Biochemical Pharmacology, 1962, Vol. 11, pp. 957–960. Pergamon Press Ltd., Printed in Great Britain.

SOME ACIDIC SUBSTANCES IN NEOPLASTIC MAST CELLS AND IN THE PINEAL BODY*

JACK PETER GREEN, MARGARET DAY† and JOSEPH D. ROBINSON, JR.

Department of Pharmacology,
Yale University School of Medicine, New Haven, Conn., U.S.A.

(*Received* 6 *June* 1962; *accepted* 12 *June* 1962)

Abstract—In neoplastic mast cells in culture the levels of histamine and 5-hydroxytryptamine reflected the levels of taurine, cysteic acid, cerebroside sulfate, and adenosine triphosphate and the capacity of the cells to synthesize heparin. No neuraminic acid could be detected in these cells. In the pineal body, high levels of neuraminic acid were found along with significant concentrations of taurine and cysteic acid, but no cerebroside sulfate or sulfomucopolysaccharides were detectable. Reserpine did not affect the levels of taurine in brain, spleen, or heart of the rat.

EXPERIMENTS designed to measure acidic substances in tissues rich in biogenic amines have had two separate but related goals. One is to account for the anionic substances that preserve electroneutrality in such tissues as brain[1] and axoplasm.[2] The other goal is to learn how endogenous and exogenous amines are bound in tissues, for evidence (recently reviewed[3]) indicates that amines may exist in loose linkage to other substances, probably organic anions. Some of the anionic substances could form complexes with exogenous amines at the nonspecific, "silent receptors",[4] the "sites of loss"[5] of administered amines, and some may contribute to the mechanism that stores endogenous amines in cells.

Among the compounds that have been implicated in the binding of amines are adenosine triphosphate (ATP), sulfomucopolysaccharides like heparin, and acidic lipids such as cerebroside sulfate. ATP is associated with the catecholamine-containing granules of the adrenal medulla[6, 7] and in the duodenal mucosa with the granules containing 5-hydroxytryptamine (5-HT)[8]; the capacity of platelets to concentrate 5-HT is proportional to its concentration of ATP.[9] Similarly, levels of heparin are roughly proportional to levels of histamine and 5-HT in neoplastic mast cells,[10] in which all three compounds have a similar intracellular distribution.[10, 11] Other sulfomucopolysaccharides have been described in amine-rich platelets[12] and in brain.[13] Cerebroside sulfate, which is also found in neoplastic mast cells as well as in brain and other tissues,[14] forms complexes with biogenic amines and with acetylcholine.[15] Similarly, amines could react with other acidic substances, such as polymers containing neuraminic acid, that form complexes with organic bases.[16]

Another organic acid that can interact with amines[17] is cysteic acid, which is found in platelets,[18] brain, and neoplastic mast cells.[14] Taurine, which has a pK_a of about

* Grants from the United States Public Health Service (GM-K3-2459-C3), the American Heart Association, and the Life Insurance Medical Research Fund supported this work.

† Recipient of a fellowship provided by a grant from the Squibb Institute for Medical Research. Present address: Department of Pharmacology, Royal College of Surgeons, London.

1·5,[19] is also found in neoplastic mast cells[14] (which can take up taurine[17]) and in other tissues rich in amines such as brain[20] and platelets.[18, 21] Taurine is a major anionic substance both in squid axoplasm[2] and in platelets.[22] In fact its concentration in platelets is so high that urinary excretion of taurine is a good reflection of platelet destruction.[18] It is of further interest that taurine has a depressant action on mammalian neurons comparable with that of γ-aminobutyric acid; cysteic acid is excitatory to neurons.[23]

It seemed of interest to measure the levels of the aforementioned acidic substances in neoplastic mast cells concomitant with measurements of histamine and 5-HT in these cells. These acidic substances have also been measured in the pineal body, which is rich in biogenic amines[24, 25] and can concentrate amines, unlike most other areas of the brain.[26]

METHODS

The P-815-X-1 and P-815-X-2 neoplastic mast cells, derived from the Dunn and Potter mastocytoma, P-815,[27] were grown in culture by methods previously described.[10, 28, 29] Bovine pineal bodies were kindly provided by Dr. Aaron B. Lerner. Cerebroside sulfate was extracted[30] and measured by determining sulfate after hydrolysis.[14] Taurine and cysteic acid were separated by chromatography[20] and measured with a ninhydrin reagent.[31] The incorporation of ^{35}S-sulfate into heparin and the extraction and chromatography of sulfomucopolysaccharides were carried out as previously described.[10, 13] To measure ATP, washed neoplastic mast cells were extracted in ice-cold 5%-trichloroacetic acid, the acid was removed by shaking the extract three times with 5 volumes of diethyl ether, and residual ether in the aqueous phase was blown off with nitrogen. ATP was determined in the extract and in standard ATP solutions, treated in the same manner, with a firefly enzyme extract. Neuraminic acid was determined[32] and calculated as N-acetylneuraminic acid. Histamine and 5-HT were determined by bioassay on the guinea pig ileum and on the heart of *Venus mercenaria*, respectively.

RESULTS AND DISCUSSION

Table 1 shows that in neoplastic mast cells the incorporation of ^{35}S-sulfate into heparin, which reflects the levels of heparin in these cells,[10] was proportional to the

TABLE 1. LEVELS OF HISTAMINE, 5-HT, AND SOME ACIDIC SUBSTANCES IN MAST CELLS IN CULTURE*

Exp.	Cell line	Histamine	5-HT	Taurine	Cysteic acid	Cerebroside sulfate	ATP	^{35}S-heparin
1	X-1	6·31	0·11	6·01	0·24	2·14	—	31,500
2	X-1	2·16	0·28	2·13	0·14	1·20	1·13	13,600
3	X-1	1·89	0·17	—	—	—	1·03	—
4	X-2	0·72	0·11	—	—	0·32	—	4,700
5	X-2	0·72	0·06	1·28	0·05	0·34	0·41	4,300
6	X-2	0·09	0·11	—	—	—	0·22	—

* All values are μmoles/10^9 cells or cpm/10^6 cells.

concentration of amines, as previously demonstrated.[10] Similarly, the levels of taurine, cysteic acid, cerebroside sulfate, and ATP reflected the levels of amines. No neuraminic acid could be detected in these cells from culture or in cells (0·4 g) grown as ascitic tumors in mice. The apparent relationship between the concentration of amines and of acidic substances may indicate (1) the need to preserve electroneutrality in the cell, (2) the presence of factor(s) that limits the levels of all these varied substances, and (3) an association between the amines and the acidic substances. These possibilities are being examined by experiments now in progress.

TABLE 2. CONCENTRATION OF SOME ACIDIC SUBSTANCES IN BOVINE PINEAL BODY*

Substance	Concentration
Neuraminic acid	5·85
Taurine	2·10
Cysteic acid	0·70

* Values are μmoles/g fresh tissue.

In the pineal body neither cerebroside sulfate, which is present primarily in white matter of brain, nor sulfomucopolysaccharides, which are present in gray matter,[13] could be detected. Table 2 shows that the pineal body had less taurine than whole rat brain (5·5 μmoles/g[20, 31]) but a greater concentration of neuraminic acid than bovine brain (gray matter has 3·92 μmoles/g[32]).

The presence of taurine in tissues rich in amines prompted a study of the effect of reserpine on the release of taurine from intact brain, spleen and heart. Twenty hr after the injection of reserpine (2·5 μg/g) the taurine content of these organs in two treated rats did not differ from that in two untreated rats.

REFERENCES

1. H. MCILWAIN, *Biochemistry and the Central Nervous System*, 2nd ed., p. 30. Churchill, London (1959).
2. G. G. J. DEFFNER and R. E. HAFTER, *Biochim. biophys. Acta* **42**, 200 (1960).
3. J. P. GREEN, *Advanc. Pharmacol.* **1**, 349 (1962).
4. J. CHEYMOL and F. BOURILLET, *J. Physiol., Paris* **51**, 433 (1959).
5. H. VELDSTRA, *Pharmacol. Rev.* **8**, 339 (1956).
6. N.-Å. HILLARP, in *Ciba Symposium on Adrenergic Mechanisms*, p. 481. Churchill, London (1960).
7. H. BLASCHKO, *Pharmacol. Rev.* **11**, (2) 307 (1959).
8. W. H. PRUSOFF, *Brit. J. Pharmacol.* **15**, 520 (1960).
9. G. V. R. BORN, O. HORNYKIEWICZ and A. STAFFORD, *Brit. J. Pharmacol.* **13**, 411 (1958).
10. J. P. GREEN and M. DAY, *Biochem. Pharmacol.* **3**, 190 (1960).
11. P. HAGEN, R. J. BARRNETT and F.-L. LEE, *J. Pharmacol.* **126**, 91 (1959).
12. T. T. ODELL, JR. and B. ANDERSON, *Proc. Soc. exp. Biol., N.Y.* **94**, 151 (1957).
13. J. D. ROBINSON, JR. and J. P. GREEN, *Yale J. Biol. Med.* In press (1962).
14. J. P. GREEN and J. D. ROBINSON, JR., *J. biol. Chem.* **235**, 1621 (1960).
15. J. P. GREEN, J. D. ROBINSON, JR. and M. DAY, *J. Pharmacol.* **131**, 12 (1961).
16. A. F. HARRIS, A. SAIFER and S. K. WEINTRAUB, *Proc. Soc. exp. Biol., N.Y.* **107**, 35 (1961).
17. J. P. GREEN and M. DAY, *Ann. N.Y. Acad. Sci.* In press (1962).
18. H. MORITA and T. OSADA, *Sang* **28**, 827 (1957).
19. A. ALBERT, *Biochem. J.* **47**, 531 (1950).
20. J. AWAPARA, *J. biol. Chem.* **218**, 571 (1956).
21. J. FRENDO, A. KOJ and J. M. ZGLICZYNSKI, *Nature, Lond.* **183**, 685 (1959).

22. E. SCHRAM, *Arch. int. Physiol.* **68**, 698 (1960).
23. D. R. CURTIS and J. C. WATKINS, *J. Neurochem.* **6**, 117 (1960).
24. N. J. GIARMAN and M. DAY, *Biochem. Pharmacol.* **1**, 235 (1958).
25. N. J. GIARMAN and D. X. FREEDMAN, *Nature, Lond.* **186**, 480 (1960).
26. B. B. BRODIE, E. O. TITUS and C. W. M. WILSON, *J. Physiol., Lond.* **152**, 20P (1960).
27. T. B. DUNN and M. POTTER, *J. nat. Cancer Inst.* **18**, 587 (1957).
28. R. SCHINDLER, M. DAY and G. A. FISCHER, *Cancer Res.* **19**, 47 (1959).
29. G. A. FISCHER and A. C. SARTORELLI, *Meth. med. Res.* **10**. In press (1963).
30. M. LEES, J. FOLCH, G. H. SLOANE-STANLEY and S. CARR, *J. Neurochem.* **4**, 9 (1959).
31. D. B. HOPE, *J. Neurochem.* **1**, 364 (1957).
32. L. WARREN, *J. biol. Chem.* **234**, 1971 (1960).