EFFECT OF NIAMID AND BENZQUINAMIDE (QUANTRIL) ON THE RELEASE OF CORTICOTROPHIN IN THE RAT

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A survey of literature concerning the effects of psychotherapeutic drugs on pituitary adrenal axis (ACTH-release) in relation to the varieties of acute stress, reveals conflicting views. Central nervous system depressants may either depress or stimulate the pituitary adrenal axis depending on the conditions under which they are examined. Effect of reserpine and chlorpromazine on ACTH release has been the subject-matter of numerous investigations.

Maickel, Westermann & Brodie (8) on the basis of their detailed studies of the various parameters of pituitary adrenal stimulation of rats have indicated that both reserpine and stress (cold exposure) could elicit ACTH hypersecretion. Large doses of reserpine or prolonged stress lowered the pituitary content of ACTH to such an extent that the animals were shown to be unable to respond to an additional pituitary stimulus.

Ashford & Shapero (1) have shown that single injection into rat of chlorpromazine, reserpine, benactyzine or phenobarbitone stimulated the release of corticotrophin. However, the chronic administration of these drugs failed to elicit this response, while stimulant effect of ether on pituitary adrenal axis was still manifested after single as well as after five daily injections of these drugs. Westermann *et al.* (12) and Costa et al (3) emphasized that central actions of reserpine like sedation, hypersecretion of ACTH and depletion of brain 5-HT were all closely related. They, while commenting on the observations of Martel *et al* (9) who demonstrated that pretreatment of rats with a potent MAO inhibitor prevented reserpine induced sedation and ACTH hypersecretion, inferred that hypersecretion of ACTH was an integral part of reserpine central action. However, the action of MAO inhibitor on pituitary adrenal axis, per se, does not seem to be recorded. Westermann *et al* (12) also showed that a Benzoquinolizine derivative RO 4-1284 (Structural analogue of reserpine) produced a short lasting central action (sedation) and elicited a correspondingly fleeting ACTH discharge.

Shulkin *et al* (11) studied the psychopharmacological effects of Benzquinamide (Benzoquinolizine derivative) a prototype of tetrabenazine, having a structural resemblance to reserpine and reported that it did not alter the normal monoamine contents of rat brain. Goswami *et al* (5) confirmed Benzquinamide to be a non-releaser of monoamines (5-HT and catecholamines) in dog periventricular grey matter.

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Earlier reports from this laboratory showed depletion of adrenal ascorbic acid in rats exposed to positive accelerative stress G forces, (7); further-more, a marked increase in plasma ACTH was observed when the rats were exposed to 28 G for 1 minute (6). It was, therefore, decided to undertake the study of Benzquinamide (Quantril) a non-releaser of monoamine and Niamid a preserver of monoamines, in relation to their effects on pituitary adrenal axis, in animals when exposed to G forces, in an attempt to further elucidate any correlation between the psychotherapeutic agents and corticotrophin out-pouring. Adrenal ascorbic acid depletion has been taken as an index of corticotrophin activity.

METHODS

100 albino rats of both sexes weighing between 100 to 175 gm. kept on standard normal diet, water adlib and constant room temperature were selected for this work. The drugs Niamid (Nialamid) 3 mgm/kg. and 50 mgm/kg. and Quantril 20 mgm/kg. were administered intraperitoneally in aqueous solution. These doses (Niamid, 3 mgm/kg. and Quantril 20 mgm/kg.) were chosen on the basis of therapeutic dose range and Niamid 50 mgm/kg on its protective effect against stress induced gastric ulcers (2) The volume of injection was kept as 1.0 ml./100 gm. body wt. Control animals received the diluent only. The acute stress was caused by subjecting rats to centrifugal accelerative force at 28 G for 1 mt. as reported earlier (6, 7).

Two groups of 17 rats each were taken. One group was treated as control and normal level of ascorbic acid of the adrenal glands was determined; where as the rats of the other group were subjected to acute stress and killed by decapitation after 1 hour of the stress and adrenal ascorbic acid level was determined. In another six groups of 11 rats each, three groups of rats were given Niamid injection 3 mgm/kg., 50 mgm/kg and Quantril 20 mgm/kg. body wt. respectively i. p. These rats were killed 1 hour after injection and adrenal ascorbic acid level was determined. Remaining three groups were similarly treated with Niamid and Quantril. One hour after injection, they were subjected to acute stress and killed after 1 hour of the stress to determine the level of adrenal ascorbic acid.

For determination of adrenal ascorbic acid, the adrenal glands were quickly removed after killing the rats, trimmed free of fat, weighed on a microtorsion balance and homogenized in 6% trichloroacetic acid. The homogenate was centrifuged and ascorbic acid was estimated in the centrifugate by the method of Roe & Kuether (10).

RESULTS

1. Effect of acute stress :—

In a group of 17 rats subjected to acute stress a marked depletion of adrenal ascorbic acid was noticed, the average value being 228.13 ± 11.02 mgm/100 gm where-as the average normal level of adrenal ascorbic acid as seen in a control group of 17 rats was 361.63 ± 8.3 mgm/100 gm. Thus, the stress alone produced 36.91% depletion of adrenal ascorbic acid.

2. Effect of Niamid :—

Injection of Niamid in doses of 3 mgm/kg. and 50 mgm/kg. alone produced marked depletion of adrenal ascorbic acid when determined after 1 hour of injection.

Niamid 3 mgm/kg. produced 27.39% depletion as the average adrenal ascorbic acid level following the injection in a group of 11 rats was 256.6±19.79 mgm 100 gm. as compared to the average figure of 361.68 mgm/100 gm. in control group. Similarly, Niamid 50 mgm/kg, alone produced a depletion of adrenal ascorbic acid to the extent of 36% in a group of another 11 rats, the average level was 231.47±11.8 mgm/100 gm.

Further exposure of the rats to stress indicated that Niamid in doses of 3 mgm. and 50 mgm/kg. did not block the stress-induced depletion of adrenal ascorbic acid as is evident from the results that in a group of 11 rats treated with Niamid 3 mgm/kg. and further subjected to acute stress after 1 hour showed the average depletion of adrenal ascorbic acid of 41.99%; the average level being 209.78±15.06 mgm/100 gm. as compared to the average level in control group of 361.68 mgm/100 gm. Similarly, in a group of rats pretreated with Niamid 50 mgm/kg. and subjected to acute stress after 1 hour, the average adrenal ascorbic acid level was 198.60±7.1 mgm/100 gm. thus producing 45.04% depletion. (See table I and Fig. 1). All the results are highly significant, P being $\angle 0.001$

TABLE I

Effect of Niamid 3 mgm/kg. and 50 mgm/kg. on Adrenal Ascorbic Acid Contents (mgm/100 g,) of Nonstressed and Stressed Rats

No. of expt.	Control	Stress	Niamid I. P. 3 mgm/kg.	Niamid I. P. 3 mgm/kg. plus stress after 1 hour	Niamid I. P. 50 mgm/kg.	Niamid I. P. 50 mgm/kg. plus stress after 1 hour
1	2	3	4	5	6	7
1.	342.8	209.0	315.3	225.0	183.3	229.2
2.	364.2	172.9	165.7	175.0	219.0	193.1
3.	373.9	141.1	350.0	157.1	230.0	176.4
4.	408.0	269.2	227.2	180.0	242.4	200.0
5.	425.0	157.1	350.0	238.4	282.3	182.6
6.	325.9	258.3	272.8	252.6	228.5	157.1
7.	375.0	250.0	304.7	252.6	293.3	160.0
8.	344.4	260.8	195.4	241.0	172.4	235.2
9.	409.0	263.6	192.3	208.3	210.8	219.0
10.	400.0	230.3	210.5	241.3	222.7	230.0
11.	358.3	288.0	238.8	136.3	261.1	200.0
12.	357.7	195.3				
13.	313.5	237.5				
14.	342.8	264.0				
15.	304.7	172.9				
16.	375.0	229.1				
17.	328.4	270.0				
Average	361.68	228.18	256.60	209.78	231.47	198.60
S. E.	±8.3	±11.02	±19.79	±15.06	±11.8	±7.1
P.		<0.001	<0.05	<0.001	<0.001	<0.001
				<0.05 Compared with column 3.		<0.05 Compared with column 6
Ascorbic acid depletion%		—36.91%	—27.39%	—41.99%	—36.0%	—45.04%

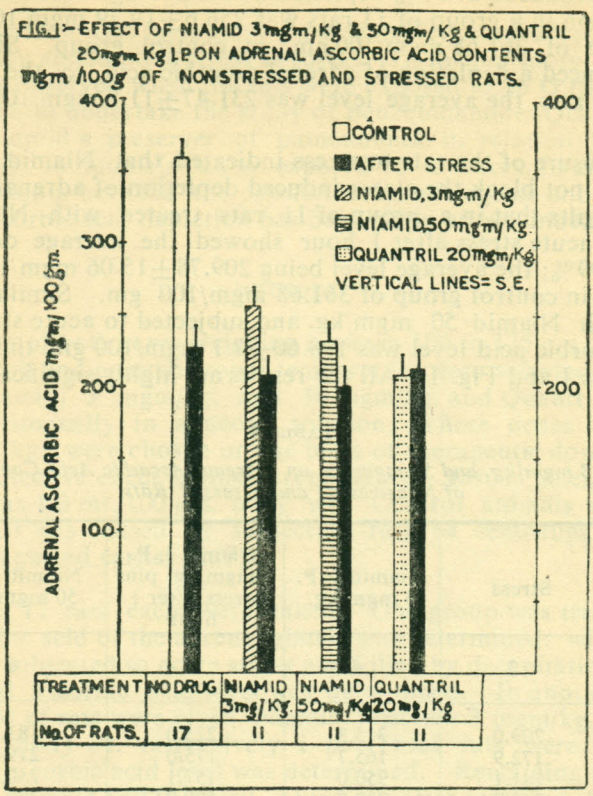

Fig. 1

3. Effect of Quantril :

Table II indicates the effects of Quantril 20 mgm/kg. given i. p. to one group of 11 nonstressed rats and to another group of 11 rats which were further subjected to acute stress after 1 hour of the injection. Quantril alone produced 42.45% average depletion in a group of non-stressed rats as the average level of adrenal ascorbic acid in this group was 208.10±22.4 mgm/100 gm. compared to the control level of 361.68/mgm100 gm. The other group further subjected to acute stress 1 hour after the injection of Quantril, did not reveal significant change as compared to the above group; the average level of adrenal ascorbic acid was 212.92±17.1 mgm/100 gm. thereby showing 41.13% depletion as compared to the control group. Thus, stress alone produced 36.91% depletion of adrenal ascorbic acid, Quantril 20 mgm/kg. alone produced 42.45% depletion where-as Quantril followed by stress produced 41.13% depletion of adrenal ascorbic acid. Hence, it is evident that Quantril treated rats when subjected to acute stress could not demonstrate further depletion of adrenal ascorbic acid suggesting the absence of release of stress induced corticotrophin. (Table II & Fig. 1).

DISCUSSION

Niamid 3 mgm/kg. and 50 mgm/kg. and Quantril 20 mgm/kg. on intraperitoneal administration to rats alone produced marked depletion of adrenal ascorbic acid indicating that these drugs produced outpouring of pituitary corticotrophin in these animals.

TABLE II

Effect of Quantril (Benzquinamide) 20 mgm/kg. on Adrenal Ascorbic Acid Contents (mgm/100 g.) of Nonstressed and Stressed Rats

No. of experiments	Control	Stress	Quantril I. P. 20.0 mgm/kg.	Quantril I. P. 20.0 mgm/kg. plus stress after 1 hr.
1	2	3	4	5
1.	342.8	209.0	235.0	209.0
2.	364.2	172.9	160.0	157.1
3.	373.9	141.1	310.0	296.2
4.	408.0	269.2	327.2	243.8
5.	425.0	157.1	183.7	329.7
6.	325.9	258.3	166.6	160.0
7.	375.0	250.0	286.9	178.5
8.	344.4	260.8	190.9	211.7
9.	409.0	263.6	121.7	170.0
10.	400.0	230.3	166.6	214.8
11.	358.3	288.0	140.5	171.4
12.	357.7	195.3		
13.	313.5	237.5		
14.	342.8	264.0		
15.	304.7	172.9		
16.	375.0	229.1		
17.	328.4	270.0		
Average	361.68	228.18	208.10	212.92
S. E.	±8.3	±11.02	±22.4	±17.1
P.		<0.001	<0.001	<0.001
				N.S. compared with column 4
Ascorbic acid depletion%		−36.91%	−42.45%	−41.13%

Maickel *et al* (8) have shown that reserpine alone in doses of 1.0 mgm/kg. caused increased adrenocortical activity as shown by marked depletion of adrenal ascorbic acid to the extent of 33.9% indicating massive liberation of pituitary corticotrophin. They have shown that reserpine had made the pituitary unresponsive to further stimulation as the drug almost depleted the ACTH contents of the pituitary, commenting that the ACTH discharge following reserpine or stress (cold exposure) is greater than its rate of synthesis.

Our present studies indicate that the animals exposed to acute stress by subjecting them to positive acceleration at 28 G for 1 mt. produced 36.91% depletion of adrenal ascorbic acid, Quantril alone 20 mgm/kg. produced 42.45% depletion whereas pretreatment of rats with Quantril followed by acute stress led to 41.13% depletion. These studies indicate that Quantril has already led to sufficient discharge of pituitary corticotrophin, little remaining behind to be subsequently discharged to the additional stress. Maickel *et al.* (8) have reported that out of the number of Rauwolfia alkaloids which

released brain amines, and caused sedation alone were found to increase the activity of adrenal cortex, isoreserpine, isoraunescine, and raunescine did not release brain amines, elicit sedation or stimulate ACTH secretion. Westermann *et al.* (12) on the basis of their detailed studies have emphasized that release of pituitary ACTH by reserpine is related to blockade of monoamine storage in brain, since only in doses that lowered the amine stores by 50% or more did the drug produce ACTH discharge, a maximum discharge of ACTH occuring after doses of reserpine which completely produced blockade of storage of brain amine. Westermann *et al.* (12) discussing the mechanism of action of reserpine, have surmised that after reserpine administration, the central action, hypersecretion of ACTH and the blockade of 5HT storage are all closely related, further concluding that the action of reserpine on the pituitary is part and parcel of its sustained action on neuronal pathways in the brain.

In contrast to this, our present studies with Quantril a compound of Benzoquinolizine series and a structural analogue of Tetrabenazine, is well documented to be a traquillizing agent, producing sedation, but unlike reserpine, although does not deplete brain monoamine (11, 5) has elicited marked release of corticotrophin, thereby causing adrenal ascorbic acid depletion when given alone. This suggests that at least there is no correlation between the monoamine depletion and hypersecretion of ACTH with Quantril as has been observed in case of reserpine.

Studies with Niamid-which is a potent monoamine oxidase inhibitor and a psychostimulant, is well documented to raise the monoamine level of brain and having a central stimulant effect, yet in 3 mg/kg. and in 50 mgm/kg. doses alone produced 27.39% and 36% depletion of adrenal ascorbic acid. These studies further emphasize that there is no correlation between the monoamine depletion and hypersecretion of ACTH as has been noticed for reserpine (12).

Niamid treated rats further exposed to acute stress showed increased depletion of adrenal ascorbic acid, with Niamid 3 mg/kg. the depletion was 41.99% from 27.39% and with Niamid 50 mgm/kg. the depletion was 45.04% from 36% thereby showing that there was further outpouring of pituitary ACTH and that the pituitary was not completely depleted of its corticotrophin when Niamid was given alone, without disturbing the adaptive mechanism to stress completely. Thus, our findings are in accord with Ashford and Shapero (1) who demonstrated that pretreatment of rats with reserpine, chlorpromazine, benactyzine and Sod. : phenobarbitone when exposed to ether stress could still cause depletion of adrenal ascorbic acid indicating further discharge of corticotrophin. However, the extent of depletion of ACTH contents of the pituitary after 3 mgm. and 50 mgm/kg. of Niamid and Quantril 20 mgm/kg. in rats can only be revealed by determining the corticotrophin contents of the pituitary glands. Studies with Niamid indicated that the animals still had their adaptive mechanism intact suggesting that either the depletion is not absolute or the synthesis of ACTH is rapid. Hence, from the present studies it emerges that Quantril in 20 mgm/kg. doses completely blocks the reaction of organism to stress or the adaptive mechanism. In absence of studies pertaining to the pituitary ACTH content it is difficult to state whether inhibition of ACTH discharge is a manifestation of a gland substantially depleted of ACTH or is a specific inhibitory effect on pathways regulating the secretion of the hormone. If, as is likely, the drug causes subtantial depletion of pituitary ACTH (prolonged stimulation of adrenocortical activity) in man more should be known about the long term physiological and biochemical consequences of tranquillizers which are being increasingly introduced in therapeutics. Niamid, a psychoenergizer, however, does not seem to disturb the

adaptive mechanism of the organism to stress to the same extent as does the psychodepressants (Tranquillizers).

Hypersecretion of ACTH produced by exposure of animals to stressful environment is usually described in terms of non-specific activation of the hypothalamopituitary system. It seems highly probable that drugs acting at the hypothalamic level psychodepressants and psychoenergizers lead to nonspecific type of stress by inducing corticotrophin discharge. The evidence so presented together with the studies of Ashford and Shapero (1), Maickel *et al.* (8) and Westermann *et al.* (12) support the hypothesis of Egdahl (4) that the secretion of ACTH is controlled both by inhibitory and stimulatory pathways in the hypothalamus; ACTH discharge might result from activation of stimulatory pathways or inhibition of inhibitory pathways. Psychodepressants Reserpine, Chlorpromazine, Benactyzine and Quantril, irrespective of their actions on monoamines, may act by depressing the inhibitory pathways, while the psychoenergizers monoamine oxidase inhibitors may act by activation of stimulatory pathways.

Since, Niamid which is a psychoenergizer, does not cause total depletion of the pituitary ACTH in the therapeutic doses, and the subsequent stress of positive acceleration being still capable of eliciting a further discharge of corticotrophin, shows that the animals were well prepared to stand the stress of even extreme gravitational forces and unlike psychodepressants, the animals were not altogether deprived of one of the primary defence mechanisms. Further work on the protective effect of Niamid against G-forces is in progress before the clinical use of the drug in pilots exposed to G-forces can be suggested. Use of psychoenergizers would also keep them mentally alert-and perhaps better equipped for any aviation hazard.

CONCLUSIONS

The following conclusions emerge from the present studies :-

1. Quantril, a psychodepressant and a non-potent releaser of brain monoamines, itself stimulates the pituitary adrenal axis to an extent that, the pituitary is not responsive to a subsequent stressful stimuli such as positive acceleration stress.

2. Niamid—a psychoenergizer and a preserver of brain monoamines, although itself a stimulant of pituitary adrenal axis, does not block the pituitary response to a subsequent stressful stimuli. Thus, the administration of Niamid does not completely deprive the animals of their corticotrophin stores thereby keeping the primary defence mechanism still intact and the animals perhaps were better equipped to bear the brunt of subsequent stress.

3. The hypersecretion of ACTH which forms an integral part of central actions of reserpine and which is closely correlated with sedation and 5HT depletion (12) does not seem to hold for Quantril which although produces hypersecretion of ACTH and sedation, does not deplete brain monoamine stores.

4. The hypothesis of Egdahl (4) that the ACTH secretion is controlled both by inhibitory and stimulatory pathways in the hypothalamus and ACTH discharge might result from activation of stimulatory pathways or inhibition of inhibitory pathways is

further substantiated. Niamid, a psychoenergizer, acts by stimulating the facilitatory pathways while Quantril, a psychodepressant, causes ACTH liberation by depressing the inhibitory pathways.

5. The possibility of Niamid in small doses being used as a protective shield to enhance the tolerance of pilots against hazards of G forces is cautiously predicted.

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