EFFECT OF RESERPINE ON PROSTATE GLAND FRUCTOSE

By

John A. Thomas*, Richard V. Andrews
and Marvin F. Hill

ABSTRACT

Small doses of reserpine (2.5 or 5 µg/day for 2, 5 or 11 days) caused significant elevations in fructose levels in the prostate glands of normal mice. This effect was not seen in the castrate mouse. In castrates given 50 µg/day of testosterone, reserpine did increase prostatic fructose levels, but less markedly than in intact mice. Adrenalectomized mice also responded to reserpine less markedly than intact animals.

The stimulatory actions of reserpine on sex accessory fructose depends upon both the dose and duration of administration of this alkaloid. These stimulatory actions of reserpine on sex accessory organs occur prior to any loss in testicular weight and might be related to the synergistic activities of ACTH and/or prolactin on prostatic tissue.

Reserpine has been reported to cause alterations in gonadal activity in male rodents (Gaunt et al. 1954; Tuchmann-Duplessis 1956; Eränkö et al. 1957; Adams & Fudge 1959; Khazan et al. 1960; Soulairac & Soulairac 1961). The effects of this agent on reproductive structures are more easily demonstrated in the female than they are in the male. Amounts of rauwolfia alkaloids in the so-called nontoxic range elicit few if any definite alterations in reproductive activity (Gaunt et al. 1963). Most previous investigations reveal a retardation of spermatogenesis (Tuchmann-Duplessis 1956; Adams & Fudge 1959; Khazan et al. 1960) and a reduction in sex accessory activity (Gaunt...
et al. 1954; Tuchmann-Duplessis 1956; Khazan et al. 1960). The regressive changes in male reproductive organs have been observed in both immature (Adams & Fudge 1959; Khazan et al. 1960) and adult rodents (Gaunt et al. 1954; Tuchmann-Duplessis 1956; Eränkö et al. 1957; Khazan et al. 1960). It is quite difficult to make any valid interpretation or comparison of the existing literature since there are considerable differences in both the doses of reserpine and the durations of administration. Moreover, these previous reports indicate differences in the interval between the last injection of this agent and the time of sacrifice.

The present studies were more concerned with the acute effects of this alkaloid on sex accessory organs and on testicular morphology. It was of interest to determine whether alterations in reproductive organs could be invoked by single or by multiple injections of reserpine. Particular attention was devoted to avoiding doses or dose schedules of this drug that produced sedation, lethargy and loss of appetite.

**METHODS**

Mature male albino mice fed a standard diet of laboratory chow and water *ad libitum* were used in these investigations. The mean body weight for all mice used in these studies was 30 g. All drugs were injected subcutaneously. Mice were sacrificed 24 hours following the last injection of drug and/or hormone. In preliminary experiments, reserpine (Serpasil®) in a dose schedule of 20 μg/mouse daily for 5 days was observed to be too toxic in that it produced sedation, lethargy, weight loss and in some instances death. Daily doses of reserpine (2.5 or 5 μg/mouse) to normal mice for periods up to 12 days caused no significant reductions in body weights. Loss of body weight was evident in normal mice receiving 10 μg of reserpine daily for a period of 11 days, but not at earlier sacrifice intervals.

Castrate mice received initial injections of testosterone (50 μg/mouse daily for 5 days) and appropriate doses of reserpine on the day of operation. All mice in the castrate series were killed 6 days post-orchiectomy. Adrenalectomized mice received daily doses of reserpine (5 μg/mouse for 5 days) beginning on the day of operation. Mice in the adrenalectomized series were also killed 6 days post-operatively or 24 hours after the last injection.

At sacrifice, sex accessory organs and gonads were weighed on a torsion balance. Anterior lobes of the prostate (coagulating gland) were homogenized in trichloroacetic acid and analyzed for their fructose (Roe 1934). Testicular tissue was quenched in liquid nitrogen, sectioned in a cryotome at 12 μm, and stained with haematoxylin and eosin.

Adrenal gland activity was assessed in both normal and castrate mice treated with reserpine (5 μg/mouse/day for 5 days). Twenty-four hours after the final injection, mice were sacrificed, their adrenal glands removed and incubated in a phosphate

* Reserpine (Serpasil®) was generously supplied by Ciba Pharmaceutical Company, Summit, New Jersey.
buffer of physiological salt solution (pH 7.2) at 37°C for 2 hours. Each incubating flask contained 1 µc of 14C labelled acetate. Corticosteroid secretory rates were estimated from analysis of the incubating medium which utilized a combination of solvent partitioning, and fluorometric determination of ethylacetate extractable steroids (Silber et al. 1958); the acid fluorescence test of Silber was employed in combination with radioactive conversion test described earlier (Andrews & Folk 1964). Values for secretory rates represent the µg of steroid detected in the medium during each hour interval and/or relative (cpm) of 14C steroid synthesized from radioacetate.

To evaluate the significance of results the 't' test was applied to the difference between the means of the respective control groups and those of the appropriate experimental groups (Snedecor 1956).

RESULTS

Table 1 shows the effects of varying doses and of different durations of reserpine on prostate gland fructose in normal mice. Reserpine caused a consistent increase in fructose concentrations. Similarly, fructose contents (µg/organ) were also shown to be increased following reserpine administration. Only prostates

<table>
<thead>
<tr>
<th>Day of Sacrifice</th>
<th>Fructose Content (µg/organ)</th>
<th>P</th>
<th>Fructose Concentration (mg/100 mg)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (24)</td>
<td>100.4 ± 8.1*&lt;sup&gt;**&lt;/sup&gt;</td>
<td></td>
<td>0.55 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>3rd day (8)</td>
<td>177.8 ± 13.4</td>
<td>0.1 %&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.71 ± 0.02</td>
<td>0.1 %&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>6th day (8)</td>
<td>131.5 ± 24.3</td>
<td>1 %&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.61 ± 0.04</td>
<td>0.1 %&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>12th day (6)</td>
<td>148.6 ± 17.1</td>
<td>1 %&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.75 ± 0.03</td>
<td>0.1 %&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>3rd day (7)</td>
<td>172.6 ± 12.0</td>
<td>0.1 %&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.66 ± 0.03</td>
<td>1 %&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>6th day (8)</td>
<td>153.9 ± 12.9</td>
<td>1 %&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.73 ± 0.05</td>
<td>0.1 %&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>12th day (6)</td>
<td>147.8 ± 24.2</td>
<td>5 %&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.71 ± 0.05</td>
<td>1 %&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>3rd day (8)</td>
<td>168.5 ± 29.6</td>
<td>0.1 %&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.70 ± 0.07</td>
<td>1 %&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>6th day (8)</td>
<td>122.4 ± 18.3</td>
<td>1 %&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.72 ± 0.25</td>
<td>1 %&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>12th day (6)</td>
<td>82.5 ± 6.0</td>
<td>0.1 %&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.66 ± 0.01</td>
<td>1 %&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Number of animals/group
*<sup>**</sup> Mean ± standard error

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from mice treated with the highest dose of reserpine (10 \( \mu g \)/mouse) and the longest duration of administration of this drug (12 days) exhibited a lower amount of fructose (\( \mu g \)/organ). Data from this same table, but expressed/final body weight showed an identical pattern. Single injections of the same doses seen in Table 1, viz 2.5, 5 or 10 \( \mu g \), failed to evoke any alterations in prostate fructose when animals were sacrificed 48 hours post-injection.

The stimulatory actions of reserpine upon prostate gland fructose were not seen in the castrate animal (Table 2). These doses and this particular sacrifice interval were effective in causing enhanced fructose levels in normal mice (Table 1), but ineffective in the castrate groups (Table 2). There are no statistical differences between castrate controls and reserpinized groups.

The effect of reserpine on the castrate treated with testosterone may be seen in Table 3. This dose of testosterone (50 \( \mu g \) daily for 5 days) administered to castrate mice was effective in maintaining normal fructose levels. It may be observed that despite the fact that fructose levels in this series are comparable to normal fructose levels, reserpine was unable to cause any further stimulation of this sugar in prostate tissue. In other words, reserpine could not enhance prostate fructose in the testosterone-treated castrate mouse. No statistical differences between testosterone-treated castrate controls and the various reserpinized groups were noted.

The relationship of the adrenal to the action of reserpine may be seen in Table 4. Reserpine caused only a slight increase in the amount of adrenal steroid in normal mice. Adrenal specific activity (cpm/\( \mu g \) steroid/mg tissue) in normal and castrate groups was inconsistently altered by the administration of reserpine (5 \( \mu g \) daily for 5 days) (unpublished). Reserpine administration to adrenalectomized mice, like those of normal mice, caused an increase in the

\[ \text{Table 2.} \]

Effect of reserpine (2.5, 5 or 10 \( \mu g \) daily for 5 days) on anterior prostate fructose levels in castrate mice. Animals were sacrificed 6 days following castration or 24 hours following the final injection.

<table>
<thead>
<tr>
<th>Reserpine</th>
<th>Fructose Content (( \mu g )/organ)</th>
<th>Fructose Concentration (mg/100 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 ( \mu g ) (12)</td>
<td>53.7 ± 8.1</td>
<td>0.408 ± 0.18</td>
</tr>
<tr>
<td>5.0 ( \mu g ) (11)</td>
<td>46.4 ± 9.4</td>
<td>0.369 ± 0.03</td>
</tr>
<tr>
<td>10 ( \mu g ) (12)</td>
<td>60.6 ± 10.3</td>
<td>0.416 ± 0.04</td>
</tr>
</tbody>
</table>

* Number of animals/group

** Mean ± standard error
Table 3.
Effect of reserpine (2.5, 5 or 10 μg daily for 5 days) on anterior prostate fructose in testosterone-treated (50 μg daily for 5 days) castrate mice. Animals were sacrificed 24 hours following the last injection.

<table>
<thead>
<tr>
<th>Testosterone</th>
<th>Reserpine</th>
<th>Fructose Content (μg/organ)</th>
<th>Fructose Concentration (mg/100 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 μg (6)*</td>
<td>--</td>
<td>119.2 ± 12.0**</td>
<td>0.633 ± 0.04</td>
</tr>
<tr>
<td>&quot; (7)</td>
<td>2.5 μg</td>
<td>142.6 ± 13.1</td>
<td>0.657 ± 0.05</td>
</tr>
<tr>
<td>&quot; (6)</td>
<td>5.0 μg</td>
<td>147.2 ± 18.1</td>
<td>0.635 ± 0.04</td>
</tr>
<tr>
<td>&quot; (7)</td>
<td>10.0 μg</td>
<td>118.3 ± 6.7</td>
<td>0.561 ± 0.02</td>
</tr>
</tbody>
</table>

* Number of animals/group
** Mean ± standard error

Table 4.
Effect of reserpine (5 μg daily for 5 days) on both the adrenal activity in normal mice and on the prostate gland of adrenalectomized mice. See methods section for further description of experimental design.

<table>
<thead>
<tr>
<th>Group</th>
<th>Reserpine</th>
<th>Anterior Prostate Fructose</th>
<th>Adrenal Gland</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Content μg/organ</td>
<td>Concentration mg/100 mg</td>
</tr>
<tr>
<td>Normals (5)*</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Normals (6)</td>
<td>5 μg</td>
<td>--</td>
<td>112.1 ± 14.5</td>
</tr>
<tr>
<td>Adrenalectomized (9)</td>
<td>--</td>
<td>128.9 ± 32.3</td>
<td>--</td>
</tr>
<tr>
<td>Adrenalectomized (9)</td>
<td>5 μg</td>
<td>128.9 ± 32.3</td>
<td>0.53 ± 0.08</td>
</tr>
</tbody>
</table>

* Number of animals/group
** Mean ± standard error

level of prostate gland fructose. The stimulatory actions of reserpine on sex accessory organs in adrenalectomized mice is, however, greatly reduced.

Testicular weights in normal mice treated with varying amounts of reserpine were not altered. Neither seminal vesicle nor anterior prostate weights were significantly altered in this same series. Histologic examination of testes of these normal mice again failed to reveal any significant morphologic alteration. Seminiferous tubules and interstitial cells in reserpinized mice were indistinguishable from those of normal controls.
The stimulatory actions of reserpine on sex accessory organs in normal mice serve to demonstrate the complexity of endocrine alterations following the administration of this alkaloid. This agent has been shown to cause inhibition of gonadotrophin secretion (Barraclough 1955) and also evoke the secretion of luteotrophin (Barraclough & Sawyer 1959). Reserpine is known to stimulate adrenocortical activity, as indicated by increased steroidogenesis (Eechante et al. 1962), adrenal hypertrophy and augmentation of compensatory adrenal hypertrophy (Gaunt et al. 1954). It is not surprising then that assessment of reserpine-induced changes in target organs influenced by the trophic hormones becomes an exceedingly difficult task. Not only are the dose and duration of reserpine administration important considerations, but available evidence suggests that alterations in each of several pituitary secretions need not be affected simultaneously by amounts of this drug.

The so-called 'chemical indicator' test for the male sex hormone, i.e. fructose, is perhaps more sensitive than gravimetric responses of sex accessory organs. The hypersecretion of sex accessory fructose in rats (Mann & Parsons 1950) and mice (Thomas & Strauss 1965) can be accomplished with greater than normal doses of male sex hormone. Other hormones, viz prolactin (Chase et al. 1957; Grayhack 1963) and ACTH (Tullner 1963), have been reported to work synergistically together or with testosterone to produce responses greater than controls or greater than that caused by testosterone alone. Both of these trophic hormones are known to be released following reserpine administration. The testosterone dependent process of fructose secretion appears to be augmented by the release of prolactin and/or ACTH. The release of these trophic hormones is undoubtedly induced by the administration of reserpine. While no direct evidence for enhanced prolactin secretion is presented, testosterone plus prolactin (NIH-ovine) causes a greater stimulation of prostatic fructose in castrate mice than does testosterone alone (unpublished). Adrenal glands of reserpine-treated mice indicate an enhancement of corticosteroid synthesis (Table 4). Although the assay for adrenal activity would not demonstrate increased secretion of adrenal androgens, it seems unlikely that increased release of adrenal androgens played any significant role in the hypersecretion of fructose since adrenalectomized mice treated with reserpine continued to exhibit somewhat enhanced sugar levels.

The effect of reserpine on testicular histology seems to be related to dose and duration of reserpine administration. Earlier studies (Tuchmann-Duplessis 1956; Adams & Fudge 1959; Khazan et al. 1960) reporting diminished spermatogenesis following reserpine used either higher doses of reserpine and/or injection periods longer than those reported in these investigations. The normal appearing seminiferous tubules observed in the present studies are consistent...
with the findings of Eränkö et al. (1957), even though these workers used higher doses and longer durations of administration. These previous studies (Eränkö et al. 1957), however, were designed in such a manner that the reserpine-treated rats were not sacrificed until 9 days after the last injection. The Leydig cells in the present studies were also normal in their morphology. Most previous studies (Tuchmann-Duplessis 1956; Eränkö et al. 1957; Adams & Fudge 1959) have reported an atrophy of interstitial cells, but again dose and duration of administration may make a comparison with these present findings somewhat difficult.

Testicular weights in mice treated with reserpine were unaltered in these studies. Others report increases (Gaunt et al. 1954; Prange & Bakewell 1966), decreases (Khazan et al. 1960) or no change (Giuliani et al. 1966; Feldman et al. 1966) on gonadal weights following reserpine. Nutrition could certainly influence the gonadal reaction if doses of reserpine were in the range that caused loss of appetite and severe lethargy. Insufficient dose and duration of reserpine could account for the lack of gonadal response to this drug. It is of interest, however, to note that cortisone therapy in small doses is capable of preventing gonadal inhibition in reserpinized rats (Chatterjee 1965). Perhaps low doses of reserpine can stimulate endogenous adrenocortical hormone and thereby retard changes in gonadal activity. Higher doses would ultimately lead to inhibition of gonadotrophin release and subsequently produce deleterious effects upon the testes.

It is not clear as to why reserpine failed to cause hypersecretion of prostatic fructose in castrate mice treated with a maintenance dose of testosterone (Table 3). The stimulatory effect of reserpine seen in normal mice (Table 1) tends to suggest that the drug has some direct action upon the interstitial cells of the testes. It seems unlikely that gonadotrophin secretion was altered in castrate mice treated with testosterone since exogenous androgen was instituted on the day of orchidectomy. Injected testosterone may simply have not provided optimal levels necessary for either ACTH or prolactin synergism; such levels may be provided by endogenous male sex hormone.

**ADDENDUM**

Taylor et al. (J. Pharmacol. Exp. Ther. 156 (1967) 483) have recently reported that reserpine causes accumulation of tissue glycoproteins. Both fucose and N-acetyl-neuraminic acid are elevated by reserpine.

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REFERENCES


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