DEGENERATIVE AND NECROBIOTIC CHANGES IN THE MYOCARDIUM INDUCED BY PERFUSION OF THE ISOLATED RAT'S HEART

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Coronary perfusion of the isolated rat's heart with oxygenated Hanks' solution containing glucose for 1-1.5 h led to the development of typical degenerative and necrobiotic changes in individual myocardial cells. These changes progressed with continued perfusion. On the addition of amino acids, noradrenalin ($4 \cdot 10^{-8}$ g/ml), and insulin (0.08 unit/ml) to the balanced salt solution, the principal cellular forms of injury were contractural changes and acute cloudy swelling of the myocardial cells.

The study of the character of degenerative and necrobiotic processes developing in the myocardium of the isolated perfused heart is interesting as a means of elucidating the role of extracardial factors and of plasma imbibition in the development of acute focal ("metabolic") lesions of the myocardium. In some recent papers, changes in the ultrastructure of the myocardium obtained in the isolated heart after alteration of the potassium and calcium ion concentrations in the perfusion fluid [8, 11, 12, 17] of ischemia and hypercapnia [15] have been described. However, the possibility of structural changes in the myocardium associated with perfusion of the isolated heart itself has not been properly evaluated, although Anichkov [1] found definite histological changes in the isolated heart after perfusion for only 30 min.

In the investigation described below, to study changes in the myocardium during normal perfusion of the isolated rat's heart, special polarization-microscopic and histochemical criteria developed for use in the early diagnosis of degenerative and necrobiotic changes in the myocardial cells in experiments in vivo [2-5] were used.

EXPERIMENTAL METHOD

Isolated hearts taken under acute experimental conditions under Nembutal anesthesia from 54 albino rats weighing 120-180 g were perfused via the coronary circulation for periods ranging from 5 min to 4 h with oxygenated physiological saline at 37°C. One of the following solutions was used as the perfusion fluid: Hank's solution (balanced salt solution with glucose in a concentration of 1 g/liter), Hank's solution with noradrenalin in a concentration of $4 \cdot 10^{-8}$ g/ml, medium No. 199 (balanced salt solution containing an assortment of amino acids, vitamins, and metabolites), and medium No. 199 with noradrenalin ($4 \cdot 10^{-8}$ g/ml) and insulin (0.08 unit/ml). To stabilize the noradrenalin, ascorbic acid (20 mg/liter) and sodium ethylenediamine tetraacetate (10 mg/liter) were added to the perfusion fluid as recommended by Iversen [9].

After the end of perfusion the heart was stopped with cold physiological saline and fixed for 48 h with 10% neutral formalin. To detect the enzymes, half of the heart was frozen at the temperature of liquid nitrogen. Paraffin sections, 6 μ in thickness were stained with hematoxylin-eosin and with picrofuchsin by

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Fig. 1

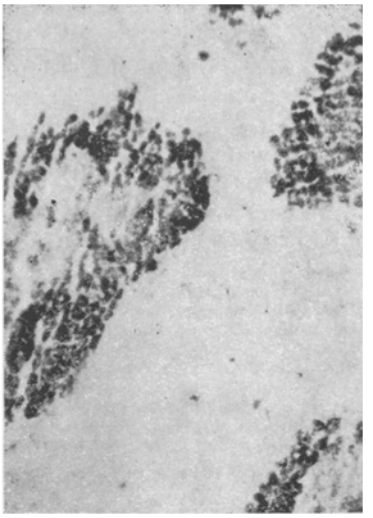

Fig. 2

Fig. 1. Area of overstretching of muscle fibers, with anisotropic disks a considerable distance apart. Photographed in polarized light, objective 25×, ocular 6.3×.

Fig. 2. Reaction for phosphorylase, positive only in some areas of the myocardium.

Fig. 3. Small foci of injury to the myocardium. Myocardial cells in a state of granular degeneration. Photographed in polarized light, objective 25×, ocular 6.3×.

Van Gieson's method; the PAS reaction was carried out in conjunction with staining for acid polysaccharides with colloidal iron and the PAS reaction was also performed after treatment of the section with diastase. Succinate dehydrogenase was demonstrated in frozen sections by Burstone's method [7], nonspecific acid phosphatase by one-stage azo-coupling using AC-BC naphthol phosphate and fast red GG, nonspecific alkaline phosphatase was demonstrated by one-stage coupling with AC-MX naphthol phosphate and fast red TP, and phosphorylase "a" and "b" and phosphorylase kinase were detected by means of modified Takeuchi's medium [7] and staining of the glycogen synthesized after incubation of the section by the PAS reaction. Stained and unstained sections were studied in polarized light.

EXPERIMENTAL RESULTS

Changes in the myocardial cells, consisting of bands of condensation formed by strongly contracted areas of the cell, with increased anisotropy and increased affinity for acid and basic dyes, were found in isolated hearts perfused with Hank's solution and with medium No. 199 for not more than 5 min. Up to three such bands could be seen in one muscle cell, and between them the normal transverse striation with appreciably weakened anisotropy was still present. These changes in the myocardial cells were definitely related to the procedure of isolation of the heart and were evidently easily reversible, because they were virtually absent during more prolonged perfusion.

Perfusion of the isolated hearts with Hank's solution for 1-1.5 h led to the appearance of contractural changes of degree I-II, in the classification of Tsellarius et al. [5], in individual myocardial cells. In the neighboring muscle cells, areas of overstretching, with a marked increase in the distance between the anisotropic disks (Fig. 1) were frequently seen. After perfusion for 2-4 h, the damage to the myocardium was more severe in character: cells were found in stage III of contracture and with acute granular degeneration. This was characterized by the breaking up of the anisotropic substance into irregularly shaped

granules, and loss of the transverse striation. The reaction for phosphorylase in hearts perfused with Hank's solution for more than 1 h was positive only in the subepicardial zone and in individual cellular regions of the remaining parts of the myocardium (Fig. 2). The reactions for succinate dehydrogenase and the nonspecific phosphatases showed no change from those in the intact heart.

If the isolated hearts were perfused with Hank's solution containing noradrenalin in a near-physiological concentration, contracture of degree III and acute granular degeneration were found after perfusion for 1-1.5 h. The number of damaged cells increased with the duration of perfusion. Muscle cells showing contractural changes stained deeply with eosin but did not give a positive PAS reaction and were resistant to diastase. When medium No. 199 was used for perfusing the heart, small foci of injury consisting mainly of myocardial cells in a state of acute granular degeneration (Fig. 3) were formed in the myocardium after perfusion for 2 h. Addition of noradrenalin and insulin to medium No. 199 led to an increase in the number of myocardial cells in a state of granular degeneration, and the degeneration itself was more severe and was evidently irreversible in character.

The degenerative and necrobiotic changes in the myocardial cells described above developed in the isolated heart exposed to no special pathogenic agent. The appearance of the first microscopic signs of degenerative and necrobiotic changes in the myocardium of the isolated heart 1-1.5 h after the beginning of perfusion suggests that these changes are the morphological equivalent of a disturbance of the metabolic stability of the myocardium, which, according to some investigators [6, 10, 13, 14, 18], invariably arises at this period. Loss of the functional stability of the isolated, perfused heart is also associated with these metabolic disorders. The use of solutions containing noradrenalin, insulin, and amino acids, in concentrations similar to those used in the present investigation, for the perfusion of the isolated heart stabilized the myocardial metabolism to some extent and had a marked effect on the processes of excitation and contraction [9, 16, 18]. On this basis, a decrease in the intensity of the damage to the myocardial cells would be expected. However, the opposite picture was observed – after the use of perfusion fluid containing noradrenalin, insulin, and amino acids, more severe forms of damage to the muscle cells, with the formation of small foci and involvement of a larger number of cells in the degenerative process, took place at a more rapid rate in the isolated heart. This paradoxical phenomenon was observed against the background of an obvious improvement in the contractile activity of the isolated heart, assessed on the basis of its appearance. Hence it can be concluded that the functional and structural disturbances of the isolated heart are based on different metabolic disorders. Those metabolic changes connected with disturbance of excitation and contraction processes are abolished by the addition of noradrenalin, insulin, and amino acids to the perfusion fluid. Those metabolic changes directly causing the development of degenerative changes in the muscle cells still persist. The increased intensity of function of the isolated heart, despite the continued operation of the factors causing the development of degenerative changes, can account for the greater severity of damage to the myocardium.

Observations on the degenerative and necrobiotic changes developing in the isolated perfused heart emphasize the nonspecific character of these changes, the absence of any connection between the nature of the pathogenic factor acting in the intact organism, and the character of the changes in the myocardial cells. Degenerative changes found in the isolated heart are very similar to those described in a number of pathological situations [2-5]. The only special feature of the necrobiotic changes in the myocardium during perfusion of the isolated heart with salt solutions is the absence of a diastase-resistant diffuse PAS reaction in the contracturally modified muscle cells. This confirms the view [4, 5] that this feature is linked with plasma inhibition, and it shows that plasma imbibition does not play a decisive role in the genesis of coagulation necrosis of the myocardial cells.

LITERATURE CITED

1. N. N. Anichkov, Russk. Vrach, 11, 2067 (1912).
2. L. A. Semenova, Dokl. Akad. Nauk SSSR, 162, 1175 (1965).
3. Yu. G. Tsellarius and L. A. Semenova, Arkh. Pat., No. 1, 44 (1966).
4. Yu. G. Tsellarius, L. A. Semenova, S. F. Tsellarius, et al., Arkh. Pat., No. 12, 3 (1968).
5. Yu. G. Tsellarius, L. A. Semenova, and L. N. Belov, Arkh. Pat., No. 11, 20 (1969).
6. M. B. Baganer, F. Lioy, and M. B. Visscher, Am. J. Physiol., 209, 519 (1965).
7. M. Burstone, Enzyme Histochemistry and Its Application in the Study of Neoplasms, Academic Press (1962).

8. J. W. Emberson and A. R. Muir, Quart. J. Exp. Physiol., 54, 36 (1969).
9. L. L. Iversen, Brit. J. Pharmacol., 21, 523 (1963).
10. J. LeLorier and F. E. Shideman, Proc. Soc. Exp. Biol. (New York), 130, 265 (1969).
11. A. R. Muir, J. Anat. (London), 101, 239 (1967).
12. A. R. Muir, J. Anat. (London), 102, 148 (1968).
13. E. Page and E. G. Page, Circulat. Res., 22, 435 (1968).
14. E. Page, B. Power, J. S. Borer, et al., Proc. Nat. Acad. Sci. (Washington), 60, 1323 (1968).
15. R. Poche, G. Arnold, and H. Nier, Arch. Path. Anat., 346, 239 (1969).
16. S. S. Schreiber, M. Oratz, and M. Rotschild, Am. J. Physiol., 211, 314 (1966).
17. D. L. Weiss, B. Surawicz, and I. Rubenstein, Am. J. Path., 48, 653 (1966).
18. J. R. Williamson, J. Biol. Chem., 239, 2721 (1964).