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THE REGIONAL DISTRIBUTION OF HISTAMINE IN BRAIN OF THE RHESUS MONKEY (*MACACA MULATTA*)*

I. ARTHUR MICHAELSON,† PETREA Z. COFFMAN and DONALD F. VEDRAL

Departments of Environmental Health and Pharmacology and Department of Anatomy, College of Medicine, University of Cincinnati, Cincinnati, Ohio, U.S.A.

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Abstract—The concentration of histamine and spermidine in fifteen different regions of rhesus monkey (*Macaca mulatta*) brain were studied using the *O*-phthaldialdehyde fluorometric method. The highest levels for histamine were found in the hypothalamus. Spermidine was found in all the regions studied and, unlike histamine, lacked significant regional localization within the central nervous system.

DESPITE numerous papers and a renaissance[1–27] in the interest in histamine in brain (see review by Green[11]), current understanding of the function of this bioactive amine in normal and pathological physiology of the central nervous system is still obscure. One school of thought maintains that the solution of these fundamental problems will be achieved by studies on the rate of formation of histamine and its metabolism, and that the use of drugs and diet capable of interfering with the biosynthesis, storage and catabolism of histamine is more promising than the static measurements of the histamine content of tissues.[28] Others[11] have suggested that the study of the effect of exogenously administered histamine on the nervous system may tell whether histamine has a function in the central nervous system. The limitations of this approach have been discussed by Green.[11] In recent years there has developed a principle, which is generally accepted without proof, that the uneven distribution of a pharmacologically active substance in the brain implies that the agent plays a role in the function of those regions in which its concentration is highest.[29]

Adam[1] has shown that the regional distribution of histamine in the central nervous system in the dog[1] and cat[3, 4] is similar to that of norepinephrine[30] and serotonin.[31] In Adam's studies,[1–4] the midbrain and particularly the hypothalamus contained high concentration of histamine relative to the other areas of the brain. Furthermore, it has been shown[1, 17, 19] that the histamine there is not mast-cell histamine.

The object of the present study was to obtain values for the concentration of histamine extractable from different parts of the monkey brain (*Macaca mulatta*) and to see how its distribution compared to that in the brain of dog[1, 16] and cat.[4, 16] We also studied the concentration of protein, ribonucleic and deoxyribonucleic acids as well as that of the polyamine, spermidine.

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MATERIAL AND METHODS

The intermediate phosphonic acid cation-exchange resin (Duolite ES-63) was a gift from the Diamond Alkali Company, Redwood City, Calif. *o*-Phthaldialdehyde (Mann Research Laboratories, New York, N.Y. was purified by recrystallization from ligroin (b.p. 30–60°). Bovine serum albumin (BSA), yeast ribonucleic acid (RNA) and calf thymus deoxyribonucleic acid (DNA)-type V were obtained from the Sigma Chemical Co., St. Louis, Mo. Diphenylamine was purified by recrystallization from ethanol, whereas acetaldehyde was purified by steam redistillation. Other liquid reagents such as methanol and glacial acetic acid were redistilled before used.

Preparation of tissues. Three normal untreated male monkeys were killed by cervical disclocation and their peripheral tissues removed for use in experiments unrelated to the present study. The whole brain was quickly removed and placed in crushed ice. Separation of the various regions of the brain was carried out on a cold glass slab. Parts of the brain were removed and placed in tared beakers sitting in ice-chips. For cerebral cortex, cerebellar cortex and corpus callosum, one monkey brain was sufficient for analysis, whereas all other areas were pooled tissues from 3 monkey brains. The tissues were weighed and homogenized in cold 0·32 M sucrose (about 1:10, w/v) in an all-glass tissue homogenizer (Kontes, Duall K-88545) and the final volume was recorded. Samples were removed from the total homogenate and handled in the following manner: for protein analysis, 0·5 ml of homogenate was transferred for freezing to tubes sitting in a slurry of dry ice and acetone. For nucleic acid analysis, 1·25 ml of ice-cold 0·5 M perchloric acid ($HClO_4$) was added to 2·5 ml of homogenate and thoroughly mixed; these tubes likewise were quickly frozen. The remaining homogenate was adjusted to 0·4N in terms of perchloric acid, mixed throughly and frozen. All fractions were kept at − 18°.

Histamine and spermidine analysis. Tissue samples extracted with 0·4 N $HClO_4$ were allowed to thaw and were then centrifuged to remove solids. The acid supernatants were neutralized with solid dibasic sodium phosphate, pH 6·1, measured with a glass electrode. The neutralized extract was then applied to ES-63 ion-exchange resin columns (size 6 × 50 mm) previously equilibrated with 0·01 M phosphate buffer, pH 6·1, and treated as described earlier.[13, 14, 32–35] After preliminary washings with 20 ml water and 60 ml of 0·02 N nitric acid (HNO_3) the columns were transferred to an automatic fraction collector (Buchler, Fractomat) and 15 ml of 0·1 N HNO_3 was applied as eluent. One-ml fractions were collected and each was reacted with *o*-phthaldialdehyde for the fluorometric estimation of histamine.[33–36] The columns were then removed from the fraction collector and eluted with 20 ml of 0·2 N HNO_3, the entire effluent being collected into one tube. A 1-ml sample was used for the *o*-phthaldialdehyde fluorometric assay of spermidine.[32–35] A second sample was diluted with an equal volume of 0·2 N HNO_3, and 1 ml was similarly assayed as a check against fluorescence quenching.

Recovery. Varying concentrations of histamine and spermidine were carried through the procedure in order to construct "internal" standard curves. These were compared to histamine and spermidine "external" standard curves. In addition, histamine and spermidine were added to brain homogenates in concentrations like those found in the tissues. Recovery of histamine and spermidine was 85 and 77 per cent respectively.

Protein. The analytical method for measuring protein was based on the Folin phenol reagent method of Lowry *et al.*[37] as modified by Oyama and Eagle.[38] Protein concen-

trations were estimated with reference to a standard curve constructed with bovine serum albumin.

Nucleic acids, RNA and DNA. The procedure used was that recommended by Monroe and Fleck,[39] an adaptation of the Schmidt–Thannhauser[40] method for RNA and of Burton's[41] diphenylamine method for DNA. The 2·5-ml sample previously adjusted to 0·2 N perchloric acid was thawed in the cold and then centrifuged to remove acid-soluble compounds of low molecular weight. The RNA in the pellet was extracted with 0·3 N KOH, DNA and protein were reprecipitated by acidification. Ribonucleic acids were prepared as directed[38] and their u.v. absorption estimated at 260 mμ. Precipitated DNA was extracted with 0·5 N perchloric acid as described by Burton[41] and estimated by reacting with dephenylamine. Estimation of tissue concentrations of RNA and DNA were made with reference to standard curves for RNA and DNA developed from procedures described above.

In control experiments rat liver and brain were homogenized in 0·32 M sucrose (1:10, w/v) and replicate aliquots were taken from both preparations. All were adjusted to 0·2 N $HClO_4$ and polymerized RNA, DNA and protein were precipitated. One pellet was treated with 0·3 N KOH, whereas the other was treated with 0·3 N KOH containing RNA and DNA at a concentration similar to that reported for that tissue[42] and carried through the analytical procedure. Recovery of added RNA and DNA was 98 and 72 per cent respectively. All values for RNA, DNA, histamine and spermidine are corrected for recovery.

RESULTS AND DISCUSSION

In order to see whether histamine or spermidine is associated with any one major chemical constituent of the tissue i.e. protein, RNA and DNA, analyses were performed on these tissue components as described in Methods. Protein (Table 1, column 1) was evenly distributed among the brain areas examined. Its concentration ranged from 99 mg/g in the cerebral peduncles to 130 mg/g in the cerebral cortex, with a mean of 119 mg/g wet wt. The relatively uniform distribution of protein among the 10 regions analyzed is attested to by a percentage deviation of only 6 per cent. The concentration of ribonucleic and deoxyribonucleic acids in those regions of the brain examined in this study is shown in Table 1, columns 2 and 3. The RNA/DNA ratio (Table 1, column 4) ranged from 0·11 in the cuneus to 0·47 in the hypothalamis. These findings are in good agreement with those found by earlier workers[43] and quoted by Davidson.[42] The earlier study[43] reported an RNA/DNA ratio of 0·52 for monkey brain, while in the present study the mean value of RNA/DNA for the 10 areas studied is 0·31 (range, 0·11–0·47).

The distribution of histamine among the 15 brain regions studied is presented in Table 2 and is compared to the distribution of spermidine. The relatively high concentration of histamine within the hypothalamus is clearly distinct from that in other areas studied. The next highest area is the cerebral peduncle which, interestingly enough, is anatomically in close proximity to the hypothalamus. The distribution of spermidine is rather even, lacking any high degree of regional localization within the central nervous system. When applying the present findings to conventional characterizations of histamine in terms of concentration per unit protein, RNA or DNA (Table 1, columns 6–8) or spermidine concentration per unit protein, RNA

Table 1. Histamine and spermidine concentration per unit protein (P), ribonucleic acid (RNA) and deoxyribonucleic aicd (DNA).

Tissue	Protein (P) (mg/g)*	RNA (μg/g)*	DNA (μg/g)*	Ratio RNA/DNA	Histamine (n-moles/g)*	Histamine (n-moles/100 mg P)	Histamine (n-moles/100 μg RNA)	Histamine (n-moles/100 μg DNA)	Spermidine (n-moles/g)*	Spermidine (n-moles/100 mg P)	Spermidine (n-moles/100 mg RNA)	Spermidine (n-moles/100 μg DNA)
Column No.	1	2	3	4	5	6	7	8	9	10	11	12
Hypothalamus	118	414	890	0·47	14·44	12·23	3·49	1·62	405	343	98	45·5
Cerebral peduncles	99	321	760	0·42	3·15	3·18	0·98	0·41	458	463	143	60·4
Colliculi					1·35				420			
Optic nerves	110	364	1090	0·33	1·33	1·21	0·37	0·21	635	577	174	58·2
Pulvinar	121	323	895	0·36	0·94	0·78	0·29	0·11	382	316	118	42·8
Cerebral cortex	124	334	1180	0·28	0·81	0·65	0·24	0·07	164	132	49	13·9
Cuneus	128	428	3980	0·11	0·78	0·61	0·18	0·02	216	169	50	5·4
Lateral geniculate body					0·79				892			
Lingula	127	622	1750	0·35	0·51	0·40	0·08	0·03	217	171	35	12·4
Caudate nucleus	123	257	1030	0·25	0·48	0·38	0·19	0·05	139	113	54	13·3
Corpus callosum	114	407	1210	0·34	0·38	0·33	0·09	0·03	590	517	145	48·6
Cerebellar cortex	130	1509	8530	0·18	<0·15	<0·115	<0·01	<0·002	243	187	16	2·9
Olfactory bulb					<0·15				367			
Olfactory tract					<0·15				463			
Cervical cord					<0·15				727			

* Per g wet wt.

or DNA (Table 1, columns 10–12), the highest specific activity for histamine is in the hypothalamus, whereas spermidine lacks similar regional localization.

In the mammalian species studied,[1, 4, 16] there appears to be a reproducible pattern of histamine concentrations among different areas of the brain. There is also a fairly good correlation between the distribution of histamine and the distribution of histidine decarboxylase, both being localized in the hypothalamus.[44] The major product

TABLE 2. COMPARISON OF THE DISTRIBUTION OF HISTAMINE AND SPERMIDINE IN THE CENTRAL NERVOUS SYSTEM OF RHESUS MONKEY*.

Region	Histamine n-moles/g† = 100%	Spermidine n-moles/g† = 100%
Hypothalamus	14·44	405
Cerebral peduncles	21·8	112
Colliculi	9·4	103
Optic nerve	9·2	157
Pulvinar	6·5	94
Cerebral cortex	5·6	41
Lateral geniculate body	5·5	220
Cuneus	5·4	53
Lingula	3·5	54
Caudate nucleus	3·3	34
Corpus callosum	2·6	145
Cerebellar Cortex	<1·04	60
Olfactory bulb	<1·04	90
Olfactory tract	<1·03	114
Cervical cord	<1·04	180

* Concentrations are expressed as a percentage of the hypothalamic level.
† Nanomoles per g wet wt.

of histamine metabolism is *N*-methylhistamine, and the enzyme imidazole-*N*-methyltransferase has a relatively high degree of localization in the hypothalamus with lower concentrations in the cerebellum and the cerebral cortex.[45] It has already been shown by Adam,[1] and confirmed in the present study, that the hypothalamus is particularly rich in histamine, whereas the cerebellum and cerebral cortex contain little or no histamine. Furthermore, it has been shown[3] that the pituitary is able to take up histamine from the blood but is unable to form it; whereas the hypothalamus is unable to take up histamine from the blood but is able to decarboxylate histidine to form histamine both *in vivo* and *in vitro*. It can reasonably be assumed that the histamine contained in the brain is formed locally, since little or no histamine penetrates the blood-brain barrier.[46]

Clearly non-mast cell brain histamine must have some role to play within the central nervous system. Histamine is as potent at activating neuronal activity as any of the known amines, whether injected into the carotid artery[16] or directly into the brain.[47] Histamine has been shown to stimulate the hypothalamus[48, 49] and, upon intracarotid injection, to inhibit as strongly as serotonin, transcollosally evoked potentials in the optic cortex.[50]. Adam and Hye[4] showed that compound 48/80 lowers the histamine in the pituitary gland (which contains mast cells) but has no effect on the hypothalamus, whereas reserpine lowers the histamine concentration in the hypothalamus but does not affect its level in the pituitary gland. Histamine is, therefore similar to serotonin and norepinephrine since these three bioactive amines, which are concentrated in the hypothalamus, can be depleted by reserpine-like compounds.

Erspamer[28] wrote that, "Histamine in the central nervous system has been ignored for a long time by several investigators as a second class amine. But this amine, however annoying the fact may be, has the same citizenship rights in the central nervous system as catecholamines and 5-hydroxytryptamine, whose function in the central nervous system is approximately as obscure as that of histamine."

It would appear from the established findings that since: 1) the distribution of histamine in brain is similar to that of norepinephrine and serotonin, 2) histamine has the ability to affect the nerve activity of certain areas in the brain, 3) brain concentrations of histamine change under the influence of certain psychoactive drugs, and 4) histamine is associated with nerve-endings isolated from the central nervous system,[12, 17–20] a careful evaluation of the role of histamine in the central nervous system is called for.

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