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Nuclear Localization of Histamine in Neonatal Rat Brain

Abstract. *The concentration of histamine in the brains of neonatal rats is considerably higher than that in adults. Subcellular fractionation studies revealed that about 90 percent of the histamine content of neonatal rat brain is confined to the crude nuclear fraction obtained by differential fractionation. Purified nuclei prepared from these fractions retained 90 percent of their histamine content. The nuclear localization of histamine in the brains of neonatal rats suggests a function for histamine in modulating the growth processes of the neonatal brain.*

Histamine appears to have various functions in different parts of the body. In many peripheral tissues most of the histamine is localized in mast cells where it presumably is involved in allergic and inflammatory processes (*1*). In the stomach of several species histamine is localized in unique chromaffin-like cells where it may function in the secretion of gastric acid (*2*). In the adult mammalian brain subcellular fractionation indicates that histamine is localized in particulate fractions enriched in pinched-off nerve endings ("synaptosomes") and may therefore have a synaptic function (*3*).

Histamine synthesis is greatly enhanced in some rapidly growing tissues, especially fetal rat liver, and it has been suggested that histamine may have an important function in certain cases of rapid tissue growth (*4*). However, in some rapidly growing tissues, such as regenerating rat liver, there does not appear to be an enhancement of the activity of histidine decarboxylase, the histamine synthesizing enzyme, although polyamine synthesis is enhanced (*5*).

In the neonatal rat brain amounts of histamine are more than five times higher than they are in the adult, and are correlated well with periods of neuronal growth (*6*). Knowledge of the intracellular localization of a chemical often helps explicate its function. Accordingly, we have examined the subcellular localization of histamine in the neonatal rat brain. We report here that in the neonatal rat brain histamine is almost wholly localized within the nuclear fraction.

Sprague-Dawley rats (*7*) were obtained at 15 days' gestation and kept in individual cages. After birth neonatal rats were maintained with their mothers until they were killed by decapitation. Brains were rapidly removed and homogenized, and crude nuclear, mitochondrial, microsomal, and supernatant fractions were obtained by differential centrifugation. In some experiments purified nuclei were prepared by sucrose gradient sedimentation according to the method of Blobel and Potter (*8*) modified so that the most dense layer contained 1.8*M* sucrose. Histamine was measured by a modification of the enzymatic-isotopic procedure of Snyder *et al.* (*9*) in which the sensitivity of the procedure is enhanced so that as little as 0.2 ng of tissue histamine can be reliably estimated (*10*).

In initial experiments histamine was measured in the telencephalon, diencephalon, and rhombencephalon of rats at six ages from 1 hour to 17 days after birth. In the telencephalon and diencephalon, the time course of changes in histamine content was closely similar to that reported by others (*6*, *11*). Histamine concentrations, expressed per unit of wet brain weight, were about 200 ng/g at birth, with a maximum of about 250 to 300 ng/g at 5 to 10 days; the amount then gradually declined by 17 days to about 50 ng/g, the same as that amount found in adult rat brain. In the rhombencephalon fluctuations in histamine concentrations were not as marked. From birth to 10 days, the concentration in the rhombencephalon was about 100 ng/g, which gradually declined by 17 days to adult levels of about 50 ng/g. These findings may relate to well-known patterns of growth in different parts of the brain. Brain maturation proceeds in a caudocephalic direction so that the rhombencephalon may have already undergone in fetal life rapid growth processes which in the telencephalon and diencephalon take place during the neonatal period.

In subcellular fractionation studies in which differential centrifugation is used, we found in the diencephalon

Table 1. Change in the subcellular localization of histamine during development of the rat diencephalon. Rats were decapitated at various ages, and brains were dissected into telencephalon, diencephalon, and rhombencephalon. The diencephalon regions from rats at various ages were homogenized with a Teflon pestle in 15 volumes of ice-cold 0.32*M* sucrose. Differential centrifugation was employed according to the following scheme. The P_1 pellet was obtained by centrifuging the homogenate for 10 minutes at 1000*g*. The resulting supernatant fluid was centrifuged for 35 minutes at 18,000*g* to obtain the P_2 pellet. The supernatant fluid from the P_2 fraction was centrifuged for 1 hour at 100,000*g* to obtain the P_3 pellet and a soluble supernatant fraction (S). Recovery values obtained when exogenous histamine was added to subcellular fractions ranged from 90 to 100 percent. The sum of the histamine content of the four subcellular fractions was 90 to 99 percent of the total histamine content of the appropriate homogenate. Data presented are the mean values obtained from four different experiments whose results varied less than 20 percent. The reaction product of the histamine assay in P_1 pellets of 3-, 7-, and 21-day-old rats had the same R_F value as 1,4-methylhistamine in three paper chromatographic systems.

Age (days)	Histamine (ng/g of tissue)					Total histamine content of tissue (%)			
	P_1	P_2	P_3	S	Total	P_1	P_2	P_3	S
3	129	12	0	0	141	91	9	0	0
5	188	7	2	3	200	94	4	1	1
7	210	23	2	2	237	88	10	1	1
13	139	16	0	7	162	87	10	0	3
21	14	18	2	26	60	23	30	3	44

Table 2. The differential retention of histamine and DNA within purified nuclei from neonatal rat brain. Whole brains of 10 to 12 6-day-old littermates were homogenized at 0° to 4°C in five volumes of 0.32*M* sucrose in TKM (50 m*M* tris-HCl, *p*H 7.5 at 25°C; 5 m*M* $MgCl_2$; 25 m*M* KCl) buffer with a Teflon pestle and centrifuged for 10 minutes at 1000*g* to obtain the P_1 pellet. The pellets were resuspended with a Dounce homogenizer in 1.5*M* sucrose in TKM buffer in the same volume as the original homogenate, and 5-ml portions were transferred into Corex tubes. A 10-ml syringe was used to underlay this suspension with about 7 ml of 1.8*M* sucrose in TKM. The tubes were centrifuged for 1 hour at 40,000*g*; the supernatant fluid was decanted, and the sides of the tubes were wiped with gauze. The pellets were resuspended by rapid vibration for 10 seconds in 2 ml of isotonic TKM buffer or the same buffer to which heparin (0.2 mg/ml) had been added. The suspension was maintained at 4°C for 15 minutes and then was centrifuged at 1000*g* for 10 minutes. The pellet and supernatant fluid were assayed for DNA (*17*) and histamine (*10*). When [^{3}H]histamine was added before homogenization in these experiments, 99 percent of the counts per minute were recovered in the 40,000*g* supernatant. In these experiments, the total amounts of DNA and histamine recovered from the purified nuclei represented about half of the values for the homogenate. Data presented are the means of five different experiments in which values varied less than 15 percent.

Treatment	Histamine (%)		DNA (%)	
	Nucleus	Supernatant fluid	Nucleus	Supernatant fluid
Purification of P_1 (*8*)	91	9	100	0
Resuspension, wash (TKM)	18	82	100	0
Resuspension, heparin	21	79	51	49

that at ages 3 to 13 days, about 90 percent of brain histamine was confined to the crude nuclear fraction (Table 1). In other experiments, the neonatal telencephalon and rhombencephalon showed the same subcellular distribution as the diencephalon. At 21 days, the distribution pattern resembled that in adult rat brain (*3*), with almost half of the histamine in the supernatant fluid and the remainder distributed between the crude nuclear and crude mitochondrial fractions.

To assess whether the apparent localization of neonatal brain histamine in the crude nuclear fraction could have been an artifact resulting from the redistribution of histamine during homogenization, we performed the following experiment. The diencephalons, telencephalons, or rhombencephalons of 7-day-old rats were homogenized together with a trace amount (0.4 ng) of [^{3}H]-histamine (specific activity 4.0 c/mmole) before differential centrifugation. In these experiments, although 90 percent of the endogenous histamine was localized in the crude nuclear fraction, 90 percent of the [^{3}H]histamine was confined to the supernatant fluid. This result seems to rule out the possibility that artifacts of redistribution could have accounted for the localization of endogenous histamine in the crude nuclear fraction.

Crude nuclear fractions prepared by differential centrifugation may be contaminated by tissue constituents other than nuclei. It is possible to prepare, by sucrose gradient fractionation, intact purified nuclear preparations which, as judged by microscopic criteria, contain only nuclei (*8*). Such purified nuclei can be prepared from a variety of tissues including liver and brain (*12*). When crude nuclear fractions were subjected to sucrose gradient centrifugation according to the technique of Blobel and Potter (*8*), 90 percent of the histamine content of the crude nuclear pellet was retained in the purified nuclear preparation as was all the DNA (Table 2). Light-microscopic examination of these purified nuclei showed that they contained only nuclei and were free of contamination with unbroken cells (*13*) or with mast cell granules, which store histamine in many tissues and sediment with the crude nuclear fraction (*14*).

To determine if histamine and DNA were stored in a similar fashion, we examined the retention of histamine and DNA within purified nuclei after they were washed with an isotonic medium with or without heparin (Table 2). Washing with the heparin-free buffer solution released 80 percent of the nuclear histamine into the supernatant fluid, whereas all of the DNA remained within the nuclear fraction. Heparin causes swelling of nuclei from a variety of tissues and a partial release of DNA (*15*). Washing with the buffer medium containing heparin did not release any more histamine from the nuclei than did washing with the buffer solution alone. However, treatment with heparin caused leakage of 50 percent of the nuclear DNA into the supernatant fluid. The differential response of nuclear histamine and DNA to these treatments suggests that histamine and DNA are not stored in the same way. It is not clear why histamine in nuclear fractions remains stored throughout the procedures of differential centrifugation and sucrose gradient fractionation, yet can be washed out of the purified nuclear preparation.

In preliminary experiments the subcellular localization of histamine in the fetal rat liver at 15 days' gestation, the time at which maximum histidine decarboxylase activity occurs in the liver, was examined by differential centrifugation. No more than 10 percent of the fetal liver histamine was localized in the crude nuclear fraction. Whether histamine in the fetal rat liver is not stored in the liver nuclei or whether it leaks out during the subcellular fractionation procedure cannot be determined from these experiments.

In rapidly growing tissues biochemical changes take place in all parts of the cell. If the elevated amounts of histamine in the neonatal rat brain are related to growth processes in this tissue, our results suggest a role for neonatal brain histamine in nuclear function. Histamine is a very basic compound. The polyamines, also highly basic, have been proposed as regulators of nucleic acid function in rapidly growing tissues (*16*), conceivably displacing histones from their binding sites on DNA. It is possible that in the neonatal rat brain histamine plays a role analogous to that proposed for the polyamines.

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Acetoxycycloheximide Enhances Audiogenic Seizures in DBA/2J Mice

Abstract. *Inborn errors of metabolism often cause epilepsy, as with certain strains of mice. Aggravating the metabolic defect with a protein synthesis inhibitor increases the symptoms. Mature animals that have "outgrown" their genetic susceptibility to audiogenic seizures are made susceptible again by acetoxycycloheximide. After a single small dose the incidence and severity of audiogenic seizures increases at 16 hours, reaches a maximum of 40 hours, and then declines gradually.*

Young mice of certain strains can be made to convulse by exposure to loud sound. Details of age-specificity in different strains have been reported (*1*, *2*). In different laboratories in different years, the incidence and severity of convulsions in DBA/2J mice have been different, but all agree that these animals "outgrow" this attribute. Attainment of anatomical and electroencephalographic maturity of cerebral cortex (*3*) coincides with the beginning of sensitivity to audiogenic seizures. Attainment of sexual maturity approximately coincides with decline in sensitivity.

Concerning the seizure susceptibility and the age-specificity, several authorities suggest that deficiency of phenylalanine hydroxylase predisposes mice to deficiency of a neurotransmitter (*4–7*). This has not been proven. Abnormal amounts of adenosine triphosphate (ATP) and adenosine triphosphatase (*6*, *8*) and low tolerance to anoxia (*1*) have been demonstrated in DBA mice, suggesting that metabolism of high energy phosphate may have a direct role in susceptibility to seizure.

Acetoxycycloheximide is a potent inhibitor of protein synthesis that interferes with the incorporation of labeled valine into protein in the mouse brain (*9*). We studied the effect of acetoxycycloheximide on audiogenic seizures in DBA/2J mice with two sets of experiments. One deals with the rates of age-specific attack and of mortality; the other deals with the time course of the drug effect in animals 40 days of age. We have extended the age of susceptibility to audiogenic seizures, have increased the severity of seizures, and have demonstrated that the time course of this effect is similar to that of other behavorial effects of inhibition of protein synthesis is by this drug.

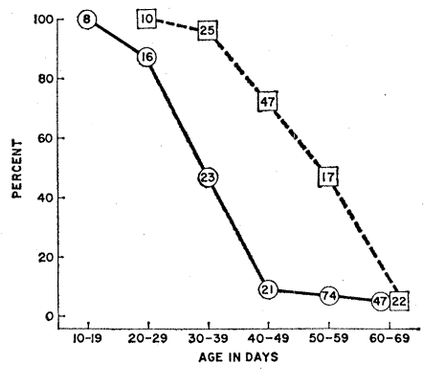

Fig. 1. Incidence of audiogenic seizure from age 17 to 69 days. Numerals in squares represent number of animals receiving acetoxycycloheximide (4 μg per gram of body weight) by injection 24 hours before testing. Numerals in circles represent number of animals receiving saline injection.

The DBA/2J mice were housed in groups on pine sawdust or cedar shavings and were fed Purina mouse chow and tap water. Only animals direct from the supplier (*10*) were used in the time course experiment. Animals direct from the supplier and offspring of unselected matings of original and first generation animals in our laboratory were used in the experiment on the relation of age to incidence.

An injection of sodium chloride solution (Cutter; U.S.P.) was used as control and diluent. The drug solution (*11*) was 400 μg of acetoxycycloheximide per milliliter of diluent. Both fluids were refrigerated until used. Each animal was caught by the tail, was assigned to drug or control group without regard to its ability to avoid capture, was weighed, and was given a dorsal subcutaneous injection (0.01 ml of solution per gram of body weight).

All animals were tested in a clear plastic cage (20 by 20 by 30 cm) with pine sawdust on the bottom and an electric doorbell under the top. The stimulus intensity was 112 db above 0.0002 dyne/cm^2 within the cage. Stimulus duration was either 1 minute or until tonic convulsion, whichever was first. Animals were observed for the several stages of audiogenic activation: stun, wild running and leaping, clonic convulsion, and death—but only clonic convulsion or death was used as criterion in this report.

In the age-specific incidence experiment, animals were tested in groups of one to four, 24 hours after injection, without regard to time of day. In the time course experiment, animals were tested singly, and all tests were done between 8 a.m. and 12 noon.

Because we were interested in the decline of sensitivity with age rather than the precise age of onset, we have few animals under 20 days of age. The susceptibility to clonic convulsion in mice, grouped by age in 10-day intervals, declines with age (Fig. 1). A parallel decline in susceptibility is seen in mide, but the curve is shifted to the animals treated with acetoxycycloheximide right by two 10-day intervals.

Figure 2 shows the incidence of death from convulsion among all animals stimulated, grouped by age in 10-day intervals. The incidence of death from convulsion in the control animals falls faster than the incidence of con-