prolactin and ACTH produced an en-

hanced plasma corticosterone response.

Effects of prolactin on adrenal steroid production in vitro

Prolactin administration to intact rats had no effect on steroid production 

by adrenal slices in vitro at rest or after ACTH (data not illustrated). Corti-

costerone and total steroid production, however, were increased after stress 

(Table 2), paralleling the changes observed in plasma corticosterone concen-

trations in vivo. Ovariectomy per se decreased corticosterone and total steroid 

production (Kitay 1968). Prolactin treatment to ovariectomized rats enhanced 

corticosterone production in vitro after stress or after administration of ACTH. 

Total steroid output remained unchanged; thus corticosterone/total steroid ratios 

were higher. Similarly, corticosterone production and corticosterone/total steroid
Table 1.
Effects of prolactin on plasma corticosterone in vivo in intact and ovariectomized rats (μg/100 ml of plasma)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Resting Level</th>
<th>Ether Stress</th>
<th>ACTH in vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td>Intact</td>
<td></td>
<td>1.9 ± 0.3(6)</td>
<td>93.1 ± 3.2(17)</td>
<td>108.6 ± 3.1(17)**</td>
</tr>
<tr>
<td>Ovariectomized</td>
<td></td>
<td>1.9 ± 0.3(8)</td>
<td>75.4 ± 3.3(7)</td>
<td>58.1 ± 2.4(8)**</td>
</tr>
</tbody>
</table>

Values in this and succeeding tables are expressed as mean±SE; number in each group shown in parentheses.

** P < 0.01 (v. corresponding control).
Table 2.

Effects of prolactin on steroid production by adrenal slices in vitro from intact and ovariectomized rats (μg/100 mg adrenal wt/h)

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Treatment</th>
<th>Ether Stress</th>
<th>Ether Stress</th>
<th>ACTH in vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact</td>
<td>Control</td>
<td>Prolactin</td>
<td>Control</td>
</tr>
<tr>
<td>Number of Rats</td>
<td>17</td>
<td>16</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>7.6 ± 0.6***</td>
<td>9.2 ± 0.5*</td>
<td>2.9 ± 0.4</td>
<td>4.4 ± 0.3***</td>
</tr>
<tr>
<td>Total Steroids</td>
<td>14.8 ± 0.8***</td>
<td>17.0 ± 0.7*</td>
<td>8.4 ± 0.9</td>
<td>9.1 ± 0.7</td>
</tr>
<tr>
<td>Corticosterone/Total (%)</td>
<td>50 ± 4***</td>
<td>54 ± 2</td>
<td>36 ± 2</td>
<td>49 ± 4**</td>
</tr>
</tbody>
</table>

* P < 0.05 (v. corresponding control)
** P < 0.01 (v. corresponding control)
*** P < 0.01 (v. ovariectomized)
ratios in adrenal homogenates were enhanced by prolactin treatment (data not illustrated), while adrenal 5α-reductase activity was simultaneously inhibited ($P < 0.01$).

**Effects of prolactin on adrenal steroid secretion rates in vivo**

Prolactin administration augmented the peripheral plasma corticosterone response of intact rats to the stress of adrenal vein cannulation (Table 3) consistent with the effect observed after ether anaesthesia alone (Table 1). Corticosterone secretion rate measured simultaneously in the adrenal venous effluent was also increased, but the variability in the sample population made the significance of the difference borderline ($P < 0.054$). DHB and THB secretion rates were not altered by prolactin treatment. However, the sum of all steroids measured (Cpd. B + DHB + THB) was increased. The stimuli of adrenal vein cannulation appeared to have further stimulated the secretion.

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
</tr>
<tr>
<td><strong>Peripheral Plasma</strong></td>
<td></td>
</tr>
<tr>
<td>Corticosterone</td>
<td>63.3 ± 4.5(11)</td>
</tr>
<tr>
<td>μg/100 ml</td>
<td></td>
</tr>
<tr>
<td><strong>Adrenal Vein</strong></td>
<td></td>
</tr>
<tr>
<td>Corticosterone</td>
<td>37.1 ± 1.8(9)</td>
</tr>
<tr>
<td>μg/h</td>
<td></td>
</tr>
<tr>
<td>DHB</td>
<td>3.5 ± 1.7(8)</td>
</tr>
<tr>
<td>μg/h</td>
<td></td>
</tr>
<tr>
<td>THB</td>
<td>6.5 ± 1.1(9)</td>
</tr>
<tr>
<td>μg/h</td>
<td></td>
</tr>
<tr>
<td>Sum (Cpd. B + DHB + THB)</td>
<td>46.7 ± 3.3(8)</td>
</tr>
<tr>
<td>μg/h</td>
<td></td>
</tr>
<tr>
<td>Cpd. B/Sum (%)</td>
<td>79 ± 3</td>
</tr>
</tbody>
</table>

* $P < 0.05$ (v. saline control)
** $P < 0.01$ (v. saline control)
of steroids by the adrenal gland in the prolactin-treated group (Δ % = 36). The output was proportionally increased, thus the Cpd. B/Sum ratio remained unchanged. Similar findings in peripheral plasma corticosterone, corticosterone secretion rate and the sum (Cpd. B + DHB + THB) were observed when ACTH was administered before adrenal venous blood collection. The data suggest that the experimental procedure was not maximally stressful and that treatment with prolactin produced similar changes to those obtained with exogenous administration of ACTH.

Prolactin treatment after ovariectomy (Table 4) lowered DHB secretion rate without changes in either corticosterone or THB output. The overall effect was a significant increase in the proportional output of corticosterone (Cpd. B/Sum). ACTH injection per se had no effect on any parameter indicating that the cannulation procedure was maximally stressful in ovariectomized rats. In contrast, when combined treatment with prolactin and ACTH was adminis-

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**Table 4.**

Effects of prolactin on adrenal steroid secretion rates *in vivo* in ovariectomized rats.

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
</tr>
<tr>
<td><strong>Peripheral Plasma</strong></td>
<td></td>
</tr>
<tr>
<td>Corticosterone μg/100 ml</td>
<td>52.0 ± 2.2(11)</td>
</tr>
<tr>
<td><strong>Adrenal Vein</strong></td>
<td></td>
</tr>
<tr>
<td>Corticosterone μg/h</td>
<td>33.4 ± 1.4(9)</td>
</tr>
<tr>
<td>DHB μg/h</td>
<td>6.9 ± 0.5(9)</td>
</tr>
<tr>
<td>THB μg/h</td>
<td>8.3 ± 1.2(9)</td>
</tr>
<tr>
<td>Sum (Cpd. B + DHB + THB) μg/h</td>
<td>42.5 ± 4.7(9)</td>
</tr>
<tr>
<td>Cpd. B/Sum %</td>
<td>69 ± 2</td>
</tr>
</tbody>
</table>

* P < 0.05 (v. corresponding saline control and saline + ACTH control)

** P < 0.01 (v. corresponding saline control and saline + ACTH control)
tered, both corticosterone secretion and the sum (Cpd. B + DHB + THB) were augmented significantly beyond the level observed with ACTH given alone. The proportional output of corticosterone was increased concomitantly, as was peripheral plasma corticosterone concentration. These data are in accord with the findings obtained with adrenal slices in vitro.

DISCUSSION

The data show that administration of low doses of prolactin to intact female rats augmented the response of plasma corticosterone concentrations to stress (ether anaesthesia or adrenal vein cannulation). These results were correlated with increased corticosterone and total steroid production by adrenal slices in vitro. ACTH treatment abolished this effect, indicating that adrenal response to ACTH itself was not altered by prolactin administration. Direct measurements from the adrenal vein paralleled in vitro results. Adrenal secretion rates of steroids were proportionally increased (Cpd. B/Sum remained unchanged). These findings may not be explained by inhibition of adrenal 5α-reductase since adrenal glands from intact animals contain little such activity (Kitay et al. 1970). The data suggest that the stimulatory influence of prolactin in intact female rats might be exerted on the release of ACTH from the pituitary gland rather than on adrenal steroid secretion per se.

Prolactin treatment to ovariectomized rats produced an apparent disagreement between in vivo and in vitro results. On one hand, plasma corticosterone response to stress was inhibited (ether anaesthesia or adrenal vein cannulation). The effect was consistent with that observed in stressed, lactating rats (e.g. Thoman et al. 1970; Endröczi & Nyakas 1974). On the other hand, both adrenal slices and adrenal homogenates from prolactin-treated ovariectomized rats produced more corticosterone after stress and after treatment with ACTH; adrenal 5α-reductase activity was inhibited concomitantly. The latter was substantiated in vivo by measurements from the adrenal venous blood showing that prolactin treatment lowered DHB secretion without altering the response of the remaining parameters. Increments in both corticosterone secretion and the sum (Cpd. B + DHB + THB) greater than those obtained with prolactin alone were obtained only after combined treatment with ACTH and prolactin. DHB secretion remained low, therefore the proportional output of corticosterone was increased, as was the peripheral plasma concentrations. These data now confirmed the findings obtained from adrenal slices in vitro and demonstrated that: 1) adrenal responsiveness to ACTH is enhanced by the effects of prolactin on adrenal 5α-reductase activity, and 2) pituitary-adrenal response to stress is dampered by prolactin treatment alone. Increments observed in the production of adrenal steroid in vitro after the effective inhibition of
adrenal 5α-reductase activity in ovariectomized rats were confirmed in vivo only after exogenous administration of ACTH.

The differences observed between intact and ovariectomized rats demonstrate that prolactin effect on ACTH secretion apparently involves an interaction with gonadal hormones. Oestradiol is known to stimulate not only prolactin but also ACTH secretion in the ovariectomized rat (Chen & Meites 1970; Kitay 1965), whereas prolactin alone inhibited ACTH secretion in the ovariectomized-stressed rat. The presence of the gonads (presumably oestrogens) prevented the inhibitory action of prolactin on ACTH secretion and in turn seemed to act synergistically with prolactin to facilitate the pituitary-adrenal response to stress.

Additional investigations are needed to determine the exact mechanisms by which prolactin interacts on adrenal function with ACTH and oestradiol and, perhaps, to elucidate more complex mechanisms by which prolactin could interact with other gonadal and/or pituitary hormones.

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REFERENCES


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