RESERPINE AND CORTICOTROPHIN SECRETION

By

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ABSTRACT

It has been shown that reserpine counteracts the depressive effect of dexamethasone on adrenal weight and function. These data have been interpreted as indicating that the main effect of reserpine on the hypophysial-adrenal axis is that of enhancing rather than depressing corticotrophin secretion. This might be achieved through the suppression of a midbrain inhibitory action. The blocking of stress reactions induced by reserpine is thought to be a result of the feedback effects of the enhanced blood levels of adrenal steroids, induced by the drug.

Reports on the effects of tranquilizing drugs on the secretion of corticotrophin (ACTH) are contradictory. A number of studies indicate that reserpine and chlorpromazine inhibit the release of this hormone in response to stressful stimuli (Olling & De Wied 1956; Munson 1963) while other suggest that these drugs can induce a persistent secretion of ACTH (Gaunt et al. 1954; Maickel et al. 1961).

The present experiments were undertaken in order to clarify further the problem of the effects of reserpine on ACTH secretion and to try to explain the discrepancies so far reported. For this purpose the effects of reserpine on the hypophysial-adrenal axis were studied in animals in which ACTH secretion had been blocked by the concurrent administration of the potent synthetic adrenal steroid, dexamethasone1 (Martini et al. 1962; Fraschini et al. 1964). It was thought that the experimental device of lowering the initial level of hypophysial-adrenal activity would enhance the stimulation of ACTH secretion induced by reserpine, assuming that the drug does actually possess an ACTH-

1. $\Delta^1$-9α-fluoro-16α-methylcortisol.
releasing activity. Changes in adrenal weight and in adrenal and plasma corticosterone concentrations were taken as indices of ACTH release (Fraschini et al. 1964).

MATERIALS AND METHODS

The experiments were performed on normal male rats of the Sprague-Dawley strain. Chronic administration of reserpine usually results in a loss of body weight which is related to the dose of the drug and to the duration of administration (Wells et al. 1956). In order to have comparable absolute organ weights at the end of the experiment it was decided not to randomize the animals but to include the heaviest in those groups which were going to receive reserpine. The rats were then divided into the following 4 groups: 1) controls, which were injected only with placebo solutions; 2) dexamethasone, which was given daily for 6 days in a subcutaneous injection of 15 μg/100 g body weight; 3) reserpine, which was given daily for 6 days in a subcutaneous injection of 25 μg/100 g body weight; 4) dexamethasone plus reserpine, which were given simultaneously, but at two different sites (see 2 and 3 for doses).

All animals were kept quiet in single screened cages for at least 24 h before sacrifice and killed only between 9.30 and 10.30 in the morning in order to avoid diurnal fluctuations of adrenal activity (Guillemin et al. 1959 a). Animals were decapitated with a guillotine 24 hours after the last treatment. The hypophyses, adrenals and testes were taken out, carefully cleaned and weighed on a torsion balance. The mixed blood from jugular vein and carotid artery was collected in heparinized tubes. Plasma and adrenal corticosterone levels were determined according to a modification (Fraschini et al. 1964) of the fluorimetric procedure of Guillemin and co-workers (Guillemin et al. 1959 b).

RESULTS

Table 1 shows that the control animals had a normal weight increase. Animals treated with dexamethasone alone showed a weight increase which was subnormal. Both groups of animals given reserpine showed a decrease rather than an increase in body weight. Body weight was more affected in those animals which received dexamethasone in addition to reserpine than in animals given reserpine alone.

If absolute values are considered, reserpine alone has no effect on the hypophysial, adrenal and testis weight. Dexamethasone induces a significant decrease in absolute adrenal weight. In the group of animals given dexamethasone plus reserpine, the adrenal weight is lower than in controls or in the reserpine group, but higher than in animals given dexamethasone alone. The picture is only slightly modified when the weight of these endocrine organs are considered on a per 100 g body weight basis; the major difference in this case is that the reserpine treated animals show an increase in adrenal weight while the group receiving reserpine plus dexamethasone has an adrenal weight similar to that of the untreated control group.
Table 1.
Effect of chronic (6 days) treatment with reserpine (25 µg/100 g body weight/day), dexamethasone (15 µg/100 g body weight/day) and with reserpine plus dexamethasone on body weight and on the weight of the endocrine structures.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals</th>
<th>Body weight</th>
<th>Hypophysial weight</th>
<th>Adrenals weight</th>
<th>Testes weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>initial g</td>
<td>final g</td>
<td>absolute mg</td>
<td>relative mg/100 g b. wt.</td>
</tr>
<tr>
<td>Controls</td>
<td>25</td>
<td>88 ± 3.1</td>
<td>112.8 ± 2.7</td>
<td>4.6 ± 0.20</td>
<td>3.8 ± 0.18</td>
</tr>
<tr>
<td>Reserpine</td>
<td>19</td>
<td>109.3 ± 2.1</td>
<td>108.6 ± 2.4</td>
<td>4.3 ± 0.16</td>
<td>4.3 ± 0.27</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>23</td>
<td>94.5 ± 4.1</td>
<td>109.0 ± 3.6</td>
<td>4.4 ± 0.30</td>
<td>4.1 ± 0.26</td>
</tr>
<tr>
<td>Dexamethasone + reserpine</td>
<td>23</td>
<td>101.8 ± 4.0</td>
<td>95.2 ± 2.2</td>
<td>3.9 ± 0.22</td>
<td>4.0 ± 0.20</td>
</tr>
</tbody>
</table>

Values are means ± S. E.
* P < 0.001 vs Controls.
** P < 0.005 vs both Controls and Dexamethasone.
Table 2.
Effect of chronic (6 days) treatment with reserpine (25 μg/100 g body weight/day), dexamethasone (15 μg/100 g body weight/day) and with reserpine plus dexamethasone on plasma and adrenal corticosterone levels.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals</th>
<th>Plasma corticosterone (μg/100 ml)</th>
<th>Adrenal corticosterone (μg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>25</td>
<td>14.7 ± 1.5</td>
<td>5.2 ± 0.7</td>
</tr>
<tr>
<td>Reserpine</td>
<td>19</td>
<td>12.7 ± 2.1</td>
<td>5.6 ± 0.6</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>23</td>
<td>4.0 ± 0.3*</td>
<td>1.9 ± 0.4*</td>
</tr>
<tr>
<td>Dexamethasone + reserpine</td>
<td>23</td>
<td>12.4 ± 1.2**</td>
<td>3.8 ± 0.9**</td>
</tr>
</tbody>
</table>

Values are means ± S. E.
* P < 0.001 vs. controls.
** P < 0.001 vs. Dexamethasone.

Dexamethasone reduces plasma corticosterone levels to values which are usually seen after hypophysectomy; corticosterone plasma levels in the reserpine group are not significantly different from those of the control group. The rats which received dexamethasone plus reserpine had corticosterone plasma levels significantly higher than those found in the dexamethasone group and practically similar to those of control animals (Table 2).

Adrenal concentration (μg/100 g of tissue) of corticosterone is decreased by treatment with dexamethasone. Reserpine, when given alone, does not affect the adrenal corticosterone concentration. The drug, however, is able to counteract the effect of dexamethasone at adrenal level; in the animals treated with dexamethasone plus reserpine the adrenal corticosterone concentration was more than twice that present in the animals treated with dexamethasone alone.

**DISCUSSION**

If it is accepted that changes in adrenal weight and in blood (Frascini et al. 1964; Corbin et al. 1965) and in adrenal corticosterone concentration (Holzbauer 1957; Corbin et al. 1965) reflect differences in the cumulative amount of ACTH secreted by the end of treatment, the data reported here indicate that dexamethasone induces a chronic suppression of ACTH secretion. Reserpine, which, in the dose used, does not stimulate ACTH release above the physiological levels in animals which did not receive dexamethasone, produces, however, a marked secretion of ACTH, if administered to animals in which a
strong inhibition of ACTH secretion has been induced by the concurrent administration of a powerful ACTH-blocking steroid.

The observation that dexamethasone, like other adrenal steroids, significantly reduces adrenal weight indicates that the steroid suppresses the release of ACTH; synthesis of new ACTH is also reduced as shown by the fact that prolonged administration of adrenal steroids depletes hypophysial ACTH stores (Farrell & Laqueur 1955; Kitay et al. 1958; Fortier 1959). Reserpine also depletes the stores of hypophysial ACTH (Kitay et al. 1959; Saffran & Vogt 1960; Maickel et al. 1961); this drug, however, counteracts the suppressive effect of dexamethasone on adrenal weight and function, i.e. enhances release of ACTH. This clearly indicates that reserpine-induced depletion of hypophysial stores of ACTH cannot be explained by a simple inhibition of the synthesis of the hormone; depletion of hypophysial ACTH following reserpine treatment is better explained by assuming that the drug stimulates both synthesis and release of ACTH and that the release is more stimulated than is the synthesis. The data reported here apparently show that reserpine is able to enhance synthesis and release of ACTH even after both these processes have been largely suppressed by the administration of potent adrenocortical steroids.

Hertting & Hornykievicz (1957) and Ashford & Shapero (1962) have reported that the hypertrophy of the adrenal glands and the acute release of ACTH they have observed following treatment with reserpine, were inhibited by the concurrent administration of cortisone or cortisol. These results are at variance with the data reported here which demonstrate that ACTH release induced by reserpine is not suppressed by one of the more potent ACTH-blocking steroids. On the contrary, reserpine is able to overcome the blockade of ACTH secretion induced by dexamethasone.

The discharge of ACTH induced by drugs which, like reserpine, mainly act on the central nervous system (CNS) may result either from activation of stimulatory pathways or from depression of inhibitory pathways. Inhibitory effects have been shown to play a significant role in the regulation of ACTH secretion (Egdahl 1960; Fraschini et al. 1964). That reserpine-induced ACTH-release might result from the suppression of an inhibitory effect is suggested by the following indirect evidence: 1) reserpine evokes mammary growth and milk secretion; this probably depends on the ability of the drug to remove the tonic inhibition exerted by the CNS on prolactin secretion (Meites et al. 1963); 2) as far as the activity of the hypophysial-adrenal axis is concerned, animals treated with reserpine resemble animals in which a complete transection at midbrain level has been performed. Both groups of animals show a chronic activation of the hypophysial adrenal axis (Fraschini et al. 1964) and do not respond to certain types of stress (adrenaline) (Van Peenen & Way 1957; Giuliani et al. 1961), while they are still activated by others (histamine, salicylate, etc.). The data reported here show in addition, that reserpine treated
animals, like midbrain sectioned animals (Fraschini et al. 1964; Martini et al. 1964), are less sensitive than normal to the ACTH-suppressing effect of dexamethasone. These facts suggest that reserpine might activate ACTH secretion through the suppression of inhibitory CNS effects; it is quite possible that the drug eliminates the tonic inhibition which the midbrain has been recently shown to exert on the hypothalamic-hypophysial axis (Fraschini et al. 1964; Martini et al. 1964).

If the most relevant effect of reserpine on the hypophysial-adrenal axis is that of stimulating ACTH secretion, how can one explain results which demonstrate that reserpine blocks stress-induced ACTH release? Up to the present they have usually been explained by the fact that reserpine depletes hypophysial ACTH stores (Kitay et al. 1959; Saffran & Vogt 1960; Maickel et al. 1961). This interpretation is no longer tenable, since recent data contrary to previous reports (Kitay et al. 1958; Kitay et al. 1959) have clearly shown that the capacity of the hypophysis to release ACTH in response to stressful stimuli does not depend on the level of its ACTH stores and that a lowered hypophysial content of ACTH does not preclude the response to stressing procedures (Vernikos Danellis 1963). The working hypothesis is proposed that inhibition by reserpine of stress-induced ACTH release might be only an indirect and secondary effect brought about by the feedback effect of the excess of endogenous corticoids produced by the primary effect of the drug, i.e. by the activation of the hypophysial-adrenal axis. Administration of exogenous corticoids has been repeatedly reported to suppress the hypophysial-adrenal response to stress (see Martini et al. 1962 for references); it is obvious that endogenous corticoids too, if produced in supra-physiological amounts, should act in the same way. An objection which might be raised against the hypothesis proposed here is that no elevated levels of plasma corticoids are found 24 hours after the end of treatment with reserpine, i.e. at a time when stress reactions are still blocked (Maickel et al. 1961). This is not a serious objection, however. It has actually been shown that there is no direct relationship between the existing blood corticoid levels and the ability of the hypothalamic-hypophysial axis to respond to stress. Fochi et al. (1960), Gavazzi et al. (1961), Smelik (1963) and Hodges & Jones (1964) have clearly demonstrated that the inhibition of ACTH secretion induced by physiological (Smelik 1963; Hodges & Jones 1964) or unphysiological steroids (Fochi et al. 1960; Gavazzi et al. 1961) lasts many hours after the blocking agent has been eliminated. These data would indicate that the inhibition of ACTH secretion is dependent on a persisting accumulation of steroids in the effective loci, rather than on their actual concentration in the blood.
REFERENCES

Vernikos Danellis J.: Endocrinology 72 (1963) 574.

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