OXIDATIVE PHOSPHORYLATION IN HOMOGENATES AND MITOCHONDRIA OF CAT HEART MUSCLE FOLLOWING SEROTONIN INJECTION INTO THE MYOCARDIUM

(UDC 616.127-005.8-092.9-02:577.17]-07:616.127-008.9)

Zh. A. Chotoev

Biochemistry Laboratory, Institute of Pharmacology and Chemotherapy, USSR Academy of Medical Sciences, Moscow

Presented by Active Member Prof. S. E. Severin
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 58, No. 10, pp. 50-53, October, 1964
Original article submitted August 15, 1963

Among the many activities of serotonin (5-hydroxytryptamine) [4, 16] its capacity to cause injury to the myocardium similar to infarction deserves attention [2, 7].

We have not found information in the literature concerning the action of serotonin on the metabolism of cardiac muscle. Data concerning the effect of serotonin on respiration and respiratory phosphorylation in other organs are few and contradictory. A number of workers have established that serotonin depresses respiration [10, 13, 17, 19] and phosphorylation [11, 12, 20] whereas others have found that it stimulates respiration and phosphorylation [8, 9, 14].

In this paper are presented certain data concerning the effect of serotonin on the respiratory process and on the phosphorylation conjugated with it in the tissues of the cat heart muscle when an "infarct" is produced by serotonin.

METHODS

Experiments were performed on cats, anesthetized with urethane (600 mg/kg) and chloralose (40 mg/kg). Under conditions of artificial respiration the thoracic cavity of the animals was opened, the pericardium incised, and the heart laid bare. A dose of 300 μg of serotonin-creatine sulfate was injected at a depth of 2-3 mm in the wall of the left ventricle. Within 15-20 min maximal injury (infarction) had developed in the myocardium at the injection site. After 20 minutes, the heart was quickly removed and perfused with cold physiological solution to remove the blood. Next, the injured portion of the left ventricle (experimental) was cut out as well as an uninjured area of myocardium from the same ventricle (control). For a normal, data were obtained on tissue taken from the left ventricle of a healthy cat (heart of healthy cat extirpated under ether anesthesia).

Homogenates and isolated mitochondria were obtained by a method described earlier [3].

The homogenate was poured out into 5 ml Warburg flasks in 0.3 ml aliquots. The flasks already held 0.3 ml phosphate buffer (pH 8.0). The final concentrations of the components in the buffered solution were: Na_2HPO_4—4.6 $\times 10^{-2}$M, $MgSO_4$—2 $\times 10^{-3}$M, KCl—5 $\times 10^{-3}$M, NaCl—1 $\times 10^{-3}$M. Creatine was used for the final phosphate acceptor at 40 μM per sample.

Oxidative phosphorylation in the homogenates was studied with endogenous substrates.

The isolated mitochondria were suspended in a solution of 0.25 M sucrose (pH 7.4) in an amount equal to double the initial tissue weight and were rapidly added to the Warburg vessel. Each vessel contained 0.5 ml of mitochondrial suspension (corresponding to 0.5-0.7 mg of protein nitrogen), 0.3 ml phosphate buffer, 0.1 ml of 0.25 M sucrose solution with respiratory substrates, and 0.1 ml (0.5 mg) hexokinase solution. The total volume of the incubation mixture was 1 ml. Concentrations of the components in the buffer solution (pH 7.4) were: K_2HPO_4—3 $\times 10^{-2}$M, $MgSO_4$ —1.5 $\times 10^{-2}$M, ATP—6 $\times 10^{-3}$M, glucose—5 $\times 10^{-2}$M.

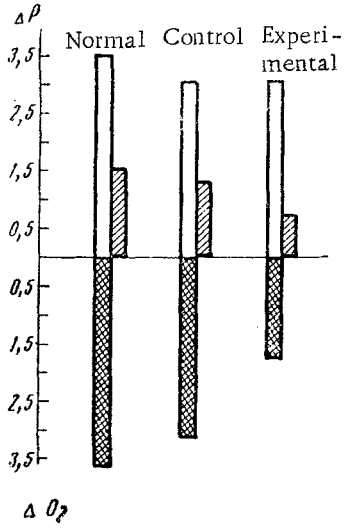

Fig. 1. Effect of serotonin on respiration and phosphorylation in homogenates of cat cardiac muscle. Cross-hatched bars—oxygen consumption in microatoms; non-hatched bars—decrease in mineral (inorganic) phosphate in microatoms; striped bars—phosphocreatine in microatoms P.

Respiratory substrates were alpha-ketoglutarate with malonate, succinate, and beta-hydroxybutyrate in a final concentration of 25 μM.

For in vitro studies of the effect of serotonin on mitochondrial oxidative processes the serotonin-creatine sulfate was injected into the Warburg vessel by dissolving in 0.1 ml of 0.25 M sucrose solution such a quantity of serotonin that the final concentration was 1×10^{-3}M.

The experiments with homogenates and mitochondria were conducted in an atmosphere of air; oxygen consumption was measured in the Warburg apparatus. Incubation was for 10 min at 26°. In experiments with mitochondria, the decrease in inorganic phosphate [18] was measured in a protein-free trichloracetic acid filtrate before and after incubation, and the phosphocreatine was measured in experiments on homogenates [1].

RESULTS

The results of the experiments, which characterize the rates of respiration and phosphorylation in heart muscle homogenates from areas damaged by serotonin (experimental) from undamaged areas (control) and from normal hearts, are presented in Fig. 1.

As these data show, the oxygen consumption of homogenates in the experimental samples is 50% less than in the normal. In control samples some very insignificant and inconstant lowering of oxygen consumption is observed. The decrease in inorganic (mineral) phosphate in the incubation medium was the same in all series of experiments.

Since the fall in inorganic phosphate in the homogenate (on endogenous substrates) depends on many factors, it is difficult to calculate the P/O coefficient.

It must be noted that in the normals and in the controls only a third (and in experimental samples even less) of the inorganic phosphate which disappeared from the medium was detected as phosphocreatine. It may be suggested that ATP which forms as a consequence of its "universal" position gives rise to concurrent relations between various simultaneously occurring reactions (kinase reactions leading to phosphohexoses, hydrolase activity leading to the splitting of ATP, its use in biosynthesis, in oxidation of fatty acids, etc).

The decrease in phosphocreatine formation in the homogenate of injured myocardial fragments may be attributed to the increased ATP-ase activity caused by serotonin [11] or may be regarded as a consequence of local anoxia which lowers the content both of ATP and of creatine phosphate [15].

The results of experiments on the appearance of serotonin effect on the processes of respiration and respiration-conjugated phosphorylation in cat heart muscle mitochondria (in the presence of various respiratory substrates) are presented in Fig. 2. As the data show, in the presence of succinate in mitochondria isolated from the injured area of myocardium, a small increment in respiration and respiratory phosphorylation is found (statistically insignificant).

It is interesting to note that the activity of beta-hydroxybutyrate dehydrase in cat heart is very low.

The results of experiments carried out on mitochondria in the presence of succinate and alpha-ketoglutarate with the addition serotonin-creatine sulfate* in vitro do not differ from results obtained in experiments on mitochondria after serotonin injection in situ.

The data set forth here permit us to conclude that serotonin inhibits oxygen consumption by homogenates and does not affect oxidative phosphorylation in isolated mitochondria (in the presence of succinate or alpha-ketoglutarate). This fact supplies the basis for the hypothesis that the study of oxygen consumption in artificially isolated

* Equivalent concentrations of creatine sulfate do not affect respiration and phosphorylation in homogenates and in mitochondrial suspensions of cat heart muscle.

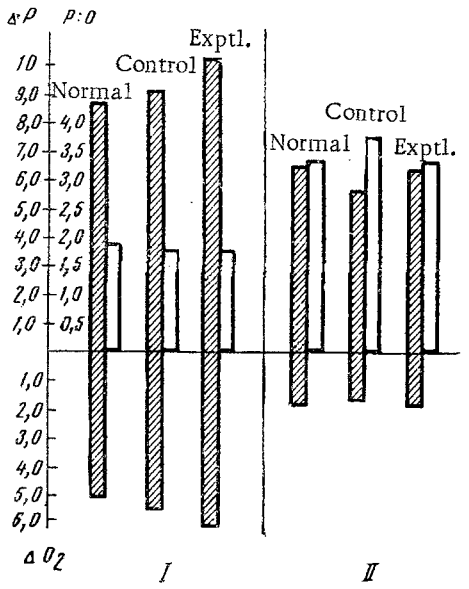

Fig. 2. Effect of serotonin on oxidative phosphorylation in mitochondria from cat cardiac muscle. Striped bars—oxygen consumption (downward from horizontal line of origin) and decrease in mineral (inorganic) phosphate (upward from line) in microatoms; open bars—P/O coefficient. I) Succinate; II) alpha-ketoglutarate + malenate.

mitochondria does not completely reflect the oxidative processes which are taking place in the entire heart muscle. A decrease in the oxygen consumption by homogenates of a damaged portion of the myocardium is evidently related to the considerable change within it of the composition of oxidizable respiratory substrates because of the intense stream of extra-mitochondrial metabolic processes (similar to what is seen in the heart in thyrotoxicosis).

In relation to the data obtained it is important to study the peculiarities of anaerobic reduction of carbohydrates which, in this instance, is apparently the basic source for the formation of respiratory substrates.

SUMMARY

Injection of serotonin-creatine-sulfate (300 μg) into the wall of the left ventricle of the cat's heart caused myocardial injury (infarction) at the administration site.

Oxidizing phosphorylation was studied in the homogenates and mitochondria obtained from the serotonin-affected area. Oxygen intake by the homogenates from the infarction area was half as much as compared with intake by the homogenates from the healthy myocardial tissue. Oxidizing phosphorylation processes were normal in the isolated mitochondria obtained from the affected myocardial area.

LITERATURE CITED

1. A. M. Alekseeva. Biokhimiya (1951), No. 2, p. 97.
2. V. V. Zakusov, Pharmakol i toksikol. (1963), No. 2, p. 131.
3. A. M. Zubovskaya. Byull. éksper. biol. (1961), No. 3, p. 55.
4. V. V. Men'shikov and L. S. Bassalyk, Kardiologiya (1963), No. 2, p. 83.
5. P. M. Samoilov, Vopr. med khimii (1963), No. 2, p. 188.
6. S. E. Severin. In book: Phosphorylation and Function [in Russian], Leningrad (1960), p. 11.
7. P. H. Bulle. Science (1947), v. 126, p. 24.
8. B. Castelli and L. D. De Giuseppe. Farmaco ed. sci. (1955), v. 10, p. 1011.
9. J. Cahn et al., C. R. Soc. Biol. (1957), v. 151, p. 82.
10. I. Földes and J. Palinkas. Arch. int. Pharmaco-dyn. (1960), v. 127, p. 331.
11. K. Kuriaki and N. Baba. C. R. Soc. Biol. (1959), v. 153, p. 1638.
12. H. Laborit et al. Int. Rec. Med. (1959), v. 172, p. 361.
13. E. Mietkiewski and I. Jankowska. Wien. klin. Wschr. (1960), Bd. 72, S. 643.
14. K. E. Moore et al., Brit. J. Pharmacol. (1961), v. 17, p. 278.
15. G. Michal and S. Naegle et al., Am. J. Physiol. (1961), v. 197, p. 1147.
16. I. H. Page. Physiol. Rev. (1958), v. 38, p. 277.
17. M. M. Rapport and M. Virno. Proc. Soc. expt. biol. (N. Y.), (1952), v. 81, p. 203.
18. J. L. Peel and B. Loughman, Biochem. J. (1957), v. 65, p. 709.
19. G. Salgarello. Boll. Soc. ital. Biol. sper. (1955), v. 31, p. 786.
20. E. Walaszek and L. G. Abood. Proc. Soc. exp. Biol. (N. Y.) (1959), v. 101, p. 37.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
