Brit. J. Pharmacol. (1954), 9, 100.

THE INTRACELLULAR DISTRIBUTION OF HISTAMINE IN DOG'S LIVER

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(RECEIVED OCTOBER 12, 1953)

Several years ago, working with portions of dog's liver which had been ground in saline and centrifuged, Trethewie (1938) demonstrated in the supernatant fluid cellular debris which was thrown down by further centrifugation. It contained histamine which could be brought into solution by heating. Feldberg and Kellaway (1938) were able to release histamine from particles found in the perfusate from dog's liver following the intraportal injection of staphylococcal toxin. The refined methods of tissue fractionation developed during the last ten years by Claude (1946), Hogeboom, Schneider, and Palade (1948) and other workers, have provided techniques enabling a further and more detailed investigation of this phenomenon. The recent demonstration by Blaschko and Welch (1953), that the pressor amines of adrenal medulla are for the most part held within particles thrown down in gravitational fields similar to those in which liver mitochondria are thrown down, suggested the possibility that histamine might be similarly localized in particles of defined size.

With these points in mind the histamine content of the particulate and non-particulate fractions obtained by differential centrifugation of sucrose homogenates of dog's liver tissue has been investigated.

METHODS

Liver tissue was obtained immediately after death from dogs which had been bled out under ether anaesthesia and the blood largely replaced by saline. Ten grams of tissue was cut into small pieces and then dispersed for 10 minutes in sucrose solution with a Potter–Elvehjem homogenizer. The volume was made up to 100 ml. The homogenate was centrifuged for 30 minutes at 900 *g* in order to remove cell nuclei, blood, and any tissue fragments which might have escaped disintegration. A portion of the resulting supernatant fluid (homogenate) was then centrifuged for 30 minutes at 22,000 *g*. The supernatant fluid was poured off and the sediment made up to the same volume with sucrose solution. In this way three fractions were obtained: the homogenate, the resuspended sediment, and the sediment-free supernatant fluid. In one experiment the resuspended sediment was centrifuged a second time at 22,000 *g* for 30 minutes in order to wash away any contaminating supernatant fluid, and the final sediment was resuspended in sucrose solution as before.

The sucrose solutions were usually 0.25 M or 0.88 M, as standard techniques for liver-cell fractionation have been devised using these particular media (Schneider and Hogeboom, 1951). In the later experiments 0.32 M-sucrose was used, which is isotonic with tissue fluids.

All manipulations were performed in the cold, using an ice-bath, and centrifugation was carried out in a refrigerated centrifuge at -1° C.

Assays were usually made on guinea-pig ileum suspended in oxygenated Locke's solution containing 6 mg./l. of hexamethonium bromide. Some samples were assayed by the depressor response produced in cats under chloralose anaesthesia. Before the assay the fractions were usually frozen and thawed in order to liberate any histamine from the particles, but in some experiments the samples were acidified immediately after their preparation and neutralized just before the assay.

RESULTS

Distribution of Histamine.—Altogether seven experiments were carried out in which dog liver homogenates were subjected to centrifugation and the fractions obtained assayed for their histamine content. The results of these experiments are shown in Table I, in which the histamine content of each fraction is expressed as micrograms of histamine dihydrochloride per gram of liver tissue. The histamine contents of the high speed supernatant fluid and sediment are also expressed as percentages of the histamine content of the homogenate from which they were derived.

In these experiments the histamine content of the homogenates varied from 8 to 23 micrograms (as dihydrochloride) per gram of fresh liver tissue. On centrifugation a fraction varying from 10–36% (mean 19%) was found in the supernatant fluid; from 52–100% (mean 76.5%) was found in the sediment. The histamine recovered in these

TABLE I

HISTAMINE CONTENT OF DOG LIVER HOMOGENATES AND OF THE FRACTIONS OBTAINED ON CENTRIFUGATION

Histamine in μg. dihydrochloride per g. liver. The histamine content of each fraction is also expressed as a percentage of the total histamine of the homogenate from which it was derived.

Expt. No.	Molarity of Suspending Medium	Method of Assay	Histamine Content of				
			Homogenate μg./g.	Supernatant Fluid μg./g.	Supernatant Fluid %	Sediment μg./g.	Sediment %
1	0·25	G.P.I.	10·6	3·8	36	7·5	72
2	0·88	G.P.I.	8	2	25	6	75
3	0·88	G.P.I.	23	1·5	6·5	20*	87
		C.B.P.	20	2	10	16*	80
4	0·25	G.P.I.	15	2·3	15	7·8	52
5	0·88	G.P.I.	19	3·5	18	19	100
		C.B.P.				20	
6	0·88	G.P.I.	39	12	31	27	69
7	0·32	C.B.P.	20	2·5	12·5 (Mean 19%)	15	75 (Mean 76·5%)

* The resuspended sediment was re-centrifuged and suspended in sucrose solution a second time.

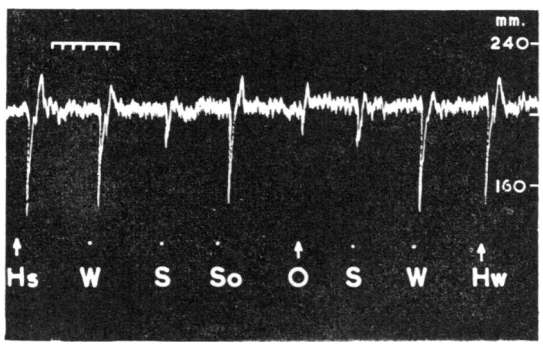

FIG. 1.—Blood pressure tracing from cat anaesthetized with chloralose, showing the effect of injecting the resuspended sediment in various media. Time, 30 sec. B.P. in mm. Hg. At Hs 1 μg. histamine dihydrochloride in 1 ml. isotonic sucrose; at W 0·5 ml. resuspended sediment made up to 1 ml. with distilled water; at S 0·5 ml. resuspended sediment made up to 1 ml. with isotonic sucrose; at So 0·5 ml. resuspended sediment made up to 1 ml. with isotonic sucrose with octylamine 10^{-3}: at O octylamine 10^{-3} in isotonic sucrose 1 ml.; at Hw 1μg. histamine dihydrochloride in 1 ml. distilled water.

two fractions varied from 67–118% of the amount in the original homogenate. There was no indication of a significant difference in the distribution of histamine when media of different tonicity were used.

The results given were obtained with fractions which had been either frozen and thawed or acidified and neutralized; however, when freshly prepared sediment was used another phenomenon was observed. When the particulate fraction, resuspended in isotonic sucrose, was further diluted with isotonic sucrose or isotonic saline solution before injection into the cat, either very little or no depressor effect was observed. On the other hand, when it was diluted with distilled water the injection was followed by a fall in blood pressure similar to that seen on injecting an equivalent quantity of acidified sediment (see Fig. 1).

When the particulate fraction was diluted with isotonic sucrose to which had been added *n*-octylamine, to give an octylamine concentration of 1 in 1,000, its injection produced the same fall in blood pressure as when it was diluted with distilled water. *n*-Octylamine in a concentration of 1 in 10,000 did not have this effect.

When the particulate fraction was diluted with isotonic sucrose to which had been added 48/80 (10 μg. in 1 ml.) its injection did not produce any greater fall in blood pressure than the slight fall which followed the injection of sediment alone in isotonic sucrose solution.

The depressor effects produced in the cat on intravenous injection of the standard histamine solution, and of the fractions obtained from dog liver, could not be obtained in a cat which had previously received 4 mg. of mepyramine.

DISCUSSION

Trethewie (1928) and Feldberg and Kellaway (1938) showed that a portion of the histamine in dog liver is contained in subcellular particles. In the experiments described in the present paper sucrose media were used. In the centrifugation procedure adopted the use of 0.25 M-sucrose results in the sedimentation of a "large granule" fraction frequently referred to as "mitochondria" and a "small granule" fraction frequently referred to as "microsomes" (Schneider and Hogeboom, 1951); with 0.88 M-sucrose only the large particles are thrown down. The lack of any marked difference in the histamine distribution between supernatant fluid and sediment, prepared in either medium, indicates that the histamine is principally in the large granule fraction. This is in agreement with Copenhaver, Nagler, and Goth (1953), who found that most of the histamine in liver is in the mitochondria.

Trethewie, and Feldberg and Kellaway, observed no fall of blood pressure on injecting the suspended particles intravenously into a cat, but were able to "release" the histamine by heating. It has been shown that the particles retain their histamine in isotonic sucrose or saline media. However, dilution of the sediment with distilled water causes a release of the histamine. It can also be released by *n*-octylamine in a concentration of 0.1%. This is the lowest concentration which Mongar and Schild (1953) found would cause the immediate release of histamine from guinea-pig lung. A lower concentration of *n*-octylamine failed to release histamine from dog-liver particles. 48/80

in a concentration of 0.01% did not cause the release of histamine from the particles; this concentration of 48/80 must have been about ten times greater than that which would be expected to obtain in the blood of cats, in which it caused a substantial fall in blood pressure (Paton, 1951).

It seems reasonable to infer that the liver particles remain intact in isotonic media so that any histamine they contain is incapable of producing its pharmacological effects; but that certain types of damage to the particles, such as freezing and thawing, acidification, heating, suspension in very hypotonic media or the addition of octylamine, cause the liberation of histamine into the media.

Summary

1. Homogenates of dog liver have been fractionated into particulate and non-particulate components by high speed centrifugation at low temperature.

2. Two-thirds or more of the histamine was found in the "large granule" fraction.

3. In isotonic media the particles retain their histamine so that its pharmacological activity is not apparent when the suspension is injected into cats.

4. The histamine can be released from the particles by a variety of procedures which might be expected to damage a surface membrane, and by the histamine releaser *n*-octylamine, but not by 48/80.

I wish to express my appreciation to Dr. H. Blaschko for his advice and help throughout the course of this work and to Professor J. H. Burn for allowing me to work in his Department during the tenure of a C. J. Martin fellowship of the National Health and Medical Research Council of Australia.

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