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THE EFFECT OF RESERPINE ON HYPOTHALAMO-PITUITARY-ADRENOCORTICAL FUNCTION IN THE RAT

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1 The effect of reserpine on hypothalamo-pituitary-adrenocortical (HPA) function in the rat was investigated by the use of direct and indirect indices of pituitary adrenocorticotrophic activity.

2 Administration of a single dose of the drug induced prolonged hypersecretion of corticotrophin (ACTH).

3 Corticotrophin release in response to the drug no longer occurred after repeated daily injections, indicating that some form of 'adaptation' occurred.

4 The increase in HPA activity normally caused by exposure to cold was prevented by reserpine once 'adaptation' to the drug had been produced.

5 Inhibition of stress-induced ACTH release was due neither to depletion of pituitary stores of the hormone, nor to a corticosteroid feedback effect.

Introduction

Although the effect of reserpine on hypothalamo-pituitary-adrenocortical (HPA) function has been extensively studied, its action on the secretion of corticotrophin (ACTH) is still not fully understood. Some reports indicate that the drug reduces the activity of the HPA system probably by depressing hypothalamic regions which control the output of ACTH (Mason & Brady, 1956; Wells, Briggs & Munson, 1956; Mahfouz & Ezz, 1958), whereas others suggest that it increases the functional activity of the system (Gaunt, Renzi, Antonchak, Miller & Gilman, 1954; Egdahl, Richards & Hume, 1956; Harwood & Mason, 1957; Saffran & Vogt, 1960; Montanari & Stockham, 1962).

The experiments described here were carried out in an attempt to clarify some of the discrepancies in the literature, by the use of direct estimates of blood and pituitary ACTH for the assessment of pituitary-adrenocorticotrophic activity in addition to the indirect indices which have been almost exclusively employed in the past.

Methods

Animals

Male Sprague-Dawley rats (Fisons Pharmaceuticals Ltd.) weighing 150-250 g were used. The animals were housed two per cage in a temperature-controlled room (22° C) for at least 5 days before the start of any treatment. Food (Diet 41B, Lane-Petter & Dyer, 1952) and water were always available *ad libitum.*

Drugs

Reserpine (Halewood Chemicals Ltd.) was dissolved in 1% glacial acetic acid in deionized water. The pH of the solution was adjusted to 4 with 4 M NaOH and the concentration to 2.5 mg reserpine/ml. Less concentrated solutions were prepared by dilution of the stock solution with vehicle (i.e. 1% glacial acetic acid in deionized water, adjusted to pH 4). The solution was injected intraperitoneally in volumes of 1 ml/kg body weight, in doses of either 1.25 or 2.5 mg reserpine/kg body weight. Controls received 1 ml/kg of the vehicle by the same route. All injections were given in the morning between 09 h 00 min and 10 h 00 minute. In contrast to the vehicle-treated animals, the reserpine-treated rats had diarrhoea for the first 3-4 days of treatment and exhibited ptosis, slight ataxia, pilo-erection and loss of body weight.

Cold stress

Cold stress was applied by placing the cages, each containing two rats, in a large, ventilated refrigerator maintained at 4° C.

Blood samples

Blood for plasma corticosterone determination only was collected under ether anaesthesia from the abdominal aorta, into a heparinized 5 ml syringe. The samples were collected within 2 min of removing the animals from their cages, centrifuged at 3,000-3,500 rev/min within 1 h of collection and the plasma stored at -10° C. The plasma corticosterone concentration was estimated fluorimetrically by the method of Zenker & Bernstein (1958).

Blood for the estimation of plasma ACTH concentration was collected from the trunk following rapid decapitation with a pair of large, sharp, scissors. The blood was strained through heparinized glass wool, collected in chilled heparinized plastic tubes and centrifuged immediately at 3,000-3,500 rev/min for not more than 5 minutes. Volumes (0.3 ml) of plasma were placed in chilled, stoppered plastic tubes which were then stored on solid CO_2 in the deep freeze. The plasma ACTH concentration was estimated by the redox bioassay method (Chayen, Loveridge & Daly, 1972) in which ACTH-induced changes in the stainable reducing activity in the zona reticularis of the guinea-pig adrenal are measured by scanning and integrating microdensitometry.

Adrenal glands

Adrenal glands were removed immediately after withdrawal of blood from the abdominal aorta, freed from peri-adrenal fat and connective tissue and weighed on a torsion balance. Each gland was transferred to a test-tube containing 12 ml of 4% (w/v) trichloroacetic acid solution and carefully ground with the aid of sand and a glass rod. Adrenal ascorbic acid concentrations were determined by the method of Roe & Kuether (1943).

Pituitary glands

After decapitation the top of the cranium was cut away and the cerebral hemispheres were carefully lifted back to expose the pituitary gland. The gland was removed, weighed on a torsion balance and homogenized in 1 ml cold 0.1 M HCl. The homogenate was placed in a chilled plastic tube, each tube containing two pituitaries from a given group of animals, and stored overnight in a refrigerator. The following day the tubes were transferred to the deep freeze and stored on solid CO_2. Pituitary ACTH content was estimated by the redox bioassay method (Chayen *et al.*, 1972).

Results

The effect of reserpine on adrenal ascorbic acid, plasma corticosterone and plasma ACTH concentration, and pituitary ACTH content

Adrenal ascorbic acid concentrations, adrenal weights and plasma corticosterone and ACTH concentrations after single intraperitoneal injections of reserpine are shown in Table 1 and Figure 1. In the vehicle-treated group the plasma ACTH concentration rose significantly ($P < 0.01$), reached a maximum value between 2.5 and 10 min and returned to the basal level within 40 min of the injection. In the reserpine-treated group the plasma ACTH concentration reached a similar

Table 1 Adrenal ascorbic acid concentrations, adrenal weights, plasma corticosterone concentrations and plasma ACTH concentrations in rats 24 h after single intraperitoneal injections of reserpine or vehicle

Treatment	*Adrenal weight (mg)*	*Adrenal ascorbic acid concentration (mg/100 g)*	*Plasma corticosterone concentration (µg/100 ml)*	*Plasma ACTH concentration (mu/100 ml)*
None	31.5 ± 0.56 (19)	425.5 ± 5.5 (56)	8.2 ± 0.3 (48)	0.56 ± 0.05 (20)
Vehicle (6)	32.6 ± 0.85	438.9 ± 15.5	8.05 ± 0.9	0.50 ± 0.1
Reserpine 1.25 mg/kg (6)	37.8 ± 2.8*	367.8 ± 25.9**	22.7 ± 2.1*	2.8 ± 0.4*
Reserpine 2.5 mg/kg (6)	36.8 ± 1.4*	363.3 ± 17.2**	20.0 ± 2.1*	3.5 ± 0.7*

Values are means ± s.e. mean.
Significantly different from control values *$P < 0.001$, **$P < 0.01$.
Numbers of animals used in parentheses.

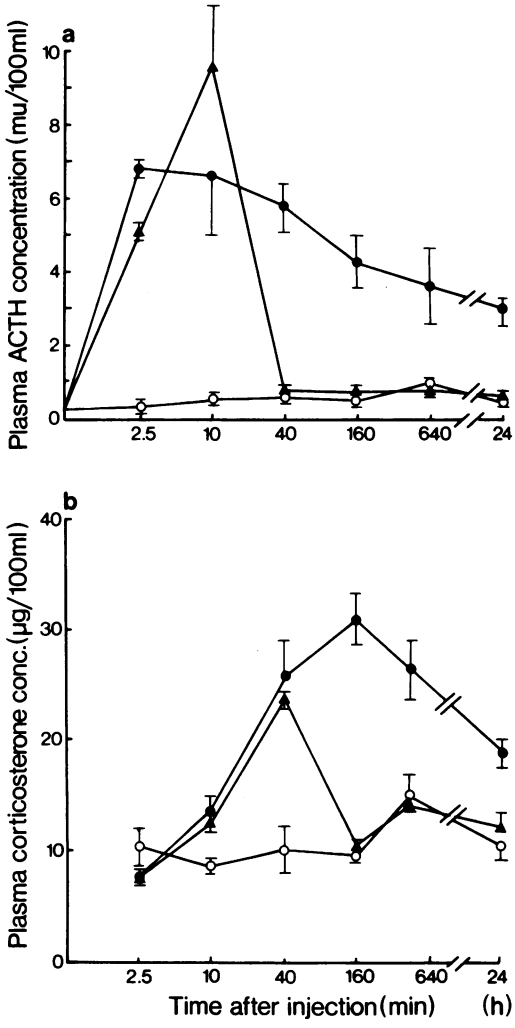

Figure 1 Plasma ACTH and corticosterone concentrations in rats at various time intervals after a single intraperitoneal injection of reserpine, 2.5 mg/kg (●) or vehicle, 1 ml/kg (▲); untreated controls (○). All injections were given at 09 h 00 minute. Each point represents the mean of at least 6 determinations. Vertical bars show s.e. mean.

peak between 2.5 and 10 min after the injection but then declined very gradually and remained significantly ($P < 0.001$) above the basal level for at least 24 hours. The plasma corticosterone concentration showed a similar response. In the vehicle-treated group it reached a peak in 40 min and then fell sharply to the control value. The initial increase in plasma corticosterone was similar in the animals treated with reserpine but instead of falling to the control level after 40 min, it continued to rise, reaching a maximum in 160 minutes. The plasma corticosterone concentration subsequently decreased gradually but remained significantly ($P < 0.001$) greater than the control value, suggesting that a single intraperitoneal injection of reserpine induces hypersecretion of ACTH for at least 24 hours.

The effects of injections of reserpine repeated at daily intervals are shown in Figure 2 and Tables 2 & 3. The first injection of the drug caused adrenal ascorbic acid depletion and an increase in the plasma corticosterone concentration 1 h after injection. This response was no longer evident 1 h

Table 2 Plasma ACTH concentration 2.5 min after the last of repeated daily injections of reserpine or vehicle

Treatment	*No. of daily injections*	*Plasma ACTH (mu/100 ml)*
–	–	0.56 ± 0.05 (20)
Vehicle (1 ml/kg)	1	5.6 ± 0.3* (6)
Vehicle (1 ml/kg)	3	4.5 ± 1.1* (6)
Vehicle (1 ml/kg)	5	5.8 ± 1.0* (6)
Vehicle (1 ml/kg)	7	3.1 ± 0.9* (8)
Reserpine (2.5) mg/kg)	1	6.8 ± 1.5* (7)
Reserpine (2.5 mg/kg)	3	1.1 ± 0.5* (6)
Reserpine (2.5 mg/kg)	5	0.96 ± 0.4† (6)
Reserpine (2.5 mg/kg)	7	0.56 ± 0.1 (6)

Values are means ± s.e. mean.
Significantly different from control values *$P < 0.001$, †$P < 0.01$.
Numbers of animals indicated in parentheses.

Table 3 Pituitary ACTH content in rats 'adapted' to reserpine and subjected to cold stress (5 min at 4°C) immediately after the last reserpine injection

Treatment	*ACTH mu per pituitary* *Before stress*	*After stress*
–	115.6 (6)	120 (6)
Reserpine, 2.5 mg/kg once daily for 7 days	66.7 (8)	60.3 (8)
Vehicle once daily for 7 days	62.0 (6)	61.0 (6)

Each value is the mean of three samples, each containing two pooled pituitaries. Numbers of animals used in parentheses.

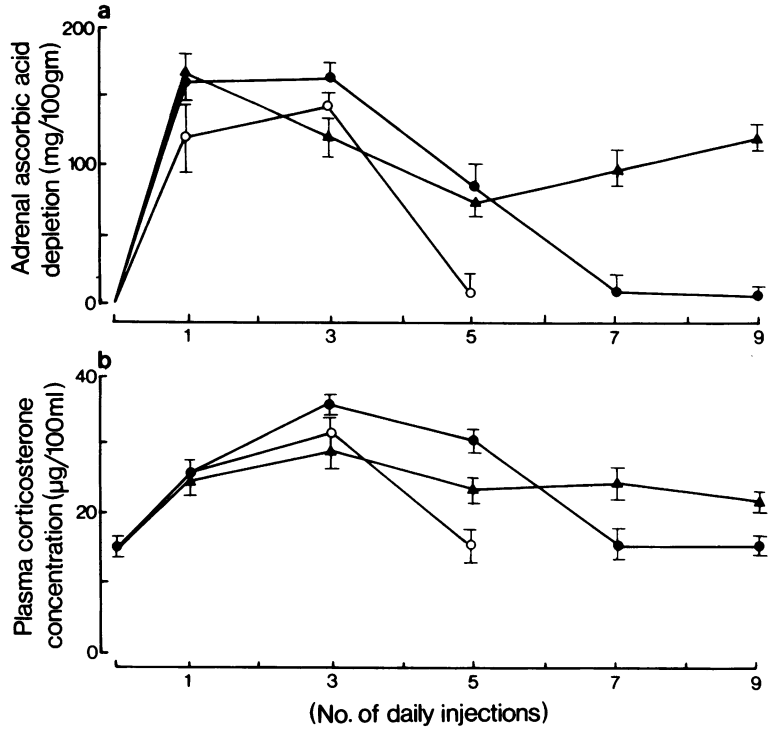

Figure 2 (a) Adrenal ascorbic acid depletion and (b) plasma corticosterone concentration after repeated daily intraperitoneal injections of reserpine, 1.25 mg/kg (●), 25 mg/kg (○) and vehicle (▲). All observations were made 1 h after the final injection. Each point represents the mean of at least 6 determinations. Vertical bars show s.e. mean.

after the 5th and 7th injection of 2.5 and 1.25 mg reserpine/kg respectively (Figure 2). Injection of the vehicle alone also caused adrenal ascorbic acid depletion and an increase in the plasma corticosterone concentration but the responses persisted even after the 9th injection. The results indicate that some form of 'adaptation' occurs in response to repeated daily injections of the drug but not to the vehicle alone. Direct estimates of plasma ACTH after repeated injections of reserpine confirm that 'adaptation' to the drug occurs and the plasma ACTH concentration was unchanged 2.5 min after the 7th injection (Table 2). The pituitary ACTH content (Table 3) after repeated injections of reserpine was similar to that in the corresponding vehicle-treated group.

The effect of cold stress on adrenal ascorbic acid, plasma corticosterone and plasma ACTH concentration, and pituitary ACTH content after treatment with reserpine

Exposure to cold (4° C for 1 h) caused a decrease in the adrenal ascorbic acid concentration and a marked increase in the plasma corticosterone concentration in untreated control rats. In rats 'adapted' to reserpine (5 daily injections of 2.5 mg/kg) and in vehicle-treated controls, exposure to cold for 1 h immediately after the final injection caused no change in adrenal ascorbic acid or plasma corticosterone concentration (Figure 3). However, the adrenal ascorbic acid was already depleted and the plasma corticosterone raised at the time of stress in the vehicle-treated group but not in the reserpine-treated rats. Twenty-four hours after the last injection, when adrenal ascorbic acid and plasma corticosterone concentrations were normal, cold stress caused a significant fall in the concentration of adrenal ascorbic acid and a rise in that of plasma corticosterone ($P < 0.01$ and $P < 0.001$ respectively) in the vehicle-treated control group. In the corresponding group of reserpine-treated rats exposure to cold caused a significant ($P < 0.001$) rise in the concentration of plasma corticosterone but no concomitant adrenal ascorbic acid depletion. Hence, if adrenal ascorbic acid depletion is taken as the index of

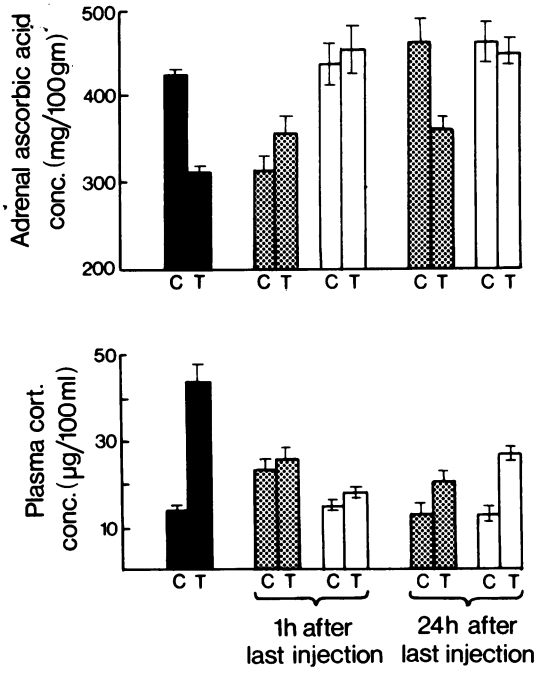

Figure 3 Adrenal ascorbic acid and plasma corticosterone concentrations in rats adapted to reserpine (2.5 mg/kg), subjected to cold stress and killed immediately afterwards. Solid columns, untreated controls; dotted columns, five daily injections of vehicle only; open columns, five daily injections of reserpine (2.5 mg/kg); C, before stress; T, 1 h at 4° C. Each column represents the mean of at least 6 determinations. Vertical bars show s.e. mean.

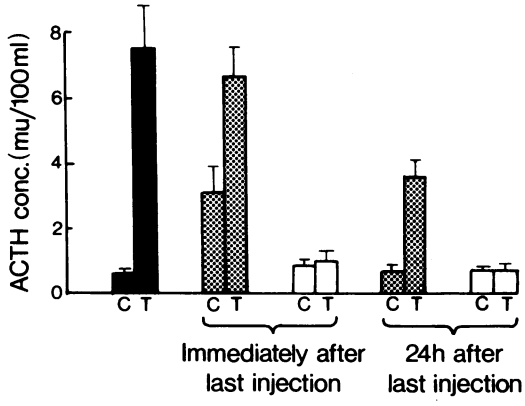

Figure 4 Plasma ACTH concentrations in rats subjected to cold stress after 'adaptation' to reserpine (2.5 mg/kg). Rats were killed immediately after cold exposure. Solid columns, untreated controls; dotted columns, seven daily injections of vehicle only; open columns, seven daily injections of reserpine (2.5 mg/kg); C, before stress; T, 5 min at 4° C. Each column represents the mean of at least 6 determinations. Vertical bars show s.e. mean.

pituitary-adrenocorticotrophic activity, inhibition of ACTH release is apparent 1 h and 24 h after the final injection of reserpine but if changes in plasma corticosterone concentration are used it is apparent only 1 h later.

Blood and pituitary ACTH were estimated in rats 'adapted' to reserpine after exposure to cold for 5 min (Figure 4 and Table 3). The stress caused a marked rise in the concentration of ACTH in the blood of untreated control animals with no concomitant change in the pituitary content of the hormone. Reserpine-treated rats exposed to cold either immediately or 24 h after the 7th injection showed no significant change in the concentration of ACTH in the plasma or its content in the pituitary gland, in contrast to the corresponding vehicle-treated control animals in which a significant increase ($P < 0.05$) in the plasma ACTH concentration occurred but no change in pituitary ACTH content.

Discussion

These results indicate that reserpine is capable of blocking the stress-induced release of ACTH, although it may powerfully stimulate secretion of the hormone when administered acutely. A persistent and prolonged hypersecretion of ACTH following a single injection of reserpine has often been observed (Wells *et al.*, 1956; Kitay, Holub & Jailer, 1959; Saffran & Vogt, 1960; Maickel, Westermann & Brodie, 1961; Eechaute, Lacroix, Leusen & Bouckaert, 1962; Montanari & Stockham, 1962; Bhattacharya & Marks, 1969). This response to a single injection of the alkaloid is reduced and ultimately disappears if the injections are repeated daily. Animals 'adapted' to the effects of the drug in this way no longer release ACTH in response to cold stress. Our results have confirmed the findings of Wells *et al.* (1956), Kitay *et al.* (1959) and Maickel *et al.* (1961) that the stress-induced release of corticotrophin may be inhibited by reserpine. They also explain the failure of Eechaute *et al.* (1962) and Montanari & Stockham (1962) to demonstrate the phenomenon because of their lack of recognition of the need for their experimental animals to become 'adapted' to reserpine-treatment before the application of stressful stimuli.

Some of our data using adrenal ascorbic acid changes as the index of ACTH release are not in complete agreement with the data obtained using

plasma corticosterone changes. A dissociation between these two indices has been reported previously (Slusher, 1958; Eskin & Mikhailova, 1968) and some of the discrepancies in the literature may also be explained on this basis. The obvious limitations of indirect indices for the assessment of pituitary-adrenocorticotrophic activity were overcome in the present work by use of the sensitive, precise and specific redox bioassay for ACTH (Chayen *et al.*, 1972) and the ability of reserpine to block stress-induced ACTH secretion was confirmed.

The results obtained with the vehicle-treated controls are interesting. Indirect indices of pituitary-adrenocorticotrophic activity suggest that the vehicle is capable of suppressing the stress-induced release of the hormone. Such inhibition of ACTH secretion was confirmed by direct estimates of plasma ACTH, which showed that injection of the vehicle alone acts as a profound stress, stimulating ACTH release, and that repeated exposure to such a stress results in a subsequent decrease in the response to cold stress. Similar observations have been reported previously (Stark, Fachet & Mihàly, 1963; Jones & Stockham, 1966) and emphasize the need for proper controls. This is a factor which has been neglected by some investigators (Mahfouz & Ezz, 1958; Kitay *et al.*, 1959).

The precise mechanism whereby reserpine exerts its effect on HPA function is uncertain. Failure to release ACTH in response to stress has been attributed to depletion of pituitary stores of the hormone (Kitay *et al.*, 1959; Maickel *et al.*, 1961). However, although it has been shown that a single injection of reserpine causes a marked fall in the pituitary ACTH content (Saffran & Vogt, 1960) our data indicate clearly that inhibition of ACTH release after 'adaptation' to reserpine occurs when pituitary stores of the hormone are not different from the control value. In any event, it is unlikely that the pituitary ACTH content provides a reflection of the functional capacity of the gland (Sayers & Cheng, 1949; Marks & Vernikos-Danellis, 1963; Vernikos-Danellis, 1963; Hodges & Jones, 1964). Moreover, the suppression of ACTH release was not due to a corticosteroid feedback mechanism as has been suggested by Giuliani, Motta & Martini (1966), because it occurred when reserpine-treatment caused no change in the concentration of corticosterone in the plasma. Probably its effects on HPA function are associated with changes in hypothalamic catecholamines or 5-hydroxytryptamine, as was suggested by Westermann, Maickel & Brodie (1962), Bhattacharya & Marks (1969) and Marks, Hall & Bhattacharya (1970).

Our results indicate that reserpine inhibits the functional activity of the HPA system and partly explain how the use of different, relatively insensitive indices of ACTH secretion and the neglect of proper controls have led to many discrepancies in the literature and to widely different interpretations of the experimental data.

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