Brain Histamine: Rapid Apparent Turnover Altered by Restraint and Cold Stress

Abstract. *Histamine content of rat brain was lowered quickly by inhibitors of histidine decarboxylase, suggesting that a portion of brain histamine turns over rapidly. Restraint and exposure to cold also reduced brain histamine levels and markedly augmented its formation in the hypothalamus.*

Histamine is distributed unevenly in the brains of most mammals, with highest concentrations in the hypothalamus (*1*). Brain histamine appears to be localized in nerve terminals, since when brain tissue is homogenized in isotonic sucrose, much of the histamine is confined to "pinched-off nerve endings" or synaptosomes (*2*).

Difficulties in the chemical estimation of brain histamine have limited research in this area. Contaminants such as spermidine interfere with the fluorometric assay for histamine in brain tissue (*3*), and purification procedures to remove these impurities are somewhat tedious (*4*). Recently we have increased the sensitivity of an enzymatic-isotopic method for measuring tissue histamine so that it can efficiently detect the low levels of histamine in mammalian brain (*5*). Here we report that inhibitors of histidine decarboxylase, the histamine synthesizing enzyme, rapidly lower brain levels of histamine. Immobilization and cold exposure also lower histamine concentration and markedly enhance the formation of radio-labeled histamine from administered radio-labeled histidine.

Sprague-Dawley male rats (150 to 200 g; Huntingdon Farms, Inc.) were killed by immersion in liquid nitrogen, a procedure which results in more consistent and higher levels of brain histamine than decapitation at room temperature (*6*). Brain regions were rapidly dissected (*7*) and homogenized in 0.05*M* Na-K phosphate buffer, *p*H 7.8. The homogenates were boiled, and centrifuged at 4°C at 20,000*g* for 10 minutes, and an aliquot of the supernatant fluid was assayed for endogenous histamine by a modification (*5*) of the enzymatic-isotopic method (*8*), which can reliably detect as little as 0.2 ng of tissue histamine. Rats received intraventricular injections of L-[^{3}H]histidine (21 curie/mmole; New England Nuclear) purified by column and paper chromatography to remove trace contaminants of [^{3}H]histamine, and were killed at varying time intervals by immersion in liquid nitrogen and decapitation. The hypothalamus was rapidly dissected and homogenized in 0.4*N* perchloric acid. [^{3}H]Histidine and [^{3}H]histamine were separated and isolated by chromatography on Dowex-50W (*6*). Endogenous histidine concentration was measured by converting histidine to histamine with a bacterial histidine decarboxylase preparation and measuring the histamine formed by the enzymatic-isotopic method (*5, 6*).

Histamine in animal tissues can be formed by aromatic amino acid decarboxylase (*9*), whose physiological role presumably relates primarily to the synthesis of 5-hydroxytryptamine and dopamine, or by a "specific" histidine decarboxylase which probably is responsible for the physiological synthesis of tissue histamine (*10*). α-Hydrazino-histidine and 4-bromo-3-hydroxybenzyloxyamine (NSD-1055) are potent inhibitors of the specific histidine decarboxylase and can lower histamine concentrations in peripheral tissues, apparently by inhibiting histamine synthesis (*10*). To determine if these drugs might affect histamine synthesis in the brain, we injected rats with intraperitoneal doses (200 mg/kg) of α-hydrazino-histidine, NSD-1055, or α-methyl-DOPA, a drug which inhibits aromatic amino acid decarboxylase but not the specific histidine decarboxylase (*10*). Thirty minutes after being injected, rats received intraventricular injections of 20 μc (1 nmole) of [^{3}H]histidine and were killed 1 hour later; their hypothalami were assayed for [^{3}H]histidine, [^{3}H]-histamine, and endogenous histidine. Both NSD-1055 and α-hydrazino-histidine inhibited the formation of [^{3}H]-histamine in the hypothalamus by more than 70 percent, while α-methyl-DOPA was without effect. None of the drugs altered endogenous levels of hypothalamic histidine, indicating that the decreased histamine formation could not have resulted simply from changes in the pool size of its precursor.

Other experiments showed that inhibitors of histamine synthesis can lower brain levels of histamine. Rats received intraperitoneal doses of α-hydrazino-histidine, NSD-1055, or α-methyl-DOPA, ranging from 100 to 500 mg/kg, were killed after 3 hours, and their hypothalami were assayed

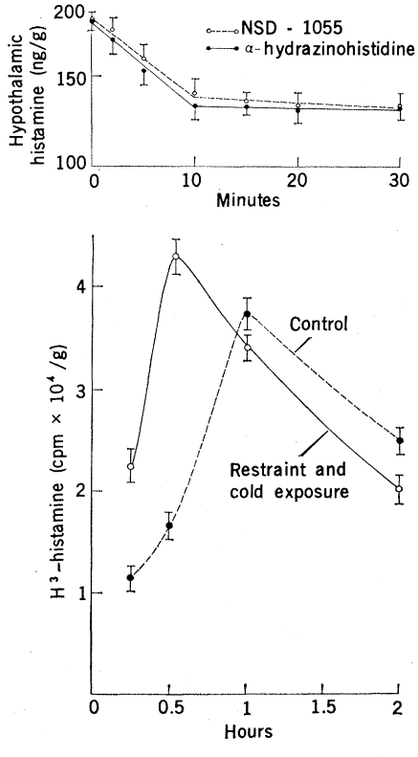

Fig. 1. (Top) Time course of the depletion of histamine in the rat hypothalamus by α-hydrazino-histidine (200 mg/kg, intraperitoneally) and NSD-1055 (200 mg/kg intraperitoneally). Each value is the mean ± S.E.M. of six determinations. Endogenous histamine was determined by a more sensitive modification (*5*) of the method of Snyder *et al.* (*8*). Tissue samples were homogenized in 10 volumes of 0.05*M* sodium phosphate buffer *p*H 7.9. (Bottom) Time course of the effect of cold and restraint stress on the formation of [^{3}H]histamine from [^{3}H]histidine in the rat hypothalamus. Rats were placed in plastic restraint boxes in a cold room (4°C) for 30 minutes before receiving an intraventricular injection of 20 μc (1 nmole) of [^{3}H]histidine. They were then returned to the restraint boxes in the cold room and killed 15 to 120 minutes later. [^{3}H]Histamine, [^{3}H]histidine, endogenous histamine, and endogenous histidine were determined in the hypothalamus (*6*). In control and stressed groups at all time intervals there was no difference in the concentration of endogenous histidine and [^{3}H]histidine in the hypothalamus. Each group consisted of eight rats.

Table 1. The effect of cold exposure and restraint on the concentration of histamine in different regions of the rat brain. Rats were restrained in plastic restraint boxes (*14*) or were placed in individual cages in a cold room maintained at 4°C, or were maintained at 4°C in restraint boxes. Each value is the mean ± S.E.M. of six determinations.

Condition	Histamine (ng/g)			
	Hypothalamus	Thalamus-midbrain	Cerebral cortex	Medulla oblongata–pons
Control	182 ± 15	61 ± 7	59 ± 6	22 ± 5
Restraint				
½ hour	160 ± 16	63 ± 9	58 ± 9	19 ± 6
1 hour	137 ± 12*	58 ± 8	48 ± 7	20 ± 5
2 hour	120 ± 11†	48 ± 8	37 ± 6*	19 ± 4
Cold				
½ hour	149 ± 16	60 ± 9	55 ± 8	22 ± 4
1 hour	129 ± 16*	51 ± 8	39 ± 5*	24 ± 6
2 hour	114 ± 14†	42 ± 8	35 ± 5*	19 ± 4
Restraint and cold				
½ hour	141 ± 11*	55 ± 8	49 ± 8	22 ± 5
1 hour	119 ± 10†	44 ± 6	38 ± 4*	24 ± 5
2 hour	117 ± 12†	42 ± 6*	36 ± 5*	20 ± 4

* Difference between control and stressed groups significant at $P < .05$. † Difference between control and stressed groups significant at $P < .01$.

for histamine. α-Hydrazino-histidine and NSD-1055 at doses of 200 mg/kg lowered histamine values by 35 to 40 percent, although doses of 100 mg/kg were without significant effect. There was no greater depletion of hypothalamic histamine with doses above 200 mg/kg. α-Methyl-DOPA failed to affect histamine levels at any dose. Thus it appears that histamine is synthesized in rat brain, at least in part, by a specific histidine decarboxylase which can be inhibited by NSD-1055 and α-hydrazino-histidine. These drugs, but not α-methyl-DOPA, can deplete a portion of brain histamine, although there appears to be a "drug-resistant" pool of brain histamine. The failure of the drugs to inhibit histamine formation completely may be partially responsible for their inability to deplete brain histamine more than 40 percent.

The time course of histamine depletion by α-hydrazino-histidine and NSD-1055 in six brain regions was assessed by killing rats at intervals from 2 minutes to 48 hours after drug administration. In the hypothalamus, histamine levels were lowered somewhat as soon as 2 minutes after drug injection, were significantly decreased at 5 minutes, and were maximally depleted at 15 minutes (Fig. 1, top). Hypothalamic histamine remained reduced about 40 percent 4, 8, 16, and 24 hours after drug injection and returned to control levels 48 hours after drug administration. The half-life for the decline of the depletable pool of hypothalamic histamine, with either NSD-1055 or α-hydrazino-histidine, was about 5 minutes. Histamine levels in the thalamus-midbrain and cerebral cortex were reduced to a similar extent and with the same time course as in the hypothalamus, while in the medulla oblongata–pons, cerebellum, and corpus striatum, histamine concentration was unaffected by these drugs (*6*). Assuming that the partial depletion of brain histamine by these drugs is related to inhibition of histamine synthesis, the rate of decline may reflect the turnover rate of brain histamine. If so, our findings suggest that a pool of brain histamine perhaps comprising about 40 percent of brain levels, turns over much more rapidly than brain norepinephrine and serotonin (*11*) but at a rate resembling the turnover rate of brain acetylcholine (*12*). Recently Menon *et al.* (*13*) reported that *p*-chlorophenylalanine and 4-thiazolylmethoxyamine markedly lowered rat brain histamine. In preliminary experiments we found that 4-thiazolylmethoxyamine depleted rat brain histamine to the same extent as NSD-1055 and α-hydrazino-histidine, while *p*-chlorophenylalanine was without effect.

To evaluate the influence of stressful procedures on levels and synthesis of brain histamine, rats were immobilized in plastic restraint boxes or placed in individual cages in a cold room at 4°C, or were maintained in the restraint boxes at 4°C, thus being subjected to both cold exposure and restraint (*14*). Thirty minutes of treatment by these procedures failed to alter histamine concentration in any brain regions examined. However, after 1 or 2 hours of cold exposure, restraint, or a combination of the two, histamine concentrations in the hypothalamus, thalamus-midbrain, and cerebral cortex were lowered (Table 1), although levels in the medulla-pons were unaffected. In preliminary experiments, we found that histamine concentration in the cerebellum and corpus striatum also was unaltered by restraint or exposure to cold. In the hypothalamus, thalamus-midbrain, and cerebral cortex, the maximal depletion of histamine was about 35 to 40 percent, similar to the maximal lowering of histamine in these areas by the histamine synthesis inhibitors. Interestingly, the brain regions whose histamine concentration was unaffected by cold or restraint were the same areas in which histamine levels were not lowered by NSD-1055 or α-hydrazino-histidine.

The role that histamine turnover might play in the partial depletion of brain histamine by restraint and cold exposure was studied by measuring the formation in vivo of hypothalamic histamine. Rats were subjected to restraint and cold exposure for 30 minutes, removed from the cold room for 5 minutes to receive an intraventricular injection of [^{3}H]histidine, and then returned to the cold room for periods varying from 15 minutes to 2 hours. The animals were then killed by immersion in liquid nitrogen, and their hypothalami were rapidly removed and assayed for [^{3}H]histidine, [^{3}H]histamine, and endogenous histidine. In control animals, allowed to move about freely in individual cages at room temperature, there was a rapid increase in [^{3}H]histamine levels with peak values at 1 hour, which fell about 30 percent in the second hour (Fig. 1, bottom). Restraint and cold exposure markedly increased the initial formation of [^{3}H]-histamine. At the earliest time interval examined, 15 minutes after [^{3}H]histidine administration and 45 minutes after the beginning of restraint and cold exposure, hypothalamic [^{3}H]histamine concentration was double control values, and by 30 minutes after [^{3}H]histidine administration it was almost triple control levels. One and 2 hours after injection of [^{3}H]histidine, [^{3}H]histamine levels in restrained and cold-exposed animals were slightly lower than those of control animals. [^{3}H]Histidine and endogenous histidine levels in hypothalamus, thalamus-midbrain, cerebral cortex, and medulla oblongata–pons of rats subjected to restraint and cold exposure for 15, 30, 60, or 120 minutes did not differ from those of control animals, indicating

that changes in the formation of [^{3}H]-histamine were not related to alterations in the size of the pools of histidine available for histamine formation. The absence of changes in [^{3}H]histidine levels accompanying the enhanced [^{3}H]histamine formation probably relates to the conversion of only a small proportion of histidine to histamine.

In control animals, maximal formation of histamine from histidine occurred in the first hour after [^{3}H]histidine administration, after which [^{3}H]-histamine levels began to decrease, presumably because at this time disappearance of synthesized [^{3}H]histamine exceeded its rate of formation. In animals subjected to restraint and cold exposure, there was a more rapid initial formation of histamine from histidine, [^{3}H]histamine values peaked earlier, and the subsequent disappearance of the radio-labeled amine appeared to be more rapid. Thus in animals subjected to restraint and cold exposure, the depletion of hypothalamic histamine was associated with a marked enhancement of its synthesis. This suggests that these stresses lowered brain histamine levels by releasing the amine at a rapid rate, and an apparent compensatory enhancement of histamine synthesis could not keep pace with the rate of amine release so that endogenous histamine levels were partially depleted.

The rapid changes in brain histamine levels elicited by restraint or cold exposure suggest that its stores are quite labile. Accordingly, we examined the effects of a number of endocrine manipulations and drugs on histamine levels. One week after hypophysectomy, ovariectomy, thyroidectomy, castration, or adrenal demedullation, hypothalamic levels of histamine did not differ from those of sham-operated controls. Moreover, in all of these conditions there was no change in the extent of depletion of hypothalamic histamine by NSD-1055, nor was there any change in the rate of conversion of intraventricularly injected [^{3}H]histidine to [^{3}H]-histamine. Reserpine in doses from 2.5 to 10 mg/kg at intervals from 1 hour to 24 hours failed to alter hypothalamic histamine levels, although other workers (*15*) have found that in the cat reserpine can partially deplete brain histamine.

Chlorpromazine (10 mg/kg) and quinacrine (10 to 100 mg/kg), drugs which inhibit the histamine methyltransferase (*16*), failed to alter hypothalamic histamine levels 1 to 4 hours after administration. Pargyline (50 mg/kg), iproniazid (150 mg/kg), and tranylcypromine (25 mg/kg), inhibitors of monoamine oxidase, also failed to alter hypothalamic histamine levels 2 or 24 hours after drug administration. Adam and Hye (*15*) reported that in the cat chlorpromazine and inhibitors of monoamine oxidase produced modest elevations of brain histamine.

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Cardiac Rate Regulated by Nutritional Factor in Young Rats

Abstract. *Milk fed by stomach tube to 2-week-old rats separated from their mothers without feeding for 16 hours transiently but fully reversed the decrease in cardiac rate which had occurred during separation. This effect was rapid in onset and was related to dosage; it was not dependent upon gastric distention, but did depend upon β-adrenergic transmission.*

The long-range effects of variations in nutrition depend upon the stage of development at which they occur (*1*). Although cardiovascular disease has long been thought to have important nutritional determinants, there have been relatively few studies dealing with early developmental stages and even fewer which employ physiological measures.

In a previous study, 2-week-old rats progressively decreased cardiac and respiratory rates during the first 12 to 16 hours after they were separated from their mothers (*2*). This decrease was independent of changes in body temperature, removal from the home nest, or alteration of litter size. Studies with autonomic blocking agents suggested that the decrease in heart rate was primarily due to decreased sympathetic tone, augmented by some vagal restraint. Milk supplied by gastric intubation at the rate of 0.8 ml every 4 hours failed to alter the rapidity or extent of the decrease in heart rates, although the pups gained 4 percent in body weight in 24 hours. Because experiments in which rat pups were supplied with nonlactating foster mothers also failed to prevent progressive cardiac slowing (*3*), the nutritional aspects of maternal deprivation were further studied. These experiments explore the cardiac rates of rat pups separated from their mothers and fed different quantities of milk. Other pups were given nonnutritive fluid to control for the effects of gastric distention. A second experiment assessed