METABOLIC DISTURBANCES AND PLASMORRHAGIA INTO MYOCARDIAL CELLS INJURED BY ADRENALIN

A. K. Nanaev and M. I. Klibaner

UDC 616.127-02:615.357.452

Serial sections through the myocardium of rats killed 1-24 h after an injection of adrenalin were used. The results of histochemical reactions for succinate dehydrogenase (test for cell injury) were compared with the results of fibrin determination by the Coons' method. The presence and character of plasmorrhagia into irreversibly damaged muscle cells, giving the characteristic appearance in the reaction with nitro-BT, and the absence of fibrin in fibers with fatty degeneration, reflected in coarse-grained formazan deposits, were demonstrated. On the basis of the results it is postulated that the reversible and irreversible injuries to the myocardium produced by adrenalin differ in their pathogenesis.

KEY WORDS: adrenalin injury to the myocardium; plasmorrhagia; succinate dehydrogenase; fibrinogenesis.

Diffusion of plasma proteins into myocardial cells during necrobiosis and necrosis has been demonstrated immunomorphologically [5]. Since plasma proteins do not penetrate into intact muscle cells [8] the suggestion has been made [5] that plasmorrhagia is connected with a disturbance of cell metabolism. Changes in oxidative processes during experimental myocardial injury following injection of catecholamines have been found during the first minutes or hours of the experiment by histochemical tests for the detection of activity of oxidoreductases [4, 9].

The object of this investigation was to compare the dynamics of plasmorrhagia into myocardial cells with changes in their metabolism as reflected in succinate dehydrogenase (SDH) activity.

EXPERIMENTAL METHOD

Injury to the myocardium was produced in noninbred male albino rats by subcutaneous injection of 0.1% solution of adrenalin hydrochloride in a dose of 0.2 ml/100 g body weight. Altogether 35 animals were used; 10 intact rats acted as the control. The rats were decapitated 1, 3, 6, 12, and 24 h after receiving the injection of adrenalin. The heart was immediately removed, placed in melting ice, and divided in the frontal plane into two halves. One half was fixed in 10% neutral formalin and embedded in paraffin wax, and sections cut from it were stained with Toluidine Blue and Schiff's reagent by McManus' method, with an α-amylase control. The other half was frozen in liquid nitrogen and serial sections were cut from it in a cryostat for immunomorphological and histoenzymological examination. In each series of five sections four (3 μ in thickness) were processed by the indirect Coons' method, and the reaction for SDH with nitro-BT was carried out [10] on the fifth section (thickness 7 μ), followed by staining for lipids with Oil Red 0. For the immunomorphological investigation sections were treated with rabbit antisera against mouse fibrinogen, and pure donkey antibodies against rabbit γ-globulin were used. The presence of common antigenic determinants in mouse and rat fibrinogen [3] made it possible to use antiserum against mouse fibrinogen to detect the antigen in rat tissue. The antisera used formed a single precipitation band with the corresponding antigen in Ouchterlony's double diffusion test in agar and immunoelectrophoresis. Control sections were treated with nonimmune rabbit serum.

Laboratory of Age Pathology, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. I. Strukov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 2, pp. 248-250, February, 1976. Original article submitted March 27, 1975.

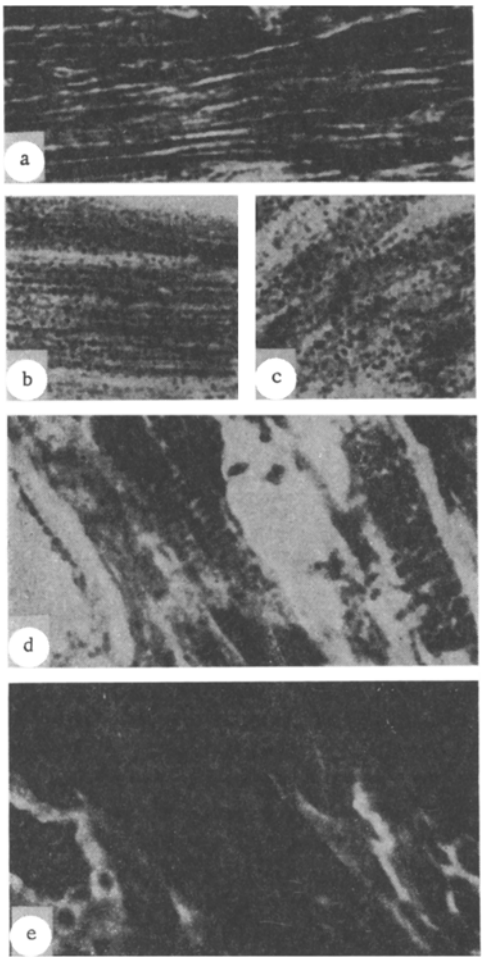

Fig. 1. Myocardial muscle cells after injection of adrenalin. a) Myocardium of control rat: predominance of linear formazan deposits (reaction for SDH; 500×); b) 1 h after injection of adrenalin: weakening of staining of linear formazan deposits and appearance of fine granules of formazan (reaction for SDH; 500×); c) 3 h after injection of adrenalin: haphazardly arranged coarse-grained formazan deposits (reaction for SDH; 500×); d) "striped" muscle cell (right) 3 h after injection of adrenalin, (left) blood vessel (reaction for SDH; 250×); e) same area (serial section) after treatment with rabbit antiserum against mouse fibrinogen by indirect Coons' method: left – blood vessel; fluorescence mainly of sarcolemmas of injured cells (250×).

EXPERIMENTAL RESULTS

After injection of adrenalin the appearance of the formazan deposits in the muscle cells changed. Some weakening of staining of the so-called linear formazan deposits, which predominate in the intact myocardium (Fig. 1a), was observed 1 h after the beginning of the experiments and there was a corresponding sharp increase in the number of fine formazan granules (Fig. 1b). This change in the character of deposits of the reaction products means increased enzyme activity compared with the control [1]. No specific fluorescence of fibrinogen (fibrin) was found at this time.

After 3 h, against the background of cells with increased enzyme activity, muscle fibers and groups of fibers were seen in which formazan was present as coarse-grained deposits (Fig. 1c). After 12 and 24 h the formazan granules increased in size and decreased in number, and staining for lipids always revealed an accumulation of lipid drops in these cells. The picture described is evidence of lower SDH activity than in the control [1]. Specific fluorescence of fibrin was absent in the cells.

At the same time (3 h) and in the same parts of the myocardium deformed areas of muscle fibers were found in which zones of a strong but diffuse reaction with nitro-BT alternated with colorless zones, giving them a striped appearance (Fig. 1d). The number of these areas increased toward the end of the first day. By this time the staining of some of these foci was reduced in intensity or had disappeared completely. Deformed cells ("striped" and colorless) stained a deep homogeneous crimson color with Schiff's reagent at all times of the experiment; the staining was not prevented by preliminary incubation of the sections with α-amylase. According to several workers [2, 4, 5], these cells are in a state of necrobiosis or necrosis.

By the use of the Coons' method specific fluorescence of sarcolemmas of the "stripped" cells was observed after 3 h (Fig. 1e). After 6 h the fluorescence in the region of the sarcolemma was stronger and individual areas of sarcoplasm also gave fluorescence. By 12 h these areas were larger, they had joined together, and the intensity of fluorescence had increased. After 24 h the muscle cells gave diffuse specific fluorescence of their whole sarcoplasm.

Comparison of the results of the histochemical and immunomorphological investigations shows that the sharp fall in oxidative processes occurring immediately after the phase of activation of metabolism in some cells, accompanied by accumulation of neutral lipids in their sarcoplasm as a result of a temporary disturbance of the utilization of fatty acids [2, 4], is not accompanied by plasmorrhagia. Plasmorrhagia was observed only in irreversibly injured cells showing the characteristic appearance in the reaction with nitro-BT. According to observations by Semenova and Martynyuk [6], disappearance is due to contracture of the myofibrils and redistribution of mitochondria in these cells.

The sharp distance between the reversibly and irreversibly injured cells as regards structure and histochemical and histoenzymological properties, is confirmed both by the results of the present investiga-

tion and data in the literature [2, 7]. This suggests a different pathogenesis for the reversible and irreversible injuries caused by injection of adrenalin. From this point of view a fact of special importance is the discovery of initial fibrin deposits along the sarcolemma of cells in which features of contraction and necrobiosis are observed. Localization of this plasma protein in this manner may perhaps lead to serious disturbances of nutrition of the cell, followed by its death. The cause of this selective deposition of fibrin on the sarcolemma of some injured cells and the absence of this phenomenon in other equally injured cells (fatty degeneration) is not yet clear. The results are in agreement with the opinion expressed by Rona and co-workers [11], who consider that the penetration of fibrin into the myocardial cell is an early sign of irreversibility of its injury.

LITERATURE CITED

1. K. M. Danilova, M. I. Klibaner, and L. I. Bardina, Byull. Éksperim. Biol. Med., No. 8, 111 (1972).
2. K. M. Danilova, M. I. Klibaner, and L. I. Bardina, Arkh. Pat., No. 8, 34 (1974).
3. A. K. Nanaev, in: Laboratory Animals in Medical Research [in Russian], Moscow (1974), pp. 139-141.
4. N. A. Probatova, Arkh. Pat., No. 7, 11 (1965).
5. V. S. Rukosuev, Arkh. Pat., No. 6, 16 (1970).
6. L. A. Semenova and R. A. Martynyuk, Byull. Éksperim. Biol. Med., No. 6, 107 (1972).
7. Yu. G. Tsellarius and L. A. Semenova, Histopathology of Focal Metabolic Injuries of Myocardium [in Russian], Novosibirsk (1972).
8. S. Freeman et al., Aust. J. Exp. Biol. Med. Sci., 44, 539 (1966).
9. A. Hecht, Einführung in Experimentelle Grundlagen Moderner Herzmuskelpathologie, Jena (1970).
10. M. M. Nachlas et al., cited by A. G. E. Pearse, Histochemistry: Theoretical and Applied [Russian translation], Moscow (1962), p. 844.
11. G. Rona et al., in: Present Status of Thrombosis (1973), p. 21.