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Effect of Hypothalamic Lesions on Tryptophan Peroxidase of the Liver.* (24706)

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Independent studies show that activity of tryptophan peroxidase in the liver of rats is under partial control of the pituitary-adrenal system(1,2,3,4). Enhanced enzyme activity follows administration of "non-specific" compounds which presumably release adrenocorticotrophin (ACTH). Augmented enzyme activity is also produced by ACTH or cortical steroids(1,2,3,4). Following adrenalectomy, non-specific agents do not produce increased enzyme activity. Tryptophan, on the other hand, augments enzyme activity by a mechanism independent of adrenal cortical hormones (1). Since release of ACTH appears to be regulated, at least in part, by the hypothalamus(5,6), it appeared of interest to determine response of tryptophan peroxidase to non-specific agents, using animals with hypothalamic lesions which block secretion of ACTH. The agent chosen was histidine since it has been more thoroughly investigated than other agents(1). The results indicate that hypothalamic lesions produce a decrease in response of tryptophan peroxidase to histidine.

Methods. Adult male rats (Long-Evans) weighing 200-300 g, fed a synthetic diet(7), were used. The Krieg stereotaxic instrument was used to produce hypothalamic lesions, designed to interrupt the supraoptico-hypophysial tract in median eminence of tuber cinereum(5,6). Only rats with successful interruptions of this tract as reflected by severe diabetes insipidus with water intake in excess of 100 ml, 24 hours following induction of lesions were used. In 6 instances, location of lesions was determined by serial sections of the hypothalamic area, and the lesions lay in median eminence of the tuber cinereum. Earlier studies show that such rats subjected to acute stress give no evidence of ACTH release(5,8). Since no discernible difference in response to histidine could be detected 2, 4 and 5 days post-operatively, the combined data were pooled. Seven days intervened between hypophysectomy and histidine injection. Five hours prior to nercopsy, half the rats in respective groups received intraperitoneal (I.P.) injections of 2 mM 1-histidine-HCl; the remaining animals were uninjected and served as controls. The rats were anesthetized with Nembutal (5 mg/100 g, IP) and decapitated. Immediately afterwards the liver was removed, chilled, and a weighed portion was used in tryptophan peroxidase activity determination(1). The method used is an indirect one and depends on kynurenine production after incubation of liver homogenates with added tryptophan as substrate, activity being expressed as number of μM of kynurenine/gram dry weight liver/hour. In some cases and immediately following removal of liver, the left adrenal was removed and analyzed for ascorbic acid concentration(5). The relevant data are summarized in Tables I and II.

Results. Effect of histidine on ACTH release. It has been assumed that histidine produces its effect on tryptophan peroxidase by stimulating secretion of ACTH, which, in turn, brings about release of cortical steroids

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TABLE I. Effect of Histidine on Adrenal Ascorbic Acid Concentration of Normal Rats and Those with Hypothalamic Lesions.*

Normal rats		Rats with hypothalamic lesions	
Control	After histidine	Control	After histidine
337	289	415	433
303	277	458	444
305	255	425	400
408	177	401	500
398	226	445	413
252	195	515	471
357	385		
329	261		
353	296		
† 338 ± 15	262 ± 21	443 ± 17	444 ± 15

* All results expressed as mg of ascorbic acid per 100 g adrenal.
† Mean ± stand. error of mean.

that increase activity of tryptophan peroxidase. To test the possibiilty that histidine does release ACTH from the pituitary, ascorbic acid depletion produced by histidine injection was evaluated in normal rats and those with hypothalamic lesions. It appears that histidine does produce a small but significant ($P<.01$) decrease in adrenal ascorbic acid in the normal rat (Table I). This is interpreted to mean that histidine produces activation of the adrenal cortex by stimulating ACTH release. However, histidine appears to be far from a maximal stimulus to ACTH discharge, since it caused a decrease in adrenal ascorbic acid concentration of only 75 mg %, whereas unilateral adrenalectomy results in decrease of 190 ± 7 mg % (McCann and Fruit, unpublished observations). In contrast to normal rats, those with hypothalamic lesions failed to show any depletion of adrenal ascorbic acid after histidine injection, indicating no ACTH discharge. This would be expected from earlier studies which demonstrated that these lesions prevent ascorbic acid depletion that normally follows a variety of stresses(5,8)

Effect of hypothalamic lesions on response of tryptophan peroxidase to histidine. In normal rats histidine produced an 8-fold increase in activity of the enzyme (Table II). In contrast, rats with hypothalamic lesions were significantly less responsive ($P<.01$) as histidine caused only a 4-fold increase in enzyme activity. Preliminary experiments with hypophysectomized rats (Table II) indicate that they respond in the same way as rats with lesions, showing a significant increase in enzyme activity after histidine ($P<.05$), but a response markedly less than that of normals ($P<.01$) and indistinguishable from that of rats with hypothalamic lesions.

After hypophysectomy or hypothalamic lesions the "resting" activity of the enzyme in uninjected rats was slightly elevated above normal (Table II).

Discussion. Our results indicate that histidine does produce a mild stimulation of the pituitary-adrenal system, and also demonstrate that when this ACTH release is blocked, either by hypothalamic lesions, or by hypophysectomy, the response of tryptophan peroxidase to histidine is reduced by about 50%. To our knowledge, this is the first demonstration that hypothalamic lesions interrupting ACTH release can alter enzyme activity in the body. In other words, those alterations in enzyme concentrations which can be induced by changes in adrenocortical function

TABLE II. Effect of Histidine on Activity of Tryptophan Peroxidase in Livers of Normal Rats, Hypophysectomized Rats and Those with Hypothalamic Lesions.*

Normal rats		Rats with hypothalamic lesions		Hypophysectomized rats	
Control	After histidine	Control	After histidine	Control	After histidine
1.1	5.8	1.3	7.3	1.5	2.5
.7	6.3	1.6	6.0	2.4	3.8
1.0	10.6	.9	3.4	2.7	5.7
.9	7.8	1.1	3.7		5.4
.9	8.3	1.6	6.1		
1.4	6.3	1.0	8.2		
1.2	5.3	1.8	4.8		
2.0	6.3	2.2	3.5		
1.5	11.9	2.1	5.2		
1.1	10.8	1.3	4.1		
1.0	11.0	2.3	7.8		
2.0	6.0	2.1	6.8		
1.4	10.5				
1.0	13.9				
1.2	12.6				
1.2	10.9				
1.1	13.3				
† 1.2 ±.03	9.3 ±.7	1.6 ±.1	5.6 ±.5	2.2 ±.4	4.4 ±.8

* All results are expressed as μmoles of kynurenine/g liver (dry wt)/hr.
† Mean ± stand. error of mean.

are controlled ultimately by the hypothalamus. The results cast no light on the mechanism whereby these alterations in apparent enzyme activity occur(1,9).

The residual response to histidine found in both hypophysectomized animals and those with lesions, is of interest since it stands in contrast to the complete block of response found by Knox in adrenalectomized rats(1). This response in our experiments appears to be similar to augmented activity of the enzyme produced by histidine in cortisone-treated adrenalectomized rats(1). It thus appears that histidine can still produce an elevation in tryptophan peroxidase activity even in the presence of a constant output of cortical steroids whereas no response occurs if steroid output is zero. The role of corticoids in response to histidine, appears to fall into the category of responses which Ingle has called the permissive action of cortical steroids(10). Presumably in our experiments, the residual response to histidine must be brought about either by another regulatory system in the body, such as the sympatho-adrenomedullary system, or by a direct effect of histidine on the liver cell. Because of the multiple agents which can bring about increase in tryptophan peroxidase, this latter possibility appears to be unlikely. Another possible explanation for residual response to histidine would be that degradation of cortical steroids might be decreased under these circumstances, thereby producing a rise in blood level of steroid in the presence of a constant output of steroids as suggested by Steenberg and Ganong(11). The elevated level of steroid could then bring about the enzyme response.

Conclusions. 1. Histidine produces a small decrease in adrenal ascorbic acid concentration in normal rats, but not in rats with hypothalamic lesions interrupting the supra-optico-hypophysial tract. 2. Hypothalamic lesions or hypophysectomy produce a decrease in the response of tryptophan peroxidase to histidine but fail to block the response completely.

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A Screening Test for Malabsorption Syndromes. (24707)

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Progress in understanding of malabsorption syndromes has been hampered by lack of simple methods of diagnosis. The I^{131} labeled fat absorption test has become a valuable tool in diagnosis of these conditions but it does not fulfill all requirements of a simple reliable screening test. Plasma becomes turbid after fatty meal primarily because of increase in neutral fat or triglyceride(1). Osmon, Zinn and Whorton(2) and Turner(3) have shown that plasma lactescence fails to develop after fat ingestion in patients with chronic pancrea-

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