Biochemical Pharmacology, 1962, Vol. 11, pp. 1049–1053. Pergamon Press Ltd., Printed in Great Britain.

TWO POOLS FOR AMINES IN NEOPLASTIC MAST CELLS*

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(*Received* 11 *June* 1962; *accepted* 17 *July* 1962)

Abstract—The distribution of endogenous and exogenous 5-hydroxytryptamine and histamine was determined in neoplastic mast cells growing in culture. In cells containing measurable amounts of these endogenous amines, most of the exogenous amines were found in intracellular fractions almost devoid of endogenous amines. In cells in which these endogenous amines could not be detected, the exogenous amines were found in the particulate material in which endogenous amines are normally present. It is suggested that the endogenous amines are held in a pool separate from the exogenous amines and that the accesibility of the exogenous amines to the pool for endogenous amines is a function of the levels of endogenous amines already existing in the pool.

IN THE preceding paper it was shown that in neoplastic mast cells, P-815-X-1, growing in culture, endogenous histamine and 5-hydroxytryptamine (5-HT) turn over at rates different from the exogenous amines.[1] This difference was most striking with histamine. Endogenously produced histamine had a half-life of 27 hr, whereas the histamine that the cells had concentrated from the medium, after an initial loss, persisted in the cells in undiminished amount for several days. One interpretation of these findings, and analogous findings with 5-HT, is that there are two pools for amines in these cells; in one pool are found the endogenous, in another the exogenous, amines. It was suggested that the exogenous amines have access to the endogenous pool. This suggestion rested on the initial loss of exogenous histamine from the cell, a loss reminiscent of the loss of endogenous amines. But the bulk of the exogenous amines is not lost from the cell and is presumably held in a pool that is not associated with a mechanism to eliminate the amines. In agreement with this postulate were comparable findings from studies on another cell line, P-815-X-2, possessing lower endogenous levels of amines, higher uptake of exogenous amines, and a slower initial loss of histamine. This hypothesis appeared amenable to direct experimentation. Density gradient centrifugation was used, and the fluctuating levels of amines in these cells were exploited to provide evidence, which is presented here, that there are two pools for amines in these neoplastic mast cells and that the intracellular localization of exogenous amines depends on the levels of endogenous amines. A preliminary report of this work has appeared.[2]

* Grants from the United States Public Health Service (GM-K3-2459-C3) and the American Heart Association supported this work which is part of a thesis submitted by A. V. Furano to Yale University School of Medicine in partial fulfillment of the requirements for the degree of Doctor of Medicine. Miss Andrea Gorske and Miss Jean Cadogan gave capable technical assistance.

† Summer Fellow (1961) of the U.S. Public Health Service.

METHODS

The X-1 cells, derived from the Dunn–Potter murine mastocytoma and growing in culture, were used in all experiments. References to the history of this tumor and the methods of growing the cells in culture appear in the preceding paper.[1] After 24 hr of incubation in the culture medium containing either ^{14}C-histamine or ^{14}C-5-HT, as described,[1] the cells were collected by centrifugation, washed three times in ice-cold 0·9% NaCl and suspended in enough ice-cold 0·3 M sucrose to give a 10 per cent suspension of cells; these were homogenized with a Teflon homogenizer in the cold. The homogenates were centrifuged at 4 °C as described previously.[3] Nuclei, cellular debris, and unbroken cells were removed by centrifugation at 900 × *g* for 20 min. The supernatant material was removed by centrifugation at 10,000 × *g* for 40 min in order to obtain the large particulate material; the latter was suspended in a small amount of 0·3 M sucrose and layered over a density gradient of sucrose, 0·3 to 2·5 M. The interfaces of these sucrose layers were marked so that the original boundaries could be identified after centrifugation, which was carried out in the SW-25 rotor in a Spinco ultracentrifuge for 1 hr at 90,000 × *g*; at the end of this time the suspension of particulate material had resolved into separate bands. A diagram was made of each tube, which was then cut at various levels with a Spinco tube-slicer to allow separate removal of the different fractions which were analyzed for the mitochondrial enzyme, succinic dehydrogenase, by a spectrophotometric method[4] and for histamine[5] or 5-HT[6] by fluorometric methods. In the same final extracts in which histamine and 5-HT were determined, the corresponding radioactive amines were measured in a liquid scintillation counter.

RESULTS

The intracellular distribution of endogenous and exogenous 5-HT

Figure 1 shows the distribution of endogenous and exogenous 5-HT in cells containing measurable amounts of 5-HT, as compared with the distribution of exogenous 5-HT in cells in which endogenous 5-HT could not be detected. In cells containing 0·25 μg of 5-HT/mg of cells and 31 μg of 5-HT in the large particulate material, the bulk

TABLE 1. INTRACELLULAR DISTRIBUTION OF SUCCINIC DEHYDROGENASE ACTIVITY; AVERAGE OF THREE EXPERIMENTS

Fraction	Activity (%)
1	0·0
2	9·4
3	72·9
4	17·5
5	0·0

of the endogenous 5-HT was found in dense particles sedimenting at two interfaces: one, F-3, between 1·2 and 1·5 M sucrose and another, F-4, between 1·5 and 1·7M sucrose. F-3 contained most of the succinic dehydrogenase activity (Table 1) and 49·3 per cent of the endogenous 5-HT in the tube; F-4 contained 42·9 per cent of the endogenous 5-HT. In these same cells only 18·8 per cent of the exogenous radioactive

5-HT was found in these layers; the rest of the exogenous 5-HT appeared in layers of relatively low density, in 0·3 to 0·8 M sucrose, F-1, a clear fraction which contained 67·7 per cent of the exogenous 5-HT, and in 0·8 to 1·2 M sucrose, F-2, which contained 13·5 per cent of the exogenous 5-HT.

In cells in which endogenous 5-HT could not be detected, the major portion of the exogenous 5-HT, 55·4 per cent, was sedimented in F-3, the dense particulate material containing succinic dehydrogenase activity. An additional 4·7 per cent of the exogenous 5-HT was found in F-4; the rest of it was found in fractions of relatively low density, F-1 and F-2.

It should be noted that cells contaning low levels of endogenous 5-HT showed a sparseness of particles at F-4 and an increase in particulate material at F-2.

The intracellular distribution of endogenous and exogenous histamine

The experiments presented in Fig. 2 are analogous to those in Fig. 1. In cells containing 0·12 μg of histamine/mg of cells and 4·48 μg of endogenous histamine in the large particulate material, all except 17·8 per cent of endogenous histamine was found in material sedimenting at F-3 and F-4, while these layers contained only 37·9 per cent of the exogenous histamine.

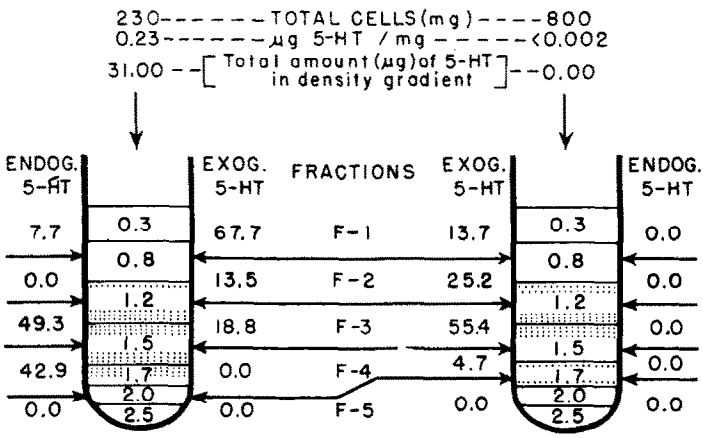

FIG. 1. The distribution of endogenous and exogenous 5-HT in particulate material of neoplastic mast cells grown in culture. The distribution in a density gradient of sucrose, 0·3 to 2·5 M, is shown on the left for cells containing measurable amounts of endogenous 5-HT and on the right for cells in which endogenous 5-HT could not be detected.

In cells in which levels of endogenous histamine could not be measured, 50·5 per cent of the exogenous histamine was found at F-3 and 8·5 at F-4. The rest of the exogenous histamine, 40·8 per cent, was found in F-1 and F-2. As noted in experiments measuring 5-HT, cells containing low levels of endogenous histamine showed a sparseness of particles at F-4 and an increase in particulate material at F-2.

DISCUSSION

The endogenous amines were found almost equally divided between F-3, which is the mitochondrial fraction, and a denser fraction, F-4. These findings are similar to

those obtained with another murine mastocytoma,[3] grown as a solid tumor, in which most of the amines were found in F-4, with relatively less in F-3. The presence of so great a percentage of 5-HT and histamine in the mitochondrial fraction of the P-815-X-1 cells may be artifactitious or it may indicate that some of the amine-contaning particles in these cells have the same density as mitochondria. It is of interest that, when the cells contained negligible amounts of endogenous amines, there was a dearth of particulate material in F-4.

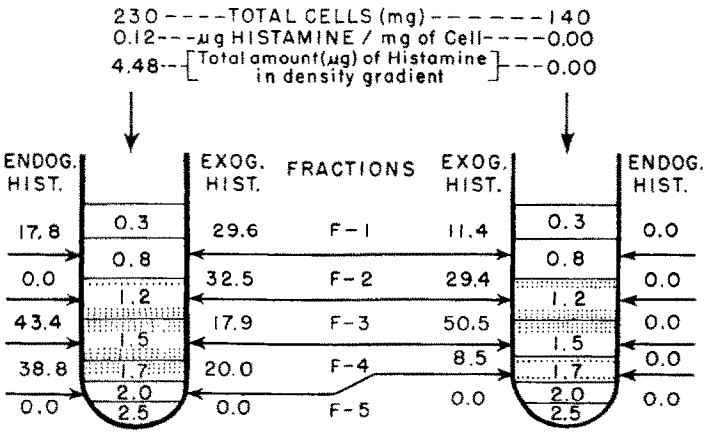

FIG. 2. The distribution of endogenous and exogenous histamine in particulate material of neoplastic mast cells grown in culture. The distribution in a density gradient of sucrose, 0·3 to 2·5 M, is shown on the left for cells containing measurable amounts of endogenous histamine and on the right for cells in which endogenous histamine could not be measured.

The presence of more than one particulate fraction containing *endogenous* amines does not confound in any way the finding that most of the *exogenous* 5-HT and histamine were found in fractions F-1 and F-2, nearly devoid of these endogenous amines—an observation demonstrating at least two pools for amines in these cells. When the same cell line contained levels of amines too low to measure, the major portion of the exogenous amines was found in F-3. Accordingly, it appears that exogenous amines have access to the pool containing endogenous amines and that binding sites for endogenous amines, when not occupied by these amines, may become available to exogenous amines.

Further support for this interpretation can be obtained by noting the quantitative differences obtained in the experiments with 5-HT and histamine. The amount of endogenous 5-HT present in the density gradient, 31 μg (Fig. 1), was considerably higher than the amount of histamine, 4·48 μg (Fig. 2). If it is true that binding sites for endogenous amines are available to exogenous amines, then in the presence of relatively low levels of endogenous amine, more endogenous binding sites should be available for the exogenous amine. The results obtained bear out this hypothesis; of the exogenous histamine (Fig. 2), 37·9 per cent was found in F-3 and F-4, while only 18·8 per cent of the exogenous 5-HT was present in these fractions.

The existence of two pools for amines helps to explain some puzzling findings, including the differences in turnover rates of endogenous and exogenous amines in

these cells.[1] When cells contain significant levels of endogenous amines, the bulk of the exogenous amines is held in a separate pool; this exogenous pool is not associated with a process for elimination of the amines, whereas the endogenous pool, which constitutes the dense particulate material in F-3 and F-4, is associated with an eliminative mechanism. As new amines are synthesized and enter the endogenous pool, they displace the amines and, therefore, the endogenous amines undergo turnover.

Some of the exogenous amines enter the endogenous pool, the amount depending on the levels of endogenous amine. The exogenous amine that enters the endogenous pool turns over; hence, the initial loss of exogenous histamine is accounted for.[1] This loss of exogenous amine depends on the amount of exogenous amine in the endogenous pool; the latter, in turn, depends on the levels of endogenous amines, as shown in Figs. 1 and 2. Cells with low levels of endogenous amine show a greater uptake of exogenous amines[1, 7] probably because there are more sites available to which the exogenous amines can be bound. Similarly, the fact that X-2 cells (which have lower levels of endogenous amines than have X-1 cells) show a greater uptake of exogenous amines,[1, 7] may rest on the availability of more free sites to bind amines. X-2 cells lose exogenous 5-HT and (initially) histamine more slowly than do X-1 cells: the slower loss of these exogenous amines from the endogenous pool is a reflection of slow synthesis of endogenous amines in X-2 cells,[8] resulting in slow displacement, i.e. turnover, of these amines.

It is possible that these observations with neoplastic mast cells may have bearing on the binding of amines in other cells. Particularly pertinent are the observations that cells containing dopamine (3-hydroxytryamine) in ungulates are mast cells[9] and that some of the previously described chromffin cells are mast cells.[10]

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