CHANGES IN THE CONTENT OF 5-HYDROXYTRYPTAMINE IN THE ENTEROCHROMAFFIN CELLS OF THE DUODENUM IN EXPERIMENTAL DIPHTHERIA

B. M. Rozenman
and K. S. Shadurskii

UDC 616.931-092.9-07:616.342-008.
939.05-092.18

In severe attacks of diphtheria the content of 5-hydroxytryptamine (serotonin) rises in some organs and tissues, and especially in the adrenals [2]. In mammals, however, apart from the mast cells in rats and mice [11], and possibly also nerve tissue [10], the only structures capable of carrying out the complete biosynthesis of serotonin are the enterochromaffin cells of the gastrointestinal tract, one of the main sources of serotonin in the body [5, 6, 9, etc.]. The study of the histochemical changes in these cells may shed light on the character of the disturbances of serotonin metabolism occurring in diphtheria.

The object of the present investigation was to study the content of serotonin in the enterochromaffin cells of the duodenum in rabbits with diphtheria poisoning.

EXPERIMENTAL METHOD

Diphtheria poisoning was produced in rabbits by means of a single injection of diphtheria toxin into the blood stream in a dose of 0.0044 ml/kg (1 M. L. D. for the guinea pig is 0.0034 ml).

The enterochromaffin cells were detected by the Masson–Hamperl argentaffin reaction in a transverse section of the duodenum, 1 cm long and 6 μ thick, made from an area of the duodenum 1 cm from the pylorus. The adjacent portion of the duodenum was used for determination of the serotonin content on a strip of the fundus of the rat's stomach by Vane's method [15].

The preparations were examined under the high power of the microscope. The enterochromaffin cells were counted and classified in accordance with the number of visible granules: 1) cells with solitary, scattered granules; 2) cells in which 1/3 of the volume was occupied by granules; 3) cells in which 2/3 of the volume was occupied by granules; 4) cells completely packed with granules.

By addition of the products of the total number of cells in each state of granulation and the numbers 1, 2, 3, and 4 (corresponding to the previously established differences in the content of granules), the index of the overall loading of the cells of the section with argentaffin material (the gravimetric granulation index) was calculated. The saturation index [4] was determined as the ratio between the gravimetric granulation index and the number of enterochromaffin cells (the mean loading of these cells with granules of secretion).

Experiments were carried out on 8 healthy rabbits and 21 rabbits in various stages of poisoning (the characteristics of the phases were given in an earlier paper [2]).

EXPERIMENTAL RESULTS

In the healthy rabbits the enterochromaffin cells of the duodenum were arranged mainly singly in the region of the crypts of Lieberkuhn, in the epithelium covering the villi, less commonly in the Brunner's glands, and sometimes in the interstitial tissue of the mucous membrane. A section 1 cm in length contained 49±8.8 cells, mostly with only a few argentaffin granules (69% of the total number of cells detected; Fig. 1, a). The gravimetric granulation index was 72±15.6, the index of saturation 1.43±0.051, and the serotonin content of the duodenal tissue was 3.912±0.996 μg/g.

Department of Infectious Diseases, Belorussian Postgraduate Medical Institute and Belorussian Research Institute of Public Health (Presented by Active Member of the Academy of Medical Sciences of the USSR, S. R. Mardashev). Translation from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 63, No. 5, pp. 41-44, May, 1967. Original article submitted September 29, 1965.

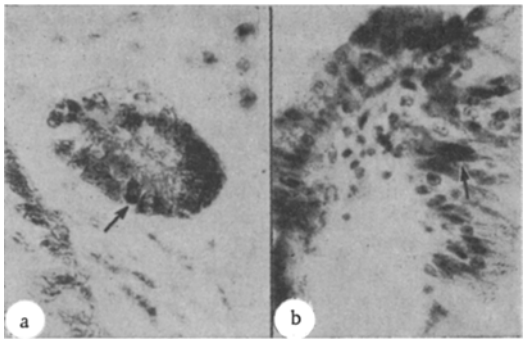

Fig. 1. Mucous membrane of the rabbits' duodenum: a) control animal; b) rabbits sacrificed in phase 1 of diphtheria poisoning. The arrows point to enterochromaffin cells. Masson–Hamperl method. 900×.

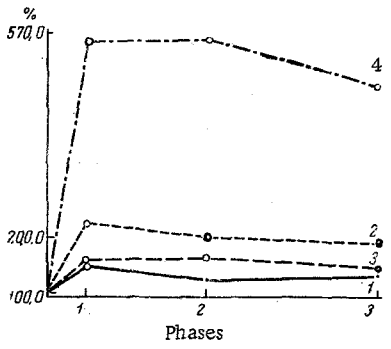

Fig. 2. Changes in the number of enterochromaffin cells detected (1), in the gravimetric granulation index (2), the index of saturation (3), and the serotonin concentration (4) in the duodenal tissue of rabbits poisoned with diphtheria toxin (in % of control). Where the difference from the control value is statistically significant, the corresponding point is indicated by a circle.

In phase 1 of poisoning the total number of enterochromaffin cells detected was increased by 51% on account of cells saturated with argentaffin granules to the level of groups 2-4 (68% of the total number of cells, compared with 31% in the control), and including those in which the granules were extremely densely packed, so that the impression of a discrete mass was lost (Fig. 1, b). This resulted in an increase in the index of saturation (by 61%) and, in particular, in the gravimetric granulation index (by 130%).

In phases 2 and 3 the number of enterochromaffin cells fell slightly, as a result of which the difference from the control values were not statistically significant. The gravimetric granulation index and the index of saturation were much higher than normal. The serotonin concentration in the duodenal tissue (determined by Vane's method) corresponded to the changes in the index of saturation and in the gravimetric granulation index.

However, on comparing the index of saturation in phases 2 and 3 of diphtheria poisoning a statistically significant decrease was found in the mean loading of the cells with argentaffin material in phase 3 (Fig. 2).

In parallel investigations conducted on the same laboratory animals the content of serotonin in the other tissues was found to increase much later, in phase 2 or 3 of poisoning [2]. The selective accumulation of serotonin in the enterochromaffin cells only, in phase 1, cannot therefore be explained by the blocking of monoamine oxidase or by the activation of decarboxylation of 5-hydroxytrptophan (5-HTP), because under the influence of monoamine oxidase inhibitors serotonin accumulates in various organs and tissues [11, 16, etc.]. while 5-HTP-decarboxylase is found in most mammalian tissues [9, 12, 13], where decarboxylation of 5-HTP also takes place with the participation of the enzyme [6, 8].

The early accumulation of serotonin in the enterochromaffin cells was evidently caused by the specific functional properties of these cells, which carry out the complete biosynthesis of serotonin. In other words, it was dependent on activation of the oxidation of l-tryptophan into 5-HTP by the specific enzyme l-tryptophan-5-oxidase [7, 14], which has an extremely limited distribution, as a result of which the oxidation of l-tryptophan and not the decarboxylation of 5-HTP is the controlling stage in the biosynthesis of serotonin [10].

Consequently, under the influence of diphtheria toxin, the accumulation of 5-HTP took place in the body.

Unlike l-tryptophan-5-oxidase, 5-HTP-decarboxylase is not a specific enzyme and it can act on other substrates besides 5-HTP [10]. One such substrate in the adrenals is 3, 4-dihydroxyphenylalanine (DOPA), one of the precursors of adrenalin. Meanwhile, 5-HTP competitively inhibits the decarboxylation of DOPA. Conversely, DOPA (like dopamine, noradrenalin, and adrenalin) inhibits the decarboxylation of 5-HTP (and its conversion into 5-hydroxytryptamine or serotonin). In other words, DOPA and 5-HTP compete in the adrenals for the same enzyme. After the injection of 5-HTP into the blood stream of animals, a considerable amount of serotonin is formed in the adrenals, and in the same granules, moreover, as those in which adrenalin accumulates [10].

From the observations described above, the following hypothesis may be put forward concerning the mechanisms of the disturbance of the biosynthesis of serotonin and the catecholamines in diphtheria poisoning.

Excitation of the sympathetic–adrenal system at the beginning of the action of the toxin triggers off the homeostatic mechanisms of the body and its accompanied by an increase in the synthesis and secretion of adrenalin by the adrenals [1, 3]. The accumulation of DOPA in these conditions inhibits the decarboxylation of 5-HTP, as a result of which the content of serotonin in the adrenals does not increase in phase 1 of poisoning, despite the excess of 5-HTP formed in the enterochromaffin cells in increased amount.

Conversely, at the end of poisoning, competition between DOPA and 5-HTP for DOPA-decarboxylase is resolved in the adrenals in favor of 5-HTP (for example, as the result of accumulation of 5-HTP in the adrenals or, of course, a decrease in the synthesis of DOPA), and the serotonin content consequently rises sharply [2] in these glands while the synthesis of adrenalin diminishes [1, 4].

LITERATURE CITED

1. V. D. Akhnazarova, Lesions of the Adrenals in Experimental Diphtheria Poisoning. Candidate dissertation, Moscow (1953).
2. B. M. Rozenma, Byull. Éksp. Biol., No. 3, 54 (1965).
3. K. F. Samsonova-Firsova, The Pathogenesis of Diphtheria Poisoning. Doctorate dissertation Kazan' (1960).
4. V. I. Talapin, The Effect of Long-Acting Hypotensive Drugs–Dimecarbine and Phemedol–on the Enterochromaffin Cells of Guinea Pigs and Albino Rats (Experimental Invesigation). Candidate dissertation Minsk (1964).
5. E. O. Benditt and R. I. Wong, J. Exp. Med., 105, 509 (1957).
6. G. Bertaccini, J. Neurochem., 4, 217 (1959).
7. C. T. Clark et al., J. Biol. Chem., 210, 139 (1954).
8. J. R. Cooper and L. Melcer, J. Pharmacol. Exp. Ther., 132 (1961).
9. V. Erspamer, Pharmacol. Rev., 6, 425 (1954).
10. B. Idem, in the book: Progress in Drug Research, New York, (1961) Vol. 3, p. 154.
11. D. Lagunoff and E. O. Benditt, Am. J. Physiol 196, 933 (1959).
12. G. P. Lewis (ed.), 5-Hydroxytryptamine, Symposium, London (1957).
13. B. Maupin, Biol. Med. (Paris), 49, 75 (1960).
14. C. Mitoma, Arch. Biochem., 63, 122 (1956).
15. J. R. Vane, Brit. J. Pharmacol., 12, 344 (1957).
16. H. Weisbach et al., J. Pharmacol, Exp. Ther., 131, 26 (1961).