INVESTIGATION OF METABOLIC MECHANISMS OF THE ISOLATED AND COMBINED ACTION OF A CHEMICAL ALLERGEN

R. V. Merkur'eva, E. I. Protsenko,
L. I. Bushinskaya, B. V. Aulika,
A. A. Kulygina, and Yu. I. Prokopenko

UDC 612.015.3-06:612.017.1

Biochemical investigation of the activity of enzyme systems located variously in the cell, either bound with lysosomes (hyaluronidase, N-acetyl-β-D-glucosaminidase, β-glucosidase) and soluble in hyaloplasm (neuraminic acid aldolase), and the study of the state of enzyme–substrate complexes of the immunologically active carbohydrate-containing biopolymer class (glycoproteins, glycosaminoglycans) were carried out in tissues of various organs (liver, kidney, small intestine, skin) and blood serum of albino rats exposed to the isolated and combined (with various doses of ultraviolet radiation) action of a chemical allergen, dinitrochlorobenzene. General and specific rules governing the metabolic reactions whose manifestation may be connected with the development of allergy of delayed type were discovered.

The investigation of the biochemical mechanisms of action of allergens acting either in isolation on the organism or in combination with physical factors may help to broaden our ideas of the rules governing the metabolic changes that characterize the development of sensitization. Such an investigation could provide a theoretical basis for the establishment of hygienic norms for the action of the corresponding external environmental factors of man.

An important role in the development of the immunological reactions of the organism is played by enzyme systems bound with the lysosomes [11, 13]. Most acid hydrolases of lysosomal origin are also known to be glycoproteins in their chemical structure, and they thus belong to an immunologically active class of proteins [16, 17].

The object of this investigation was to study enzyme systems located variously in the cell (bound with lysosomes, soluble in the cytosol), and also of enzyme–substrate complexes (carbohydrate-containing biopolymers) using an experimental model of the action of a chemical allergen in isolation and also in combination with a physical factor, namely various doses of ultraviolet irradiation (UV). In the modern view, UV can either depress or intensify the phenomena of sensitization (depending on the dose used). Information in the literature on the effect of UV irradiation on lysosomal enzymes is limited in amount and contradictory in nature [9, 18].

EXPERIMENTAL METHOD

Five groups of albino rats (54 animals altogether) were used. The animals of group 1 were exposed to the continuous action of a threshold dose of a chemical allergen, dinitrochlorobenzene (DNCB, 19.6 mg/kg by mouth); the animals of groups 2 and 3 exposed to the combined action of the same concentration of DNCB and of various doses of UV irradiation (0.75 and 3 erythema doses respectively); and the rats of group 4 received 0.75 erythema dose of UV irradiation only. The duration of the experiment was 30 days. The liver, kidneys, small intestine, skin, and blood serum of the animals were used as the test objects. At the end of exposure to

Laboratory of Biochemistry and Laboratory of Radiant Energy, A. N. Sysin Institute of General and Communal Hygiene, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. I. Sidorenko.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 10, pp. 1221-1223, October, 1976. Original article submitted February 20, 1976.

the various factors activity of the following lysosomal hydrolases was determined: hyaluronidase [12], N-acetyl-β-D-glucosaminidase [1], and β-glucosidase [7]. Activity of the enzymes was determined in liver homogenate and in the lysosomal fraction with or without the addition of the detergent Triton X-100 [8], so that the degree of stability of the bond between the enzymes and the lysosomal membranes could be judged. To investigate the enzyme–substrate complex glycosaminoglycans–hyaluronidase–N-acetyl-β-D-glucosaminidase, besides the enzymic methods indicated above, hexuronic acids also were determined [14], for their level reflects the total concentration of glycosaminoglycans. The state of glycoprotein metabolism was judged from the carbohydrates in their composition (hexoses, aminosugars) [5], and by the enzyme–substrate system: neuraminic acid (NA)–NA aldolase [4]. Hyaluronidase activity and the content of protein-bound carbohydrates of glycoproteins (hexosamines, NA, hexoses) in the blood serum of the rats was determined by methods indicated above. Considering that the ratio between catabolic and energetic processes in the blood cells can serve as a cytochemical criterion of allergy of delayed type [10], the activity of acid phosphatase [15], one of the lysosomal enzymes, and also of succinate dehydrogenase [6] in the blood lymphocytes was determined cytochemically.

The serum hyaluronidase activity was expressed in conventional units. The unit of activity was taken to be the quantity of N-acetylglucosamine liberated by the action of a 5% solution of bovine testicular hyaluronidase (330 USP units/mg, Reanal, Hungary) from hyaluronic acid during incubation for 18 h at 37°C in acetate buffer, pH 3.7.

EXPERIMENTAL RESULTS

In response to the isolated action of the chemical allergen (DNCB) while no change occurred in the activity of the lysosomal glycanohydrolases (hyaluronidase and N-acetyl-β-D-glucosaminidase) in the rats' liver a statistically significant ($P < 0.01$) accumulation of glycosaminoglycans was observed (as reflected in the hexuronic acid level), which underwent gradual hydrolytic degradation through the action of those enzymes. This relationship in the enzyme–substrate system suggests intensification of glycosaminoglycan synthesis in the liver, more especially because an increase in the content of other representatives of the carbohydrate components of glycosaminoglycans was found simultaneously in the liver and the small intestine, namely hexosamines, the level of which on average was 75% higher than in the rats of the control group. Meanwhile there was a statistically significant ($P < 0.001$) decrease in hyaluronidase activity in the blood serum to 0.89 ± 0.1 unit compared with the control (1.38 ± 0.1 unit). The disturbance of glycosaminoglycan metabolism was accompanied by opposite changes in the content of glycoprotein carbohydrates: a decrease in the NA level, especially in the small intestine (by 70%), and the accumulation of protein-bound hexoses in the liver of the experimental animals (20% higher than in the control). These changes possibly reflect quantitative or structural reorganization of the polysaccharide moiety of the immunoglobulins participating in the phenomena of sensitization.

The combined action of DNCB and 0.75 erythema dose of UV irradiation was accompanied by the development of a metabolic reaction not observed when these factors acted in isolation: a statistically significant increase in hyaluronidase activity in the tissue of the small intestine (by 57%) compared with the rats of the control group. This fact could possibly be explained by the action of an adaptive mechanism linked with the biological role of hyaluronidase in tissue and vascular permeability. Meanwhile the decrease in the level of hexuronic acids in the small intestine and liver (by 18.5 and 17.9% respectively) and also in the level of protein-bound carbohydrates, namely hexoses (on the average by 36% in the liver) and NA (by 22, 39, and 28% in the kidneys, small intestine, and liver respectively) may be attributable to disturbance of the metabolism of carbohydrate-containing biopolymers; the normalizing effect of UV irradiation, in the dose used, on the metabolic changes caused by the action of the chemical allergen was not observed in this case.

The effect of the combined action of DNCB and a higher dose of UV irradiation (3 erythema doses) on individual enzyme systems varied. For instance, it caused no significant changes in hyaluronidase activity in the liver, small intestine and blood serum, in β-glucosidase activity in the liver, and in acid phosphatase activity in the blood lymphocytes. Meanwhile no solubilization effect of the β-glucosidase of the liver lysosomes was found. At the same time, a metabolic reaction not previously manifested during the isolated action of these two factors was established: a decrease in N-acetyl-β-D-glucosaminidase activity in the liver of the experimental rats (by 46%; $P < 0.01$). This phenomenon was combined with a decrease in activity of an enzyme not bound with the subcellular structures of the cell, NA aldolase (a soluble enzyme of the cytosol), in the liver (by 65%) and by a fall of the NA level (by 90%) compared with the corresponding values in the rats of the control group.

A disturbance of glycoprotein metabolism, manifested as a change in the content of protein-bound carbohydrates, must evidently thus be included among the general metabolic reactions of the organism to a chemical

allergen. Since many members of the carbohydrate-containing biopolymer group are immunologically active proteins and, in the opinion of some workers, natural inhibitors of the immunological response [2, 10], these structural changes in the carbohydrate moiety of the glycoproteins may be connected with the development of allergy of delayed type. Indirect evidence of the onset of such allergy in response to the combined action of DNCB and high doses of UV irradiation is given by cytochemical observations pointing to opposite changes in the activity of catabolic enzymes (increased acid phosphatase activity) and enzymes of energy metabolism in the lymphocytes (depression of succinate hydrogenase activity) in individual animals. The biological significance of the role of lysosomal enzymes in immunological reactions evidently lies in the important role of lysosomal hydrolases (at the level of the blood cells and of vitally important organs and systems as a whole) in the enzymic breakdown of substances with antigenic properties.

LITERATURE CITED

1. G. V. Vikha, E. D. Kaverzneva, and A. Ya. Khorlin, Biokhimiya, No. 1, 33 (1971).
2. I. E. Kovalev, I. D. Ionov, and A. A. Burkin, Zh. Mikrobiol., No. 1, 18 (1974).
3. L. K. Katosova, "Cyto- and histochemical study of lymphocytes and certain organs during immunization and experimental hypersensitivity of delayed type," Author's Abstract of Candidate's Dissertation, Moscow (1971).
4. R. V. Merkur'eva and E. A. Bazanov, Vopr. Med. Khim., No. 1, 95 (1968).
5. R. V. Merkur'eva, T. Ya. Balaba, and L. A. Povel'nenko, Vopr. Med. Khim., No. 2, 118 (1969).
6. R. P. Nartsissov, Arkh. Anat., No. 5, 85 (1969).
7. A. A. Pokrovskii, in: V. S. Asatiani, Enzymic Methods of Analysis [in Russian], Moscow (1969), p. 471.
8. A. A. Pokrovskii, M. Ya. Kon', and V. N. Solov'ev, Byull. Éksp. Biol. Med., No. 3, 49 (1974).
9. K. A. Samoilova, in: Ultraviolet Radiation [in Russian], Moscow (1971), pp. 98-104.
10. E. N. Tareev, V. V. Sura, V. T. Troyanova, et al., Sov. Med., No. 9, 5 (1969).
11. E. L. Becker, H. S. Showell, P. M. Henson, et al., J. Immunol., 112, 2047 (1974).
12. W. M. Bonner and J. Cantey, Clin. Chim. Acta, 13, 746 (1966).
13. R. T. Dean, Biochem. J., 138, 407 (1974).
14. Z. Dische, J. Biol. Chem., 167, 189 (1947).
15. A. F. Goldberg and T. Barka, Nature, 195, 297 (1962).
16. A. P. Kendal, F. Minuse, H. F. Maassab, et al., Am. J. Epidem., 98, 96 (1973).
17. C. Lombart, T. Okumura, and G. A. Jamieson, FEBS Letters, 41, 30 (1974).
18. J. F. Woessner, in: International Review of Connective Tissue Research (ed. by D. Hall), Vol. 3, Academic Press, New York (1965), pp. 232-236.