

EFFECT OF RESERPINE ON THE 5-HYDROXYTRYPTAMINE AND ADENOSINETRIPHOSPHATE OF THE DOG INTESTINAL MUCOSA

BY

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Homogenates were prepared of the mucous membrane of the small intestine from dogs previously treated with high doses of reserpine. The granular material sedimented from these homogenates was centrifuged over a density gradient. The dense fraction usually rich in adenosinetriphosphate and 5-hydroxytryptamine contained little amine and no nucleotide; on the other hand, the less dense material, believed to consist mainly of mitochondria, contained amounts of adenosinetriphosphate similar to those found in animals not treated with reserpine.

Observations have recently been reported (Prusoff, 1960) which suggest that in the mucous membrane of the dog small intestine the 5-hydroxytryptamine present is associated with adenosinetriphosphate. The granular fraction containing the mitochondria as well as that containing the storage elements for 5-hydroxytryptamine was rich in nucleotide. The "large" granule sediment prepared from a homogenate of the tissue in isotonic (0.3 M) sucrose was resolved by ultracentrifugation over a sucrose density gradient and the distribution of both 5-hydroxytryptamine and adenosinetriphosphate was studied. The observations of Baker (1959) were confirmed, in which much of the amine was recovered not in the true mitochondrial fraction but in a particulate layer of greater density below the mitochondria. The distribution of the adenosinetriphosphate differed from that of the amine: both the mitochondrial layer and the dense layer rich in amine contained adenosinetriphosphate. In three experiments the mean molar ratio, 5-hydroxytryptamine:adenosinetriphosphate, in the dense layer was found to be 2.6; this is of an order similar to that of about 4 found in the chromaffin granules of the adrenal medulla (Blaschko, 1959). This finding suggested that in the storage of amine in the intestine the part played by the nucleotide was analogous to its role in the storage of the catechol amines in the chromaffin tissue.

To obtain further information on the relationship between adenosinetriphosphate and stored amines it was decided to investigate particulate fractions of mucosa from the small intestine of dogs in which the 5-hydroxytryptamine content had been greatly reduced by previous administration of reserpine. The results obtained are in support of a close association of the amine with adenosinetriphosphate in this tissue.

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METHODS

Reserpine dissolved in a 20% solution of ascorbic acid was administered intraperitoneally to dogs on three successive days in a dose of 0.5 mg/kg body weight. On the fourth day pentobarbitone sodium was given intraperitoneally and the dog was bled. The small intestine was quickly removed and a homogenate of the mucous membrane prepared as already described (Prusoff, 1960). Following the separation of unbroken cells, nuclei and coarse cell debris by centrifugation at 900 *g* for 20 min, the supernatant was spun at 11,000 *g* for 30 min in order to sediment the non-microsomal cytoplasmic particles. The sediment was resuspended in 0.3 *M* sucrose and layered over a sucrose density gradient prepared the previous day (Fig. 1). The tubes for the Spinco ultracentrifuge swing-out head (SW39) contained at the bottom 1.0 ml. 2.0 *M* sucrose, with 1.0 ml. 1.6 *M* sucrose layered over this, and on the top 1.5 ml. 1.5 *M* sucrose solution. The fractions, after ultracentrifugation for 1 hr at 100,000 *g*, were collected with the help of the Schuster cutter.

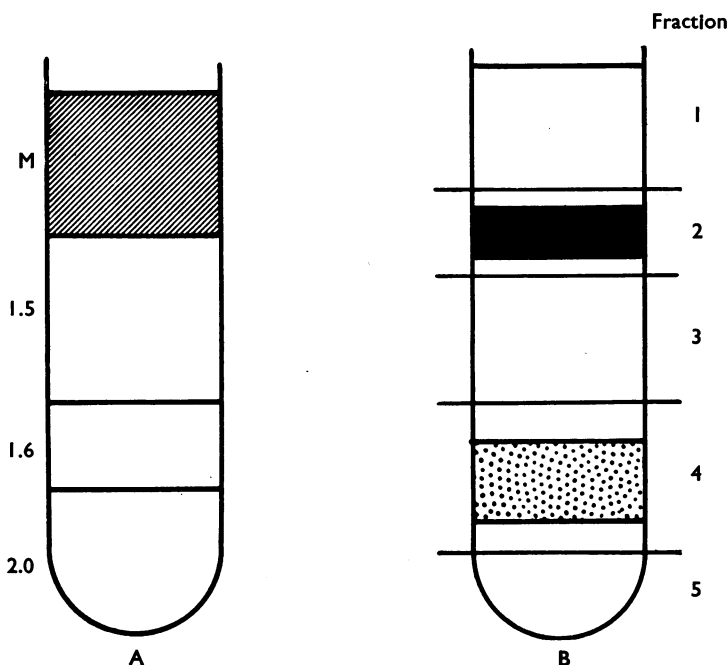


Fig. 1. Centrifugation of non-microsomal cytoplasmic particles obtained from the small intestine of dogs treated with reserpine. (A) Sucrose density gradient before centrifugation. (B) Distribution of particulates in density gradient after centrifugation for 60 min at 100,000 *g*.

5-Hydroxytryptamine was extracted with *n*-butanol by the method of Udenfriend, Weissbach & Clark (1955), as modified by Cargill-Thompson, Hardwick & Wiseman (1958) and Baker (1959), and assayed on the rat fundus preparation (Vane, 1957). Adenosinetriphosphate was estimated by the firefly luminescence method, as used by Holton (1959).

RESULTS

The two dogs on which these experiments were carried out showed the characteristic picture of animals treated with large doses of reserpine.

Although the appearance of the density gradient tubes after ultracentrifugation was essentially similar in both experiments, there were slight differences in the lower

half of the tube which determined slight variations in the choice of levels of cutting. In experiment no. 1 the appearance of the gradient tube was as shown in Fig. 1. There was a very dense and thick opaque layer at the top of the gradient (fraction 2); above this was a clear layer, corresponding to the vol. of fluid in which the suspension of large granules had been suspended before centrifugation (fraction 1). Below fraction 2 there was a relatively clear layer in which small amounts of opaque material were suspended; this was collected as fraction 3. Immediately below there was one well-defined, fairly narrow opaque layer in the 1.6 M sucrose layer (fraction 4). At the bottom of the tube, approximately corresponding to the 2.0 M sucrose layer, there was a clear area (fraction 5).

In the second experiment, fractions 1 and 2 each had the same appearance as in the first experiment, but below fraction 2 a slightly narrower clear zone (fraction 3) was followed by two bands of opaque material; these were therefore separately collected as fractions 4a and 4b; a similar distribution had occasionally been seen in earlier experiments. Fraction 5 was the clear area at the bottom of the tube, as in the first experiment.

TABLE 1
5-HYDROXYTRYPTAMINE (5HT) AND ADENOSINETRIPHOSPHATE (ATP) IN FRACTIONS OF SMALL INTESTINE OF RESERPINE-TREATED DOGS AFTER DENSITY GRADIENT CENTRIFUGATION

Concentrations expressed as n moles/g tissue

| <i>Expt. no. 1</i> Fraction no. | 5HT | ATP | Ratio: 5HT/ATP |
|---------------------------------------|-------|-------|-------------------|
| 1 | 0.001 | None | — |
| 2 | 0.069 | 1.0 | 0.069 |
| 3 | 0.026 | None | — |
| 4 | 0.026 | <0.05 | — |
| 5 | None | None | — |
| <i>Expt. no. 2</i> Fraction no. | 5HT | ATP | Ratio: 5HT/ATP |
| 1 | 0.075 | None | — |
| 2 | 1.64 | 2.54 | 0.65 |
| 3 | 0.07 | None | — |
| 4a | 0.30 | None | — |
| 4b | 0.25 | None | — |
| 5 | 0.46 | None | — |

The results of these experiments are shown in Table 1. In both experiments the total amounts of 5-hydroxytryptamine recovered were low (<4%) in comparison with those previously observed in non-reserpinized dogs. The degree of depletion achieved in experiment 1 was more severe. In particular, fraction 4 (or fractions 4a and 4b), usually rich in amine (Prusoff, 1960), contained no or little amine; in both experiments the amounts of amine recovered in fraction 2 were higher. The content of adenosinetriphosphate of fraction 2 was somewhat low but within the range seen in non-reserpinized dogs, whereas the amounts of nucleotide present in the fraction normally rich in 5-hydroxytryptamine (fraction 4) were extremely low. This is shown in Table 2, in which the adenosinetriphosphate contents of fractions 2 and 4 are given for three normal (Prusoff, 1960) and two reserpine-treated animals. It may be mentioned here that fraction 4 had not disappeared under the influence of reserpine: macroscopically an opaque layer could be seen of an appearance quite similar to that in the animals not treated with reserpine.

TABLE 2
EFFECT OF RESERPINE, ADMINISTERED *IN VIVO*, ON THE ADENOSINETRIPHOSPHATE (ATP) CONTENT OF "MITOCHONDRIA" AND "5-HYDROXYTRYPTAMINE (5HT)-RICH GRANULES" FROM THE DOG SMALL INTESTINE

| Treatment | Expt. no. | ATP in n moles/g tissue | | Ratio M/G |
|-----------|-----------|-------------------------|-----------------------|-----------|
| | | Mito-chondria (M) | 5HT-rich granules (G) | |
| None | 1 | 3.96 | 1.98 | 2.0 |
| None | 2 | 5.52 | 1.73 | 3.2 |
| None | 3 | 0.93 | 0.97 | 1.0 |
| Reserpine | 1 | 1.01 | <0.05 | >20 |
| | 2 | 2.54 | None | — |

DISCUSSION

Earlier experiments (Prusoff, 1960) had shown that by density gradient centrifugation two distinct fractions could be obtained from the mucous membrane of the dog's small intestine that were rich in adenosinetriphosphate. Only one of these fractions was also rich in 5-hydroxytryptamine. In this fraction the molar ratio, amine:adenosinetriphosphate, was similar to that found in the adrenal medulla. It was suggested that most of the nucleotide in this fraction was connected with amine storage, whereas the nucleotide in the upper layer of the density gradient was not associated with 5-hydroxytryptamine.

The present experiments support this interpretation. After treatment with reserpine the layer normally rich in 5-hydroxytryptamine contained even less amine than the "mitochondrial" layer. Table 2 shows that the nucleotide believed to be associated with 5-hydroxytryptamine had disappeared, whereas the adenosinetriphosphate content of the "mitochondrial" fraction had remained at normal levels. This is in agreement with the idea that only part of the total nucleotide is directly concerned with amine storage.

Although 5-hydroxytryptamine and adenosinetriphosphate contents of the fraction normally rich in amine had disappeared after reserpine treatment, there was no concomitant change in the opaqueness of this fraction. There are several possible explanations. The release of amine and loss of adenosinetriphosphate may occur without disruption of the granule. This would imply that the density of these granules is not due to their amine and/or nucleotide content. Another possibility is that this fraction is still heterogeneous and that the amine-carrying granules represent only a fraction thereof. Electron-microscopic examination of this fraction did in fact reveal a lack of homogeneity (Meek & Barer, personal communication).

In the chromaffin tissue of the adrenal medulla, loss of catechol amines after reserpine is accompanied by a loss in adenosinetriphosphate (Schümann, 1958; Kirpekar, Goodlad & Lewis, 1958; Hillarp, 1960; Burack, Weiner & Hagen, 1960). Thus, in both the chromaffin and the "enterochromaffin" cell, reserpine reproduces a loss in nucleotide as well as a loss of amine. This raises the possibility that the disappearance of adenosinetriphosphate is the common cause of the releasing action of reserpine, an idea already discussed by Bein (1957, 1960). It must be remembered, however, that reserpine also leads to a release of amine from blood platelets, but there appears to be no concomitant disappearance of adenosinetriphosphate

(Born, Ingram & Stacey, 1958). Nevertheless, although there is good reason to believe that in the platelets the nucleotide is also involved in storage of amine, the low molar ratio, 5-hydroxytryptamine:adenosinetriphosphate, makes it likely that in the platelets there is a great excess of nucleotide that may not be involved in amine storage.

The many analogies between the chromaffin and the enterochromaffin cells are strengthened by the results that have been described here.

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