THE EFFECT OF RESERPINE AND CHLORPROMAZINE ON THE RESPONSE OF THE RAT TO ACUTE STRESS[1]

MOHAMAD MAHFOUZ AND ESMAT A. EZZ[2]

Department of Pharmacology, Faculty of Medicine, University of Alexandria, Egypt

Received for publication January 23, 1958

It is generally accepted that the suprarenal cortex is activated as a part of the phenomena occurring in response to subjection of an organism to acute stress. This results in depletion of the adrenal ascorbic acid, which occurs within a period less than one hour. This depletion is shown to occur after injection of ACTH (Sayers *et al.*, 1944) as well as after the exposure of an organism to various stressful stimuli such as hemorrhage (Sayers *et al.*, 1945), scalding (Harkins and Long, 1945; Harkins, 1945), exposure to cold (Levin, 1945), ether anesthesia (Bowman and Muntwyler, 1937), ether plus scalding, and ether plus rapid exsanguination (Syndor and Sayers, 1954). The depletion of adrenal ascorbic acid in response to stress does not occur in hypophysectomized rats (Sayers *et al.*, 1945). Depletion of adrenal ascorbic acid also occurs after the injection of normal rats with posterior pituitary hormones; however, this does not occur in hypophysectomized animals (Martini and DePoli, 1956). The effects of injection of epinephrine and surgical trauma on ACTH secretion are blocked by an appropriate hypothalamic lesion (Hume, 1952; Porter, 1953; McCann, 1953).

The aim of this work was to investigate the action of reserpine and chlorpromazine on the response of the rat to acute stress. Chlorpromazine has been reported to inhibit the depletion of adrenal ascorbic acid induced by various stressful stimuli (Hamburger, 1955; Aron *et al.*, 1953; Olling and de Weid, 1956; Sevy *et al.*, 1957). Christy *et al.* (1956) found the stress of insulin hypoglycemia in humans to be inhibited by pretreatment with 100 to 400 mgm. of chlorpromazine. On the other hand, Holzbaur and Vogt (1954) failed to demonstrate such action, and they even found that chlorpromazine itself caused adrenal ascorbic acid depletion in unanesthetized rats. This latter finding was also demonstrated by Olling and de Weid (1956). However, Sevy *et al.* (1957) failed to demonstrate this action.

Sevy *et al.* (1957) found that adrenal ascorbic acid depletion with vasopressin was unaffected by chlorpromazine. This suggests that the drug acts on the prepituitary phase.

The effect of reserpine on the response to stress has not been investigated. Schneider *et al.* (1955) believed that reserpine may facilitate the action of cortical inhibitory centers which depress the hypothalamus.

METHODS. The degree of response to stress and any modification in such a response was assayed by the degree of cortical activity. Cortical activity was assayed by determining the degree of adrenal ascorbic acid depletion since Sayers (1950) found it to be a very reliable index of adrenal cortical activity in acute experiments.

Male albino rats from the same litter were used. All rats were approximately of the same age. The rats were kept under standard conditions with respect to diet, humidity, and room temperature, which was kept constant at 22°C. ± 2°. The day before the experiment, the rats were isolated for 24 hours during which no food was given.

Forms of stressful stimuli. These were selected on the basis of their similarity to forms of stress encountered by humans. The following three were used: 1) Continuous ether anesthesia plus rapid exsanguination: This simulates the stress of surgical operations. Rats were put in a tight glass box designed to standardize the conditions of stress with respect to the concentration of ether and time of exposure. The box has an inlet tube at the bottom and an exit tube at the top. An O_2-CO_2 mixture was passed at a constant pressure through a Wolf bottle containing ether. After 5 minutes, rats were removed from the box and bled from the tail. The site of incision was heparinized to allow

[1] Presented in part at the XX International Physiological Congress, Brussels, Belgium, July 30–August 4, 1956.

[2] Present address: Argonne Cancer Research Hospital, The University of Chicago, Chicago 37, Illinois.

free and rapid bleeding of 8 ml./kgm. b.w. The tail was then tied and the rat returned to the anesthesia box for 30 minutes before decapitation and removal of the adrenals. 2) Heat stress: This was considered to simulate conditions of hyperpyrexia or heat stroke. The rats were put in a wide brass jar closed with a glass cover and possessing an inlet and outlet for aeration. Air was supplied by an aerator at a constant and adequate rate. The jar was immersed in a water bath maintained at a constant temperature of 38°C. The rats were kept at this temperature for one hour, after which they were decapitated immediately and the adrenals removed. 3) Cold stress: This form of stress is becoming more important with the introduction of artificial hibernation and cooling techniques. The rats were put in a refrigerator at a constant temperature of +3°C. for one hour prior to decapitation and adrenalectomy.

Estimation of the adrenal ascorbic acid. After decapitation, the adrenals were dissected, freed from fat and immediately weighed to the nearest 0.1 mgm. Both glands were weighed together. They were ground in a small mortar containing approximately 2 gm. of acid-washed sand and 5 ml. of 4 per cent trichloracetic acid. The contents were transferred to a small bottle, tightly sealed and left over night in a refrigerator. The following morning, the contents of the bottle were transferred to a centrifuge tube and the bottle washed with 5 ml. of 4 per cent trichloracetic acid which was added to the centrifuge tube. After centrifuging for 5 minutes, the supernate was filtered. Analysis for ascorbic acid was then performed by the method of Roe and Keuther (1943).

Drugs and dosage. The drugs were diluted with saline and administered intramuscularly one-half hour before application of acute stress. Half of the dose was injected into each thigh. Twenty rats receiving no drug and not subjected to any stress served as controls. Eight rats received 8.0 microgm. of reserpine/kgm. body weight, were sacrificed one-half hour later without being exposed to stress and their adrenal ascorbic acid was estimated. Another group of eight rats received 2.0 mgm. of chlorpromazine/kgm., were sacrificed one-half hour later without being exposed to stress and their adrenal ascorbic acid was estimated. The rats exposed to stress were divided into three groups: one receiving no drug, another receiving chlorpromazine, and the third receiving reserpine. The rats that received ACTH were divided into three groups: one group received ACTH alone, the second received ACTH one-half hour after the usual dose of chlorpromazine, and the third group received ACTH one-half hour after the usual dose of reserpine. In each case, the rats were sacrificed one-half hour after receiving ACTH.

RESULTS AND DISCUSSION. The results are shown in table 1. These results indicate that 1) both drugs diminished ascorbic acid depletion in response to stress; 2) neither drug prevented the adrenal ascorbic acid depleting action of ACTH; 3) reserpine was highly effective in the low doses used; and 4) neither drug caused adrenal ascorbic acid depletion when given intramuscularly to unanesthetized rats.

In considering our results, we are assuming that the amount of depletion of adrenal ascorbic acid is proportional to the degree of stress (Sayers and Sayers, 1947). In these experiments, all groups of stressed rats were subjected to stressful stimuli of the same degree and duration whether or not drugs had been given. Therefore, variations in the degree of adrenal ascorbic acid depletion should be due to pretreatment with the drug used.

The action of the drugs used may have been on one or more of the following phases of the response to stress: 1) prepituitary phase of response to stress, 2) pituitary elaboration of ACTH, 3) interference with the action of ACTH on the cortex. The last possibility is eliminated experimentally since neither drug prevents ACTH from causing ascorbic acid depletion. This is supported by the results obtained by Olling and de Weid (1956) and Sevy *et al.* (1957) with chlorpromazine.

Regarding the possibility that the drugs act on the prepituitary phase, consideration must be given to the fact that the nature of this phase is still disputed. According to Sayers (1950), the organism responds normally with reactions mediated by sympathetic nervous receptor (sympathetico-adrenal discharge). This leads to increased tissue metabolism. Corticoids are used up and the pituitary-cortical system is stimulated from the periphery as a result of diminution of the concentration of the corticoids in the tissue fluids. Stimuli may travel as an alternative route via hypothalamic centers to adrenal hypophysis and through it to the adrenal cortex. Against this theory is the work of Bush (1953) which led him to conclude that "a fall in the peripheral concentration of adrenal hormones does not stimulate the release of ACTH directly." Also, severe stress causes adrenocortical stimu-

TABLE I

Inhibition of pituitary adrenocorticotrophic activity by chlorpromazine and reserpine following subjection of rats to various stressful stimuli

Treatment	No. of Rats	Pretreatment with Chlorpromazine, i.m.	Adrenal Ascorbic Acid	No. of Rats	Pretreatment with Reserpine, i.m.	Adrenal Ascorbic Acid
		mgm./kgm.	*mgm./100 gm. fresh tissue*		*μgm./kgm.*	*mgm./100 gm. fresh tissue*
Controls	20	None	337 ± 6.7*	20	None	337 ± 6.7
	8	2	341 ± 10.3	8	8	329 ± 15.5
Heat (exposed to 38°C. for one hour	9	None	190 ± 15.7	8	None	207 ± 11.3
before sacrifice)	16	2	323 ± 9.8	14	8	346 ± 13.3
Cold (exposed to 3°C. for one hour	6	None	220 ± 19.0	13	None	243 ± 6.7
before sacrifice)	14	2	333 ± 10.7	12	8	342 ± 12.1
Ether anesthesia with exsanguination of 8 ml. of blood per kgm.	12	None	270 ± 13.6	8	None	290 ± 9.5
	14	2	345 ± 6.9	12	8	331 ± 11.7
ACTH, i.m., 40 mgm./kgm.	6	None	209 ± 8.6	8	None	233 ± 9.2
	10	2	226 ± 5.5	8	8	225 ± 11.5

* Average ± standard error.

lation even in face of the most intense overdosage of corticoids (Selye, 1953). It was suggested by Schnitzer (1954, 1955) that the first mediator in response to stress is histamine liberated from injured tissues; but the work of Guillemin (1955) showed that epinephrine, norepinephrine, acetylcholine and histamine cannot be sole humoral agents responsible for pituitary stimulation by systemic stress.

In the last few years, the hypothalamic-hypophyseal system has received much attention as the probable pathway by which central neural or neuro-humoral stimuli regulate ACTH secretion. The existing evidence for this is summarized by Harris *et al.* (1954). It points to a humoral rather than a nervous mechanism. The hypothalamus elaborates posterior pituitary hormone that is formed in the neurons of the hypothalamic nuclei (Bargmann *et al.*, 1949, 1951; Hild and Zetler, 1953; Sharrer and Sharrer, 1953). Also, Adams and Sloper (1956) demonstrated the elaboration of the postpituitary principle by the hypothalamus in man, rat and dog. The findings of Martini and DePoli (1956) are consistent with the hypothesis that the posterior pituitary hormones act as a humoral transmitting agent that is released by the hypothalamus in the hypophysial portal system and which activates the anterior pituitary to discharge ACTH.

Considering this and the work of Das *et al.* (1954) and Sevy *et al.* (1957), we can postulate that chlorpromazine and reserpine depress the response of the rat to acute stress by inhibiting the hypothalamic centers.

Another possibility is that these drugs, through their action on the autonomic nervous system, may depress the alarm reaction and thus diminish the response. Whether this action is beneficial or detrimental to welfare of the organism is difficult to determine. However, it was evident that the rats subjected to stress after receiving either of the drugs were in much better condition than rats exposed to the same stress without the benefit of the drugs mentioned.

SUMMARY

Both chlorpromazine and reserpine inhibited the response of the rat to acute stress as measured by the degree of adrenal ascorbic acid depletion. Reserpine was extraordinarily effective in blocking activation in the low doses used. Neither drug prevented the adrenal ascorbic acid depleting action of exogeneous ACTH. The possible mechanisms of action of chlorpromazine and reserpine are discussed. Our results, sup-

ported by the results of other authors with chlorpromazine, are suggestive of an action on the prepituitary phase of response to stress, possibly on the hypothalamus.

ACKNOWLEDGMENTS. The authors wish to express their appreciation to Dr. George T. Okita, Mr. Ahmed Mokhtar and Mr. Mohamed Sarya for their kind help; and to Dr. Fouad Bishara, Ciba Company representative, for generous supplies of reserpine.

REFERENCES

ADAMS, C. W., AND SLOPER, J. C.: J. Endocrin., **13**: 221, 1956.
ARON, E., CHAMBON, Y., AND VOISIN. A.: Bull. Acad. Med. (Paris), **137**: 417, 1953.
BARGMANN, W., AND HILD, W.: Acta Anat., **8**: 264, 1949.
BARGMANN, W., AND SHARRER, E.: Am. Scientist, **39**: 255, 1951.
BOWMAN, D. E., AND MUNTWYLER, E.: Proc. Soc. Exper. Biol. & Med., **35**: 557, 1937.
BUSH, I. E.: XIX Cong. Internat. Physiol., 31 Aug.–4 Sept., Montreal, p. 254, 1953.
CHRISTY, N. P., KNIGHT, M., LONGSON, D., AND JAILER, J. W.: J. Clin. Endocrinol. and Metab., **16**: 913, 1956.
DAS, N. N., DASGUPTA, S. R., AND WERNER, G.: Bull. Calcutta Trop. Med., **1**: 566, 1954.
GUILLEMIN, R.: Endocrinology, **56**: 248, 1955.
HAMBURGER, C.: Acta Endocrinol., **20**: 383, 1955.
HARKINS, H. N., AND LONG, C. N. H.: Am. J. Physiol., **144**: 661, 1945.
HARKINS, H. N.: Physiol. Rev., **25**: 531, 1945.
HARRIS, G. W., AND FORTIER, C.: Fourth Annual Report on Stress, Montreal, Canada, p. 106, 1954.
HILD, W., AND ZETLER, G.: Ges. Exptl. Med., **120**: 236, 1953.
HOLZBAUER, M., AND VOGT, M.: Brit. J. Pharmacol., **9**: 402, 1954.
HUME, D. M.: Ciba Foundation Colloquia on Endocrinology, **4**: 87, 1952.
LEVIN, L.: Endocrinology, **37**: 34, 1945.
MARTINI, L., AND DEPOLI, A.: J. Endocrin., **13**: 229, 1956.
MCCANN, S. M.: Am. J. Physiol., **175**: 13, 1953.
OLLING, CH. C. J., AND DE WIED, D.: Acta Endocrinol., **22**: 283, 1956.
PORTER, R. W.: Am. J. Physiol., **172**: 515, 1953.
ROE, J. H., AND KEUTHER, C. A.: J. Biol. Chem., **147**: 399, 1943.
SAYERS, G., SAYERS, M. A., LEWIS, H. L., AND LONG, C. N. H.: Proc. Soc. Exper. Biol. & Med., **55**: 238, 1944.
SAYERS, G., SAYERS, M. A., LIANG, T. Y., AND LONG, C. N. H.: Endocrinology, **37**: 96, 1945.
SAYERS, G., AND SAYERS, M. A.: Endocrinology, **40**: 265, 1947.
SAYERS, G.: Physiol. Rev., **30**: 241, 1950.
SCHNITZER, A.: Internat. Arch. Allerg., **5**: Suppl. 87, 1954.
SCHNITZER, A.: München. med. Wchnschr., **97**: 466, 1955.
SCHNEIDER, J. A., PLUMMER, A. J., EARL, A. E., AND GAUNT, R.: Ann. New York Acad. Sci., **61**: 17, 1955.
SELYE, H.: Annual Report on Stress, **3**: 38, 1953.
SEVY, R. W., OHLER, E. A., AND WEINER, A.: Endocrinology, **61**: 45, 1957.
SHARRER, E., AND SHARRER, B.: Handbuch der Mikroskopischen Anatomie des Menschen. Von Mollendroff und Bargmann eds., Vol. **6**, p. 953, Springer, Berlin, 1953.
SYNDOR, K. L., AND SAYERS, G.: Endocrinology, **55**: 621, 1954.