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MODIFICATION OF STRESS RESPONSES IN HISTAMINE-DEPLETED RATS

BY

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Ellinger (1948) suggested that histamine might be a mediator of the stress response. Attempts by Tepperman, Rakieter, Birnie & Diermeer 1951) to test this hypothesis showed that the antihistaminics, phenoxadrine and tripelenamine, inhibit adrenal ascorbic acid depletion produced by histamine but not that produced by carbon tetrachloride. Nasmyth (1954; 1955) showed that histamine depletion modifies the influence of morphine on the ascorbic acid content of adrenal glands, and suggested that released histamine plays a role in the action of compound 48/80 in producing adrenal ascorbic acid depletion. Selye, Jean & Cautin (1960) and Cass & Marshall (1962) have presented data which suggests that stress causes histamine release in the rat. Wells, Briggs, and Munson (1956) have shown that exogenous histamine is a potent and reproducible stressing agent.

Paton (1951) demonstrated that compound 48/80 is a potent histamine liberator. The liberation of histamine by compound 48/80 takes place only from mast cells (Riley, 1956). This compound elicits a marked adrenal ascorbic acid depletion in rats (Nasmyth, 1955) after a single dose.

The purpose of the present work was to clarify the role of endogenous histamine in the events leading to the activation of the pituitary-adrenal axis in rats following various stressors. At the same time the mechanism of the stress produced by compound 48/80 was investigated.

METHODS

Male rats of the Holtzman strain weighing between 180 and 280 g. were used throughout this study. They were maintained on commercial laboratory chow and water *ad libitum*. Rats were used not earlier than one week after being received from the supplier and were maintained in a room with controlled temperature (24° C), and alternating 12 hr periods of light and darkness. The administration of drugs, stressing procedures, and decapitation were always performed in the animal room. In all experiments rats were killed between 8.30 a.m. and 11.30 a.m. within 30 sec of removal from the cage. Animals (10 to 12) were maintained in community cages. Free-flowing blood after decapitation was collected in test tubes containing crystals of potassium oxalate. After centrifugation to remove red cells the plasma was placed in vials and frozen at −15° C. Adrenal glands were removed and immediately frozen until analysed for ascorbic acid.

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Adrenal ascorbic acid was determined by the method of Maickel (1960). The method of Guillemin, Clayton, Lipscomb & Smith (1959) was used for the determination of corticosterone in plasma using an Aminco Bowman Spectrophotofluorometer at the following settings: excitation wavelength of 475 mU, fluorescent wavelength at 550 mU ; slit 0.5 ; and sensitivity 40.

Rats were stressed by the following procedures: Chlorpromazine hydrochloride 10 mg/kg, intraperitoneally ; Placing in individual cages in a refrigerator at 4° C for two hr (cold stress) ; Formaldehyde, 0.2 ml., 10% solution subcutaneously : Histamine 20 mg/kg, intraperitoneally ; Compound 48/80, 200 μg/animal intraperitoneally. Controls received saline 1 ml./kg, intraperitoneally.

Histamine depletion was accomplished using the histamine liberator, compound 48/80, as reported by Riley & West (1955). Compound 48/80 was administered to rats twice daily (9 a.m. and 9 p.m.) via the intraperitoneal route starting at 100 μg per dose and increasing the dose by 100 μg each day until a dose of 500 μg was reached on the fifth day. Control animals received saline. Thirty-six hr after the final administration of saline or compound 48/80 the rats were subjected to the various stressors and then killed. Groups of rats were treated with compound 48/80 as mentioned plus inhibitors of histidine decarboxylase as follows: D-2-hydrazine-3-(4(5)-imidazole) propionic acid HCl (α-HH, MK-785) 100 mg/kg, intraperitoneally every 12 hr for four days starting on the third day of compound 48/80 treatment. Other animals received 4-bromo-3-hydroxybenzyl oxyamine-dihydrogen phosphate (NSD-1055) 100 mg/kg, intraperitoneally every 12 hr for two days starting on the fifth day of compound 48/80 treatment ; control animals received saline.

Levels of significance were calculated using Student's " t " test.

Histamine was used as its diphosphate, doses were calculated as the free base.

In other experiments the ability of UML-491 and dexamethasone pretreatment or hypophysectomy to inhibit adrenal ascorbic acid depletion and/or plasma corticosterone elevation by compound 48/80 or histamine was assessed.

RESULTS

Effect of various stressors on plasma corticosterone and adrenal ascorbic acid in rats pretreated with compound 48/80

Rats treated with compound 48/80 exhibited prostration, oedema of the paws and nose, itching (as evidence by pawing of the face), and cyanosis accompanied by polydipsia within 5 min of drug administration. A 10% mortality was noted, most deaths occurring on the second or third day of treatment. Rats treated with the histamine liberator did not gain weight during the five days' treatment, while the body weight of saline controls increased by 10 to 15%. The mean adrenal weight of 29 histamine-depleted rats (45.0 ± 1.32 mg) was significantly greater than that (36.5 ± 1.25 mg) of 18 control animals ($P<0.01$).

The response of histamine depleted rats to chlorpromazine, cold exposure, formaldehyde, compound 48/80, and histamine itself was assessed by measuring plasma corticosterone and adrenal ascorbic acid levels.

The data obtained with the different stressors are presented in Table 1. There was a significant ($P<0.05$) difference between the level of adrenal ascorbic acid in rats pretreated with compound 48/80 and those pretreated with saline. This suggests that the ability of these stressors to affect depletion of adrenal ascorbic acid is inhibited in histamine-depleted rats. Such differences are not of a similar magnitude nor statistically significant when plasma corticosterone levels are used as an index of adrenocortical activation.

Data in Table 1 reflect the inability of compound 48/80 itself to produce plasma corticosterone elevation and adrenal ascorbic acid depletion in histamine-depleted rats, while the administration of histamine can still elicit these effects.

Effect of chlorpromazine on plasma corticosterone and adrenal ascorbic acid levels of rats pretreated with compound 48/80 and histidine decarboxylase inhibitors

As shown in Table 2 the action of chlorpromazine in producing plasma corticosterone elevation and adrenal ascorbic acid depletion in rats pretreated with compound 48/80 plus histidine decarboxylase inhibitors is similar to that obtained with compound 48/80 alone (Table 1).

TABLE 1

EFFECT OF VARIOUS STRESSORS ON PLASMA CORTICOSTERONE AND ADRENAL ASCORBIC ACID LEVELS IN HISTAMINE-DEPLETED RATS

Rats were pretreated with compound 48·80 for 5 days, as described under Methods; thirty-six hr later (12 hr in the case of formaldehyde) these animals received either chlorpromazine (10 mg/kg, intraperitoneally) formaldehyde (0/2 ml., 10% solution, subcutaneously, histamine (20 mg/kg, intraperitoneally), compound 48/80 (200 ug/animal, intraperitoneally), or were subjected to cold (4° C.) exposure for 2 hr duration. Control animals received saline (1 ml./kg, intraperitoneally). In parenthesis, under Treatment, duration of experiment

	Treatment	Plasma Cortiscosterone (μg/100 ml.±S.E.)	Adrenal Ascorbic acid (mg%±S.E.)	Number of animals per group
A. Chlorpromazine (CPZ)	48/80+CPZ (60)	53·7± 6·1	290·3±15·1	15
	Saline+CPZ (60)	59·7± 3·3	229·3±13·9	13
	48/80+CPZ (120)	45·7± 5·4	294·9±15·1	10
	Saline+CPZ (120)	59·9± 4·5	209·9±16·5	9
	48/80+saline (60)	31·1± 3·2	385·5± 4·0	5
	Saline+saline (60)	23·8± 4·3	417·8±27·3	5
B. Cold exposure	48/80+cold	55·5±13·3	313·4±19·7	5
	Saline+cold	58·6± 6·1	209·2±16·9	5
C. Formaldehyde	48/80+formaldehyde	70·0± 5·4	339·8± 2·4	5
	Saline+formaldehyde	67·8± 1·8	238·3±11·7	5
D. Compound 48/80	48/80+48/80	35·4± 7·5	397·3±20·9	10
	Saline+48/80	54·9± 2·9	281·0±11·6	10
	48/80+saline	31·1± 3·2	385·5± 4·0	5
E. Histamine	48/80+histamine	67·1± 6·8	328·8± 3·4	5
	Saline+histamine	64·9± 3·5	323·1±25·1	5
	48/80+saline	31·1± 3·2	385·5± 4·0	5

TABLE 2

EFFECT OF CHLORPROMAZINE ON PLASMA CORTICOSTERONE AND ADRENAL ASCORBIC ACID LEVELS IN RATS PRETREATED WITH COMPOUND 48/80 AND HISTIDINE DECARBOXYLASE INHIBITORS

Rats were treated for 5 days with compound 48/80, as described under *Methods*, and were treated twice daily for four days with α-HH (100 mg/kg per dose beginning on the third day of 48/80 administration, intraperitoneally), or twice daily for two days with NSD-1055 (100 mg/kg per dose beginning on the fifth day of 48/80 administration, intraperitoneally) and subsequently with chlorpromazine (10 mg/kg, intraperitoneally) or saline (1 ml./kg, intraperitoneally) twelve hr after the last administration of the histidine decarboxylase inhibitor. In group 5 saline (1 ml./kg, intraperitoneally) was administered in lieu of compound 48/80 and α-HH. Rats were killed 60 min after chlorpromazine or saline. In parenthesis number of animals per group

Treatment	Plasma corticosterone (μg/100 ml.±S.E.)	Adrenal ascorbic acid (mg%±S.E.)
48/80+α-HH+CPZ	49·6±7·3 (6)	309·0±13·5 (6)
48/80+α-HH+saline	16·6±6·2 (6)	503·3±17·7 (6)
48/80+NSD-1055+CPZ	77·5±7·9 (9)	389·4±15·5 (7)
48/80+NSD-1055+saline	31·3±6·1 (5)	526·0±22·4 (5)
Saline+saline+CPZ	74·3±6·2 (7)	253·6±12·0 (7)

Studies on the mechanism of the stress produced by compound 48/80

Dexamethasone pretreatment: Data presented in Table 3 indicate that dexamethasone blocks the action of histamine but not that of compound 48/80.

UML-491 (1-methyl-d-lysergic acid butanolamide tartrate) pretreatment: Data presented in Table 4 indicate that this compound inhibits ($P<0.05$) the plasma corticosterone elevation produced by compound 48/80, but fails to block adrenal ascorbic acid depletion caused by the histamine liberator.

Hypophysectomized rats: The administration of compound 48/80 or histamine to hypophysectomized rats did not affect plasma corticosterone elevation or adrenal ascorbic acid depletion as shown in Table 5.

TABLE 3

BLOCK OF HISTAMINE AND COMPOUND 48/80 STRESS BY DEXAMETHASONE IN THE RAT

Fourteen hr after receiving dexamethasone (300 ug/rat, intraperitoneally) or saline (1 ml./kg, intraperitoneally), rats received compound 48/80 (1 mg/kg, intraperitoneally), histamine (20 mg/kg, intraperitoneally), or saline (1 ml./kg, intraperitoneally) and were killed 30 min thereafter. Five rats were used for each treatment

Treatment	Adrenal ascorbic acid (mg%±S.E.)
Dexamethasone+48/80	386·4±32·2
Dexamethasone+histamine	463·3±36·5
Saline+48/80	400·6±18·9
Saline+histamine	337·8±19·9
Saline+saline	487·6± 5·9

TABLE 4

EFFECT OF UML-491 PRETREATMENT ON PLASMA CORTICOSTERONE ELEVATION AND ADRENAL ASCORBIC ACID DEPLETION BY COMPOUND 48/80 IN RATS

Five min before treatment with compound 48/80 (200 ug/rat, intraperitoneally) or saline (1 ml./kg, intraperitoneally) rats received UML-491 (0·5 mg/kg, intraperitoneally) or saline (1 ml./kg, intraperitoneally). All animals were pretreated for five days with saline (1 ml./kg, intraperitoneally), and were killed 30 min after compound 48/80 or saline

Treatment	Plasma corticosterone (μg/100 ml.±S.E.)	Adrenal ascorbic acid (mg %±S.E.)	Number of animals
UML-491+saline	23·8±4·2	382·8±36·1	10
UML-491+48/80	40·8±5·1	295·5±28·2	10
Saline+48/80	59·2±5·9	264·0±25·5	9
Basal levels	14·8±2·7	423·0±24·6	11

TABLE 5

BLOCK OF COMPOUND 48/80 AND HISTAMINE-INDUCED STRESS IN HYPOPHYSECTOMIZED RATS

Histamine (20 mg/kg, intraperitoneally), compound 48/80 (1 mg/kg, intraperitoneally), or saline (1 ml./kg, intraperitoneally were administered approximately 24 hr after hypophysectomy. Rats were killed 60 min after treatment

Treatment	Plasma corticosterone (μg/100 ml.±S.E.)	Adrenal ascorbic acid (mg %±S.E.)	Number of animals
Histamine	4·6±0·6	456·2±4·2	6
Compound 48/80	2·0±0·7	436·4±5·0	5
Saline	2·6±0·1	429·2±22·0	6
Intact controls	8·8±0·9	446·0±38·8	6

DISCUSSION

Compound 48/80 at doses and time sequences used in these experiments has been reported to decrease the histamine content of subcutaneous connective tissue from 32 to 1 μg/g and the histamine content of the ear from 44 to 3 μg/g in rats (Riley & West, 1955). The abdominal skin histamine content of rats is reduced from 58.1 to 7.5 μg/g (Nasmyth, 1955) by similar treatment with compound 48/80. It must be emphasized that complete histamine depletion is not achieved with compound 48/80 alone or together with histidine decarboxylase inhibitors (Levine, Sato & Sjoerdsma, 1965). Compound 48/80 liberates only mast cell histamine and even this histamine pool is not completely emptied ; there are significant amounts of non-mast cell histamine in the rat which cannot be liberated by compound 48/80 and this histamine pool may play a role in the stress response (Cass & Marshall, 1962). In any study on the role of histamine in mediation of the stress response the determination of a minimum or threshold amount of histamine required to produce adrenocortical activation is difficult because of the experimental impossibility of producing animals completely depleted of histamine as has been stated by Kahlson, Rosengreen & Thunberg (1963).

Of the stressors used in this study, chlorpromazine, cold exposure, and formaldehyde are known to liberate histamine in the rat by disruption of mast cells (Le Blanc, 1963 ; Bray & Van Arsdel, 1961 ; Jamieson & Van den Brenk, 1961).

The release and/or depletion of histamine by stressors could be explained by at least two mechanisms. First, after a stressful stimulus which triggers ACTH secretion either by a direct stimulation of the pituitary or by stimulation of afferent pathways to the hypothalamus, there may be a release (followed by tissue decrease) of histamine. A second mechanism may be a consequence of the increase in plasma corticoids produced after adrenocortical stimulation. Schayer (1956) reported that depletion of histamine may occur because of interference with its tissue binding by corticoids. The finding of Selye *et al.* (1960) that corticosterone pretreatment can prevent gastric ulcer production by compound 48/80 in the rat appears to support the second mechanism.

The fact that known histamine liberators such as morphine and chlorpromazine produce adrenocortical stimulation after a single dose while they fail to produce such stimulation after repeated administration and, moreover, can block adrenocortical stimulation by other stressors may be due, in part, to histamine depletion.

Data shown in Table 1 tend to confirm that compound 48/80 produces stress by histamine release. It appears that compound 48/80 only produces stress, as measured by adrenal ascorbic acid depletion and plasma corticosterone elevation, in the presence of releasable histamine. In a similar experiment Nasmyth (1955) found that in rats depleted of histamine (with compound 48/80 at the same dose schedule used in this experiment), the adrenocortical activation produced by compound 48/80 (5 mg/kg, subcutaneously) was modified but not to the degree shown in the present study. Data showing significant plasma corticosterone elevation and adrenal ascorbic acid depletion by administered histamine in histamine-depleted rats suggest that the absence of a response to compound 48/80 is not due to an altered sensitivity to histamine.

Levine *et al.* (1965) have reported that the histidine decarboxylase inhibitors used

herein do not further lower tissue histamine content after treatment of rats with compound 48/80. Results shown in Table 2 confirm the observation of Levine *et al.* (1965).

While histamine is undoubtedly not the only substance or its release the only mechanism involved in mediation of the stress phenomenon, the action of various stressors in rats pretreated with compound 48/80, or compound 48/80 in combination with histidine decarboxylase inhibitors, suggest a role of endogenous histamine in the stress response, as evidenced by adrenal ascorbic acid depletion.

De Wied (1964), Ashford & Jones (1963), and Cann & Stephenson (1963) have demonstrated the blockade by dexamethasone of histamine stress in the rat. Results obtained in the present study (Table 3) confirm the dexamethasone block of histamine stress but do not indicate a block of compound 48/80-induced stress. This suggests that compound 48/80 may induce stress by means in addition to release of histamine. The possibility also exists that the dose of compound 48/80 used liberated enough histamine, at an appropriate site, to overcome the dexamethasone block.

The apparent failure of UML-491 and dexamethasone pretreatment to block adrenal ascorbic acid depletion by compound 48/80 may suggest different control mechanism for plasma corticosterone elevation and adrenal ascorbic acid depletion in the rat. Further, these data cast doubt on the question of a correlation between plasma corticosterone and adrenal ascorbic acid as stress indicators.

In the present study, an interval of 5 min elapsed between the administration of UML-491 and compound 48/80. When an interval of one hr was used, no antagonism could be shown. The use of other UML-491 pretreatment intervals is indicated for further analysis of compound 48/80 antagonism. The possibility of a direct effect of compound 48/80 on the adrenal glands can be eliminated as shown by the experiment with hypophysectomized rats. Further experiments with central nervous system lesioned rats should be carried out to eliminate the possibility of a direct effect of compound 48/80 on the hypophysis. The high corticosterone reading in hypophysectomized rats found in this experiment is not surprising. Yates, Leeman, Glenister & Dallman (1961), Hedner (1961) and other workers have also reported high residual fluorescence in plasma from hypophysectomized or/and adrenalectomized rats.

SUMMARY

1. The effect of various stressors on adrenal ascorbic acid and the plasma corticosterone levels in histamine depleted rats has been studied.

2. Pretreatment of rats with compound 48/80 significantly inhibited adrenal ascorbic acid depletion induced by chlorpromazine, formaldehyde, and cold exposure. Such pretreatment blocked completely the adrenal ascorbic acid depletion and plasma corticosterone elevation induced by compound 48/80, but did not affect that caused by exogenous histamine.

3. Pretreatment of rats with compound 48/80 and histidine decarboxylase inhibitors inhibited the adrenal ascorbic acid depletion caused by chlorpromazine.

4. UML-491 inhibited the plasma corticosterone elevation, but did not affect the adrenal ascorbic acid depletion produced by compound 48/80 when administered five min prior to the histamine liberator.

5. Dexamethasone blocked adrenal ascorbic acid depletion by histamine but failed to block adrenal ascorbic acid depletion by compound 48/80 when administered 14 hr prior to histamine or compound 48/80.

6. Hypophysectomy blocked plasma corticosterone elevation and adrenal ascorbic acid depletion by histamine or compound 48/80.

7. These data indicate that histamine released may be of significance in mediating the ascorbic acid depletion produced by stressors such as cold exposure, chlorpromazine and formaldehyde.

8. A significant conclusion of this study is the lack of a quantitative inverse relationship between changes in plasma corticosterone and adrenal ascorbic acid levels after exposure of the rat to various stressors. The measurement of stress by a single parameter is thus not an absolute indication that a complete response to stimulation of the pituitary-adrenal axis has taken place.

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REFERENCES

ASHFORD, A. & JONES, J. I. (1963). The assay of corticotrophin in hypophysectomized and in hydrocortisone-treated rats. *Br. J. Pharmac. Chemother.*, **20**, 95–98.

ASHFORD, A. & SHAPERO, M. (1962). Effect of chlorpromazine, reserpine, benactyzine, and phenobarbitone on the release of corticotrophin in the rat. *Br. J. Pharmac. Chemother.*, **19**, 458–463.

BRAY, R. E. & VAN ARSDEL, P. P. (1961). In vitro histamine release from rat mast cells by chemical and physical agents. *Proc. Soc. Exp. Biol. Med.*, **106**, 255–259.

CANN, M. C. & STEPHENSON, N. R. (1963). Observations on the blockage of the pituitary production of corticotropin as indicated by adrenal ascorbic acid depletion. *Can. J. Biochem. Physiol.*, **41**, 1084–1087.

CASS, R. & MARSHALL, P. B. (1962). Effect of adrenocortical hormones on tissue histamine and 5-hydroxytryptamine in the rat. *Archs. Int. Pharmacodyn. Ther.*, **136**, 311–332.

DE WIED, D. (1964). The site of the blocking action of dexamethasone on stress-induced pituitary ACTH release. *J. Endoc.*, **29**, 29–37.

ELLINGER, F. (1948). The use of adrenal cortical hormone in radiation sickness. *Radiology*, **51**, 394–399.

GUILLEMIN, R., CLAYTON, G. W., LIPSCOMB, H. S. & SMITH, J. D. (1959). Fluorometric measurement of rat plasma and adrenal corticosterone concentration. *J. Lab. Clin. Med.*, **53**, 830–834.

HEDNER, P. (1961). Experiences with a fluorometric method for determining corticosteroids in man and rat. *Acta Pharmac. Tox.*, **18**, 65–74.

JAMIESON, D. & VAN DEN BRENK, H. A. (1961). Relation of mast cell changes to hypothermia in the rat. *Biochem. Pharmac.*, **7**, 35–46.

KAHLSON, G., ROSENGREN, E. & THUNBERG, R. (1963). Observations on the inhibition of histamine formation. *J. Physiol. (Lond.)*, **169**, 467–486.

LEBLANC, J. (1963). Histamine and cold adaptation. *Proc. Soc. Exp. Biol. Med.*, **112**, 25–26.

LEVINE, R. J., SATO, T. L. & SJOERDSMA, A. (1965). Inhibition of histamine synthesis in the rat by hydrazino analog of histidine and 4-bromo-3-hydroxy benzyloxyamine. *Biochem. Pharmac.*, **14**, 139–149.

MAICKEL, R. P. (1960). A rapid procedure for the determination of adrenal ascorbic acid. Application of the Sullivan and Clarke method to tissues. *Analyt. Biochem.*, **1**, 498–501.

NASMYTH, P. A. (1954). Factors influencing the effect of morphine sulfate on the ascorbic acid content of rat's adrenal glands. *Br. J. Pharmac. Chemother.*, **9**, 95–99.

NASMYTH, P. A. (1955). Histamine release and the " stress " phenomenon. *Br. J. Pharmac. Chemother.*, **10**, 51–55.

PATON, W. D. M. (1951). Compound 48/80: A potent histamine liberator. *Br. J. Pharmac. Chemother.*, **6**, 499–508.

RILEY, J. F. (1956). Ciba Foundation Symposium on Histamine, pp. 45–46. Boston: Little, Brown & Co., ed. WOLSTENHOLME, G. E. W., and MILLAR, E. C. P.

RILEY, J. F. & WEST, G. B. (1955). Histamine liberation in the rat and mouse. *Archs. Int. Pharmacodyn. Ther.*, **102**, 304–313.

SCHAYER, W. R. (1956). Formation and binding of histamine by rat tissue *in vitro*. *Am. J. Physiol.*, **187**, 63–65.

SELYE, H., JEAN, P. & CAUTIN, M. (1960). Prevention by stress and cortisol of gastric ulcers normally produced by compound 48/80. *Proc. Soc. Exp. Biol. Med.*, **103**, 444–446.

TEPPERMAN, J., RAKIETER, N., BIRNIE, J. H. & DIERMEER, H. F. (1951). Effect of antihistaminics drugs on the adrenal cortical response to histamine and to stress. *J. Pharmac. Exp. Ther.*, **101**, 144–152.

WELLS, H., BRIGGS, F. N. & MUNSON, P. L. (1956). The inhibitory effect of reserpine on ACTH secretion in response to stressful stimuli. *Endocrinology*, **59**, 571–579.

YATES, F. E., LEEMAN, S. E., GLENISTER, D. W. & DALLMAN, M. F. (1961). Interaction between plasma corticosterone concentration and adrenocorticotropin-releasing stimuli in the rat: evidence for the reset of an endocrine feed back control. *Endocrinology*, **69**, 67–80.