patients on whom repeated determinations were made, the character of the curve has remained essentially unchanged over periods as long as 2 years. The time between myocardial infarction and when the initial curve was obtained, also varied from a few months to over a year. The fact that the abnormal features of the lipoprotein curve can disappear might account for the 3 patients (out of 228) with normal curves with a history of previous myocardial infarction. Our observations would therefore indicate that the character of the curves is relatively constant. At present we cannot state whether such curves occur prior to myocardial infarction. Lipoprotein curves were made from 2 elderly patients who died within a few days after a myocardial infarction. The results showed relatively normal curves, suggesting abnormal lipoprotein distribution occurs at some interval after infarction. Our results, however, are based almost entirely on patients who have survived myocardial infarction as compared with those who have not had one.

Summary and conclusions. Data are presented which indicate that an abnormal type of cholesterol lipoprotein curve is associated with a history of recovery from a previous myocardial infarction. The type of curve obtained from a given individual is relatively constant over years. As yet we are unable to say whether the abnormal type of curve develops prior to, or after, initial infarction, or whether degree of abnormality is of any prognostic value. The relationship which exists suggests the need for further studies as to diagnostic and prognostic value of this method in the study of myocardial infarction.

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Fixation of 5-Hydroxytryptamine by Brain Mitochondria.* (24824)

E. WALASZEK AND L. G. ABOOD

Depts. of Psychiatry and Biochemistry, University of Illinois College of Medicine, Chicago

In investigating some physical and biochemical properties of brain mitochondria, it seemed desirable to know whether any neurohumoral amines present in the brain were attached to mitochondria and involved in their physiology. There is evidence to suggest that histamine(1), nor-adrenalin(2) and acetylcholine(3) are associated with mitochondria elsewhere in the organism; but no comparable study has been made with brain mitochondria. Since recent interest in possible role of 5-hydroxytryptamine (HT)† in brain function, and an extremely sensitive bioassay was available, such study was undertaken with this neurohumoral amine. Our report deals with localization of HT in rat brain mitochondria and its possible relationship to mitochondrial metabolism. A preliminary account of this work has been presented(4).

Methods. Mitochondria were prepared from whole rat brain by methods comparable to those described previously(4), with special precautions to eliminate blood cells and cellu-

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† The following abbreviation were used: HT, 5-hydroxytryptamine; P/O, oxidative phosphorylation. HT was kindly supplied by Dr. R. K. Richards of Abbott Labs., Chicago.

TABLE I. Distribution of HT in Cellular Fractions of Whole Rat Brain. Values are expressed in terms of μg/HT/g wet wt of whole brain and are an average of 3 experiments agreeing within 6%.

Cellular fraction	Amt HT, μg/g	% total
Supernatant	.027	12
Nuclear-debris	.052	22
Mitochondria	.149	63
Microsomes	.002	0
Whole brain	.235	100
% recovery		98

lar debris. A 5% homogenate of brain in 0.25 M sucrose, containing 10^{-4} M sodium versenate was subjected to 3 successive centrifugations at 1200 x g, to remove nuclei, erythrocytes, and cellular debris. The carefully decanted supernatant was then centrifuged at 10,000 x g for 20 minutes, to yield the mitochondria fraction. The mitochondrial residue was washed once by homogenizing in 0.25 M sucrose-versene and centrifuging at 12,000 x g for 20 minutes. Sedimentation at 75,000 x g then yielded the "microsome" residue and "supernatant" fraction. All operations were performed at 0°. For a given experiment, mitochondria were prepared from 4 rat brains and usually divided into 8 parts. Each fraction was then incubated with a particular drug in ice bath 30 minutes. After centrifugation at 15,000 x g and washed once in 10 ml of isotonic sucrose-versene, the mitochondria were treated with 20 volumes of acetone to extract HT. The residue was again extracted with 90% acetone, and extracts combined and dried *in vacuo*. After the dried residue was suspended in 2 ml distilled water, the lipids were removed by extracting twice with 10 ml of petroleum ether. The water layer was then dried *in vacuo* and ready for bioassay. Estimation of concentration of HT was made by the method of Amin *et al.*(6), using isolated rat uterus at 29° and compared to standard solution of 5-hydroxytryptamine creatine sulfate. Some samples were assayed on the isolated rat uterus in superfusion(7) for greater sensitivity. Measurement of P/O† of brain mitochondria was described previously(5). All agents were homogenized with the mitochondrial preparations and preincubated at 0° 20 minutes before determining P/O. Mitochondrial preparations were tested for P/O before use, and those not of optimal activity were discarded.

TABLE II. Effect of Reserpine and Other Agents on HT (μg) Content of Mitochondria from 1 g Rat Brain. Mitochondria were incubated with reserpine for 20 min. at 0°, then centrifuged at 15,000 × g and washed once. HT was then extracted from supernatant and mitochondria.

	Supernatant	Mitochondria
Control	.027	.200
	.060	.200
	.010	.120
	.035	.135
Reserpine	.150	.020
	.075	.015
	.125	.020
	.135	.045
Dist. water	.080	.100
Detergent*	.125	.010

* .02% sodium lauryl sulfate.

Results. Results on distribution of HT among various cytoplasmic fractions of rat brain are presented in Table I. The mitochondrial fraction contained 63% of total HT in rat brain, while the nuclear-debris fraction contained 22% and supernatant 12%.

TABLE III. Release and Binding of HT in Rat Brain Mitochondria. Concentration of reserpine and HT was 10^{-6} M. Results are average of 2 experiments agreeing within 10%.

	Original HT	Net change in HT	
	(μg/g)		% change
Control	.15		
After reserpine	.02		−87
After HT	.40	.25	255
Reserpine, then HT	.30	.28	300

Reserpine at concentration of 10^{-6} M almost completely released HT from mitochondria (Table II). Suspending mitochondria in distilled water for 15 minutes caused only a partial release of the bound HT, while exposure to .02% solution of a detergent resulted in almost complete loss of mitochondrial HT (Table II).

Freshly prepared mitochondria exhibited the capacity to bind over twice their original content of HT (Table III). After depletion of HT by pre-treatment with reserpine, mitochondria absorbed twice the original amount of HT; however, this value of 0.30 μg/g was 0.10 μg less than total amount of HT absorbed without treatment with reserpine.

Various agents other than reserpine were

TABLE IV. Effect of Various Agents on HT Binding of Rat Brain Mitochondria. Values are expressed in terms of μg/HT/g wet wt of whole rat brain, and represent avg of 2 experiments with each agent.

Agent	Conc., M	Control	After agent	After agent + HT	Agent + HT after reserpine depletion
LSD-25	10^{-5}	.09	.09	.18	.10
Reserpine	10^{-6}	.09	.003	.18	
Phenyl ether	"	.15	.14	.30	.02
Dibenamine	10^{-5}	.09	.09		.13
DNP	"	.15	.16	.40	.38
None		.15		.42	.30

tested for possible effect on binding and release of HT in rat brain mitochondria (Table IV). LSD-25, phenyl ether, and dinitrophenol were without effect on mitochondrial concentration of HT. Dinitrophenol did not affect binding of HT, while LSD, reserpine, and phenyl ether produced some inhibition. After pre-treatment with reserpine, binding of HT by mitochondria was almost completely inhibited by phenyl ether, and inhibited over 50% by dibenamine, while LSD and dinitrophenol produced no change.

The various agents were tested for effect on oxidative phosphorylation of rat brain mitochondria (Table V). SKF 501, phenyl ether, and dibenamine all produced about 50% inhibition at concentrations slightly over 10^{-5} M. LSD-25‡ and reserpine caused some inhibition at 5 x 10^{-5} M, while HT produced 26% inhibition at 10^{-4} M. The more potent inhibitors compared in effectiveness with dinitrophenol.

Discussion. Binding of HT by rat brain mitochondria is not unexpected, since other neurohumoral amines, such as adrenaline(1), histamine(2), and acetylcholine(3) are likewise present. Although the role of various neurohumoral amines in the central nervous system is uncertain, their effects on peripheral nervous system and smooth muscle have been extensively studied(8,9,10).

That dibenamine, phenyl ether, and reserpine interfere with HT binding by mitochondria is interesting, since some of these agents block contraction of smooth muscle by HT (11). All 3 agents will inhibit oxidative phosphorylation at concentrations of 10^{-5} M; but since substances exist which do not affect phosphorylation but block HT, and other inhibitors of oxidative phosphorylation (such as dinitrophenol) do not block HT, there seems to be no distinct relationship between "uncoupling action" and HT blockade. On the other hand, the mechanism of inhibition of phosphorylation by substances such as reserpine, dibenamine, and phenyl ether is different from that of dinitrophenol, which fails to block the action of HT(12). It is conceivable, therefore, that some overlapping exists between the phosphorylative portion of mitochondria and receptor sites for HT. Further evidence for a possible relationship between

TABLE V. Inhibition of Oxidative Phosphorylation (P/O) of Rat Brain Mitochondria by "Diphenyl" Agents Affecting HT Contraction of Smooth Muscle. Homogenation of agent with mitochondria and pre-incubation at 0° for 20 min. was necessary for maximal inhibition. Results are avg of 3 experiments agreeing within 7%.

Agent	Conc., M	ΔO, μatoms	ΔP, μmoles	P/O	% inhib. P/O
Control		6.1	16.6	2.7	
Reserpine	2 × 10^{-5}	5.5	12.8	2.3	12
SKF-501	3 "	3.8	4.6	1.2	56
LSD-25	5 "	5.8	12.0	2.2	18
Phenyl ether	5 "	4.0	4.1	1.0	67
Dibenamine	2 "	5.2	9.1	1.7	37
Dinitrophenol	5 "	6.1	9.0	1.5	45
HT	10^{-4}	5.8	2.9	2.0	26

‡ Studies with LSD-25 were performed with crystalline form of LSD-25, generously supplied by Sandoz Pharmaceutical Co. With respect to oxidative phosphorylation of brain mitochondria, the solution preparation supplied in 100 μg amounts in sealed vials by Sandoz proved 50 times more potent an inhibitor. Dr. Julia Apter has personally communicated that entirely different results are obtained in electroretinogram studies employing crystalline and solution preparations of LSD-25.

phosphorylation and binding of HT is that concentration of HT in platelets is partly dependent upon the ATP present(13).

That the major fraction of HT is bound to the mitochondria appears to conflict with the report of Hughes *et al.*(14) that the amine is not specifically bound to any cytoplasmic fraction. Whether this difference can be due to the fact that these authors used rabbit instead of rat brain remains to be determined. Since the slightest mistreatment of mitochondria during isolation alters both their phosphorylative efficiency and HT binding, precautions were necessary to obtain good results. Our experience has been that oxidative phosphorylation of rabbit brain mitochondria has appreciably lower specific activity than rat brain, in addition to being more labile. Baker (15) reported that most of the HT of the gastric mucosa is present in the mitochondrial fraction, and that HT is released upon exposure to a hypotonic medium.

The following can be stated with regard to the mitochondrial "receptor site" and the nature of the binding of HT. From the point of view of chemical constitution, the similarity of action to reserpine of such agents as SKF 501, dibenamine, and phenyl ether, is not unexpected(12). It had been previously reported that oxidative phosphorylation could also be restored after brain mitochondria were washed free of reserpine(12). In isolated mitochondria, reserpine does not appear to produce alteration in "receptor sites," whereas *in vivo*, capacity of brain to bind HT was inhibited long after elimination of administered reserpine(16). As far as isolated mitochondria are concerned, the only agent studied which brought about irreversible change in either HT binding or oxidative phosphorylation(12) was SKF 501. Although the nature of the binding is obscure, it appears that either mitochondrial structure or some peculiar constituent is in some way involved.

Summary. Distribution of 5-hydroxytryptamine (HT) in various cytoplasmic fractions of rat brain has been determined, and the neurohumor was confined almost exclusively to the mitochondrial fraction. Numerous inhibitors of oxidative phosphorylation were tested for ability to release HT from mitochondria or to prevent binding. Reserpine completely depleted mitochondria of HT, but did not alter their ability to reabsorb HT, while phenyl ether and LSD partly prevented mitochondrial fixation of HT.

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