Studies on the Metabolism of 5-Hydroxytryptamine (Serotonin) VI. Hydroxylation and Amines in Cold-Stressed Reserpinized Rats (33025)

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Earlier (1) it was demonstrated that in tryptophan-deficient rats depleted of their cerebral 5-hydroxytryptamine (5-HT) and norepinephrine (NE) by a single injection of reserpine, the NE rapidly returned to normal while 5-HT remained at a persistently low level. The 5-HT "depleted" animals responded to reserpine with the same intensity of sedation as their controls. It seemed, therefore, that reserpine-produced sedation per se was not directly related to the variability or dynamic states of cerebral 5-HT. Evidence for this view was recently presented (2). Nevertheless, the results of others (3–5) implied that reserpine-produced sedation was due to the loss of brain 5-HT since rats and rabbits stressed by acute cold exposure, when reserpinized, evinced very slight changes in their cerebral 5-HT content and no sedation even though their catecholamines were depleted. Results of others (6–9), however, revealed a significant lowering of the cerebral 5-HT and sedation in acutely cold-exposed rats given reserpine. In view of the controversial nature of the reported findings, we have reexamined the effect of acute cold stress, as well as the effect of reserpine on the cerebral 5-HT and NE content of tryptophan-sufficient and -deficient rats. During the course of these experiments, it was observed that multiple stresses significantly affected the hepatic phenylalanine and tryptophan hydroxylase activity. Germane to this is the observation that administration of glucocorticosteroids led to a fast inductive increase in the hepatic hydroxylase activity (10). In order to gain more information on the effect of single and multiple stresses on the metabolism of biogenic amines and to stay within the context of the above problems the experiments reported in this paper were designed.

Methods and Materials. Sprague-Dawley male rats (130 gm av wt.) were divided into two groups. The first group was kept on tryptophan-sufficient diet while the second group was kept on a tryptophan-deficient diet. The conditions and diet were as previously described (11). In the studies on the effect of acute cold stress, the animals were placed in a cold room at 4°C for 8 hours. At 4 hours some of the animals were intraperitoneally (i.p.) injected with a solution of pure crystalline Serpasil phosphate (CIBA, Ltd.) (5 mg/kg) containing purified randomly labeled tritiated reserpine (New England Nuclear Corp.) and were kept for an additional 4 hours prior to sacrifice. Animals were decapitated in the cold room and the tissues were immediately processed there for the different analyses. The livers of animals were removed for the preparation of phenylalanine and tryptophan hydroxylase (12). Tyrosine and 5-hydroxyindoles were assayed by the nitrosonaphthol method (13). The brains of the animals were analyzed for 5-HT (14) and NE content (15). Reserpine-^{3}H was recovered from brain by differential extraction (16) and the radioactivity was determined by liquid scintillation counting. In many instances the subcortical areas of the rat brains were assayed for tryptophan-5-hydroxylase activity according to the following procedure. Brain tissue was homogenized (3:1 v/w) in 0.05 *M* Tris acetate buffer, pH 7.4, containing 10^{-5} *M* EDTA and 10^{-2} *M* mercaptoethanol and spun for 30 min at 28,000*g*. The supernatant was assayed in a system containing 80 μg of pargyline (courtesy of Abbott Laboratories), 18 μg L-tryptophan-3-^{14}C (1 μC), 353 μg of $DMPH_4 \cdot HCl$ (2-amino-4-hydroxy-6,7-dimethyl-tetrahydropteridine), an aliquot of supernatant containing 2 mg of protein, and 0.05 *M* Tris acetate buffer, pH 7.4, to bring the total volume to 1.0 ml. This mixture was incubated in air at 37°C for 30 min. At this point the reaction was stopped by adjusting the pH to 9 and adding DL-5-HTP and guinea pig kidney d ecar-

TABLE I. Recovery of Reserpine-^{3}H from Rat Brain Following its Intraperitoneal Administration.[a]

Time (hours)	Control		Cold exposed	
	% of total	μg $\pm$ SE as reserpine	% of total	μg $\pm$ SE as reserpine
2	0.07	0.45 $\pm$ .03	0.05	0.34 $\pm$ .09
4	0.08	0.52 $\pm$ .06	0.06	0.40 $\pm$ .04

[a] Intraperitoneal Serpasil phosphate (5 mg/kg). Each group represents the average of four animals.

boxylase as described by Lovenberg *et al.* (17). Following 20 min incubation under nitrogen, the reaction was stopped by immersing the reaction mixture in boiling water for 5 min. After 10 min centrifugation the supernatants were passed through 100–200 mesh Amberlite CG-50 (H^+ form) columns (30 $\times$ 6 mm) at a rate of 7 drops/min. After this the columns were washed with 100 ml of hot water followed by elution of 5-HT-^{14}C with 4 ml of 4 *N* acetic acid. Aliquots of the acid eluate were counted in a liquid scintillation counter (all samples were run in duplicates).

Results and Discussion. In order to show that the results obtained by others (3) with acutely cold-exposed and reserpinized animals were not due to differences in the amount of reserpine reaching the brain, a group of tryptophan-sufficient animals was cold stressed and given 5 mg/kg i.p. reserpine. At various time intervals they were sacrificed and the cerebral reserpine content was determined. The values obtained were compared to those from reserpinized tryptophan-sufficient rats kept at room temperature. The results given in Table I indicate that the amount of recoverable reserpine from the brains of acutely cold-stressed rats is statistically not significantly different from the controls.

A reexamination of the effect of acute cold stress on the 5-HT and NE levels of tryptophan-sufficient and tryptophan-deficient rats with and without the administration of reserpine is given in Table II. Simple cold stress notwithstanding, there was sedation corresponding to rating of 10–12 (18) in all reserpinized animals over the 4-hour period. The cerebral 5-HT levels of the reserpinized and cold-stressed rats were reduced by about 40% compared to those of the cold-stressed, but unreserpinized animals. In reserpinized animals without cold stress, cerebral 5-HT levels were 55% lower than in the controls. Therefore, it seems, in accord with Ingenito (9) that acute cold stress for 4 hours does not prevent the lowering of cerebral 5-HT and NE levels usually brought about by reserpine treatment. In these experiments cold exposure alone did not decrease either 5-HT or NE levels as reported by Toh (19) who observed a 37% drop in cerebral 5-HT content of rats after 30-min exposure to 1°C.

Excess dietary L-tryptophan or glucocorti-

TABLE II. Comparison of the 5-Hydroxytryptamine and Norepinephrine Content of Rat Brain in Various Conditions.[a]

Condition	No. of animals	μg/gm $\pm$ SE	
		5-HT	NE
Tryptophan sufficient	6	0.42 $\pm$ .05	0.49 $\pm$ .04
+ cold exposed	7	0.45 $\pm$ .09	0.44 $\pm$ .14
+ reserpinized[b]	6	*0.17* $\pm$ .06	*0.06* $\pm$ .001
+ cold exposed and reserpinized[b]	9	*0.24* $\pm$ .04	*0.06* $\pm$ .005
Tryptophan deficient	16	*0.20* $\pm$ .05	0.43 $\pm$ .12
+ cold exposed	7	0.27 $\pm$ .08	0.39 $\pm$ .12
+ reserpinized[b]	6	*0.09* $\pm$ .04	*0.10* $\pm$ .01
+ cold exposed and reserpinized[b]	11	*0.13* $\pm$ .03	*0.11* $\pm$ .01

[a] Conditions as given in the text. The italicized values indicate statistically significant differences from the controls.

[b] Intraperitoneal Serpasil phosphate (5 mg/kg).

TABLE III. Effect of Various Stresses on the Phenylalanine (ϕHS) and Tryptophan (THS) Hydroxylase Systems of Rat Liver.[a]

Condition	No. of animals	mμmole/gm per min[b] ± SE	
		5-HTP	Tyrosine
Tryptophan sufficient	6	43 ± 3.4	1060 ± 175
+ cold exposed	7	45 ± 4.1	1120 ± 211
+ reserpinized	6	32 ± 9.9	870 ± 223
+ cold exposed and reserpinized	9	*28* ± 4.7	780 ± 83
Tryptophan deficient	16	36 ± 4.0	640 ± 100
+ cold exposed	7	*22* ± 5.5	*489* ± 79
+ reserpinized	6	34 ± 4.6	783 ± 215
+ cold exposed and reserpinized	11	*25* ± 2.5	*560* ± 96

[a] Conditions as given in the text.

[b] Duplicate determinations. The italicized values indicate statistically significant differences from the controls.

costeroid administration was observed to increase hepatic hydroxylation of phenylalanine or tryptophan (10). This report led us to investigate the effect of depletion of dietary tryptophan alone or in combination with various stresses, on hepatic hydroxylase. The results in Table III reveal that hepatic hydroxylation of both phenylalanine and tryptophan was unaffected in tryptophan-sufficient animals, when acutely cold exposed or reserpinized. However, tryptophan-sufficient animals under simultaneous double stress of cold exposure and reserpine showed a statistically significant decrease in hepatic hydroxylation of tryptophan only. A careful examination of the factors involved in Table III would indicate that of the three stresses (i.e., tryptophan deficiency, acute cold exposure, and reserpine) only two are necessary to affect hepatic hydroxylation significantly, and that the potentiating or *sine qua non* factor is cold exposure.

Under identical conditions tryptophan deficiency had no effect on cerebral tryptophan hydroxylase (Table IV). In both tryptophan-sufficient and -deficient rats, however, the cerebral tryptophan-5-hydroxylase activity was apparently stimulated either by cold stress or administration of reserpine. The differences between any of the stress groups and their controls are statistically highly significant ($p < 0.01$). There is a trend, albeit statistically not significant, indicating that reserpine had a more pronounced inductive effect on cerebral tryptophan-5-hydroxylase in these animals than did acute cold exposure.

It is well known that peripherally administered reserpine depletes 5-HT from brain as well as from other tissues; however, it does

TABLE IV. Cerebral Tryptophan-5-hydroxylase Activity under Various Stresses.

Condition	No. of animals/group	5-HTP synthesized[a] (mμmole/gm per min ± SE)	
		Tryptophan-sufficient	Tryptophan-deficient
	12	5.94 ± .43	5.94 ± .33
Cold exposed	10	*8.58* ± .59	*8.25* ± .63
Reserpinized[b]	6	*9.57* ± .01	*8.91* ± .63
Cold exposed and reserpinized[b]	16	*9.57* ± .03	*9.90* ± .02

[a] Duplicate determinations. The italicized values indicate statistically significant differences from the controls.

[b] Intraperitoneal Serpasil phosphate (5 mg/kg). Other conditions as given in text.

not deplete cerebral tryptophan although it does lower the trytophan level of rat liver (20). Furthermore, reserpine does not inhibit cerebral tryptophan-5-hydroxylase (20,21). In light of these reports, although no conclusions can be drawn as to why similar stresses would decrease hepatic tryptophan hydroxylation while increasing cerebral production of 5-HTP, it is conceivable that the increased cerebral tryptophan-5-hydroxylase activity found in reserpinized animals is due to a cerebral compensation for the extreme loss of dynamic tryptophan:5-HTP:5-HT equilibrium caused by 5-HT depletion.

Summary. Tryptophan deficiency did not affect either penetration or cerebral retention of reserpine-^{3}H or tryptophan-5-hydroxylase activity of brain or liver. Administration of reserpine or acute cold exposure significantly increased tryptophan-5-hydroxylase activity in the brain in both sufficient and deficient rats while cold stress, in combination with another stress, was found to be an absolute requirement in decreasing hepatic hydroxylation of either tryptophan or phenylalanine. Cold exposed and reserpinized rats responded with a statistically significant depletion of both cerebral 5-HT and NE with concomitant deep sedation.

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