DETERMINATION OF CHOLINESTERASE ACTIVITY IN THE MYOCARDIUM AT TISSUE pH BY THE METHOD OF POTENTIOMETRIC TITRATION

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UDC 612.173.1.015.14.014.462.6-088.1:543.257.1

The cholinesterase (CE) activity may be studied by histochemical, manometric, biological, titrometric, potentiometric, and colorimetric methods. After assessing the advantages and disadvantages of these methods, it may be concluded that the biological method is insufficiently accurate, the manometric method is very laborious and requires cumbersome apparatus (a Warburg apparatus), the histochemical method suffers from a complicated quantitative analysis of the results, and the colorimetric method possesses low sensitivity (0.1-0.2 pH) and gives variable results depending on the indicators used.

The most common feature of all the method listed above is the measurement of the activity of the enzyme in buffered solutions at a certain (often optimal) pH value. The use of buffered solutions means that small changes in enzyme activity taking place in vivo as a result of changes in the pH of the heart tissue cannot be detected. Measurement of the CE activity at a fixed pH value also interferes with the detection of changes in the enzyme activity associated with changes in the pH of the heart tissue.

Because of these disadvantages, at M. E. Raiskina's suggestion, the author has attempted to develop a method of measuring CE activity at the pH of the myocardium measured in vivo.

The potentiometric method was considered to be most suitable for this purpose, for it enables the CE activity to be determined within wide limits of pH with equal accuracy (0.01 pH), However, this method did not satisfy all requirements, because the initial pH of the tissue shifted to the acid side during incubation. To overcome this defect, the potentiometric method was supplemented with stage-by-stage titration with alkali to maintain the initial pH.

Principle of Determination of CE Activity. The principle of potentiometric determination of CE is based on measurement of the change of pH toward the acid side during hydrolysis of acetylcholine by the CE of the heart muscle. In the modification now used, the CE activity was determined by the potentiometric titration of the acetic acid liberated during hydrolysis of acetylcholine.

Apparatus Required. The apparatus devised permits parallel determination of CE in twelve samples. The basic elements of this apparatus, illustrated in Fig. 1, are a water bath made of organic glass and the temperature of which is kept constant at 38 ± 0.1° (1) with a heater (2) and a contact thermometer (3); to the vertical wall of the water bath are fixed twelve 1-ml pipets (10) with taps. The pipets are connected by a distributor (6) to the bottle (4) with the 0.01 N solution of NaOH for titration. To prevent contact between the alkali and the CO_2 of the air, the bottle is closed with a tube (11) containing soda-lime. Small glass jars with stoppers of organic glass, in the holes of which are fixed a glass electrode, a potassium chloride bridge, and the bottom end of the pipet, are fixed in three special holes in the water bath. The fourth hole in the stopper is intended for maintaining the atmospheric pressure in the small glass jar at the moment of titration. All the glass electrodes (of type G202C and G222C, manufactured by the firm of "Radiometer," Denmark) are connected to a pH meter (9) through a high-ohmic selector (8) made by the author. The input and output resistance of the selector is 10^{13} Ω, and this ensures accuracy of the measurements. To measure the pH, a pH-meter 27 with a wide scale (from pH 6.8 to 8.2) or a pH-meter 22 with a scale extender (type PHA62B, made by the firm of "Radiometer," Denmark) was used. The potassium chloride bridges, filled with agar-agar solution with a saturated solution of KCl, were branches from the vessel (5) containing a type K401 calomel electrode. This electrode was connected to the pH meter.

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 62, No. 8, pp. 115-118, August, 1966. Original article submitted November 20, 1964.

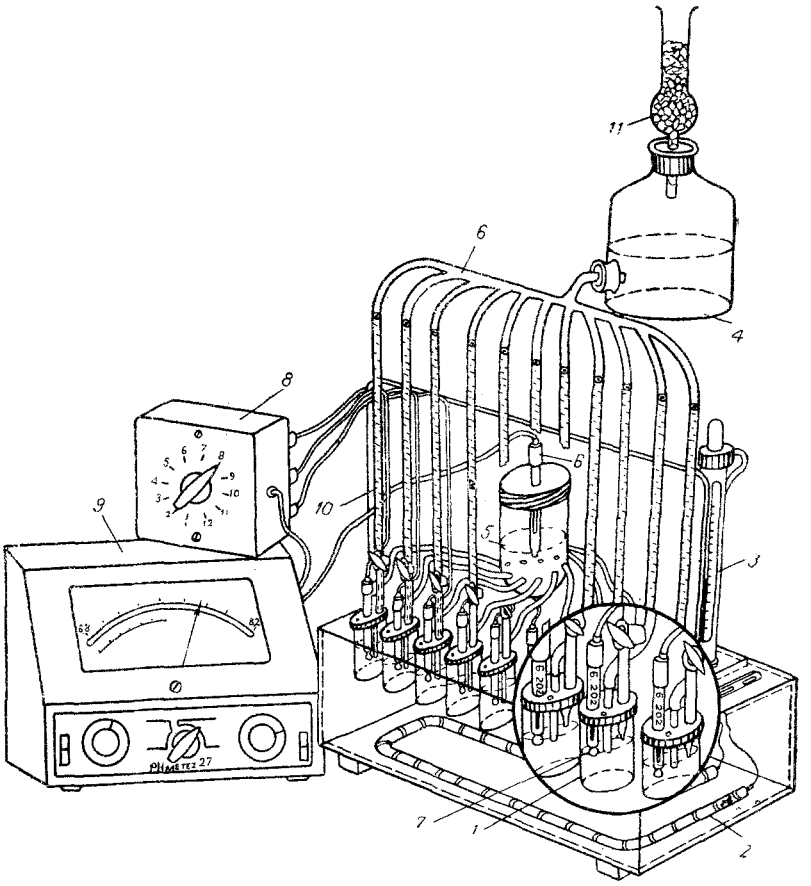

Fig. 1. Scheme of apparatus for measuring the cholinesterase activity of the myocardium. Explanation in text.

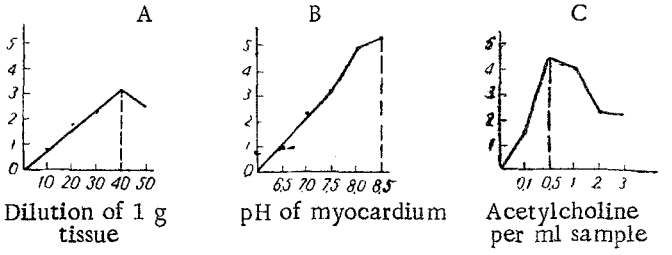

Fig. 2. Relationship between cholinesterase activity (in mg acetylcholine/g protein) and dilution of tissue (A), pH of myocardium (B), and acetylcholine concentration (C).

Immediately before the experiment, the pH meter was calibrated with each of its glass electrodes separately, using two precision buffer solutions of pH 6.5 and 4.0 (20°). The position of the calibration tumbler switch for each electrode was noted and was used throughout the experiment.

Determination of optimal conditions for measuring CE activity. Development of the method began by determining the optimal conditions for CE activity. The relationship between the dilution of tissue and the CE activity (at pH 7.5 and acetylcholine concentration 1 mg/ml sample) is shown in Fig. 2A. It is clear from Fig. 2 that the optimal dilution of tissue was 1:40, and this was used in the subsequent investigations.

The relationship between the pH of the homogenate and CE activity (with tissue diluted 1:40 and acetylcholine concentration 1 mg/ml) is shown in Fig. 2B. It is clear from Fig. 2 that the optimal pH is 8.0-8.5. A pH of 8.5 was chosen for the subsequent investigations.

The relationship between the acetylcholine concentration and the CE activity (with tissue dilution 1:40 and pH 8.5) is shown in Fig. 2C. The optimal concentration of the substrate may be seen to be 0.5-1.0 mg/ml. An acetylcholine concentration of 0.5 mg/ml was chosen.

These results confirm those obtained by other investigators for the optimal conditions for studying CE activity. For instance, according to Augustinsson [5] and Chaudhuri [9], the optimal pH for true CE is 7.5-8.0, and for pseudocholinesterase 8.0-8.5. A series of investigations has shown that the optimal concentration of acetylcholine lies between 0.5 and 1.0 mg/ml [5,6,10,11], for in these conditions CE combines with two molecules of acetylcholine at once [4].

Course of the Determination. Cats were anesthetized with a mixture of urethane (0.4 g/kg) and chloralose (0.06 g/kg) intraperitoneally. After thoractomy, the animals were maintained on artificial respiration. In some experiments, the pH of the myocardium was measured on the excised heart. In others, while the animals were alive, glass electrodes with ball points 4-5 mm in diameter were sutured into the wall of the right and left ventricles by a method developed in the author's laboratory. After the pH of the right and left ventricles had been determined, the heart was quickly excised, a cannula inserted into the aorta, and the heart washed out with cold 0.15 M solution of KCl. The right and left ventricles were separated and minced with scissors on ice and in a glass homogenizer. The homogenate of tissue from the right ventricle was transferred into a weighing bottle (1), and the homogenate of the tissue from the left ventricle was divided into two equal parts, and transferred into two weighing bottles (2 and 3). The homogenate in the weighing bottles 1 and 2 was made up with 0.01 N HCl solution or 0.01 N NaOH solution to the pH values of the right and left ventricles, respectively, determined in vivo. The pH of the homogenate in the weighing bottle 3 was adjusted to 8.5.

The contents of each weighing bottle were divided among four small glass vessels, each containing 4.5 ml homogenate. To this was added the acetylcholine solution, the final concentration of which was 0.5 mg/ml of sample. A few drops of 1% neostigmine solution were added to the two control samples to suppress enzymic hydrolysis of the acetylcholine. The glass vessels were closed with the stopper and placed in the hole in the water bath. The glass electrodes and the potassium chloride bridges were lowered into them and the pipet, previously filled with 0.01 N NaOH solution placed above the level of the fluid. The time and pH at the beginning of incubation were noted. After 30 min the liberated acetic acid was titrated with alkali to the initial pH and the incubation was continued for a further 30 min. With a smaller number of samples, the subtitrations should be carried out every 10-15 min. At the end of incubation, titration to the initial pH was again performed.

The CE activity was judged from the volume of alkali used up in the titration of the acetic acid. The CE activity was expressed in milligrams acetylcholine hydrolized by 1 g tissue per hour of incubation, and calculated per gram of tissue and per gram protein. The protein content of 1 ml homogenate and 1 g tissue was determined by the method of Robinson and Hogden [14], based on the biuret reaction.

CE Activity in the Myocardium of the Cats. The method described above was used to study the CE activity in the right and left ventricles of cats. The activity of the enzyme in the right ventricle was higher than in the left, and the corresponding values were 29 ± 4.1 and 18 ± 2.3 mg acetylcholine/g protein ($P < 0.05$). This result is in agreement with the findings of other authors [1,6-8,12,13,15], who found that the highest activity of the enzyme is present in the auricles and atria, by comparison with the ventricles, and that the CE activity in the right heart is higher than in the left.

The values obtained in the present investigations for the CE activity were rather lower than those obtained by other authors [2,3,4,16-18]. The reason for this is that they made their determination at the optimal pH, whereas in these experiments they were made at the initial pH of the tissue (mean 7.24-7.26). A direct correlation is present between the CE activity and the pH of the tissue. The coefficient of correlation is 0.80-0.82 for the right and left ventricles ($P < 0.02$).

The determination of CE at the optimal pH 8.5 gave a higher value of enzyme activity. The values of CE activity in optimal conditions were twice as high as at the pH of the tissue (7.24), the actual figures being 36 ± 3.6 and 18 ± 2.3 mg acetylcholine g protein. The significance of the differences between the mean values of the CE activity at tissue pH and optimal pH confirms the correlation relationship discovered above between the pH of the myocardium and the enzyme activity.

A high degree of correlation was found between the CE activity and pH of the myocardium, and this justifies the determination of the enzyme activity at the initial values of the pH of the myocardium and not in a buffer solution at optimal pH, as was done in the earlier investigations.

LITERATURE CITED

1. G. D. Smirnov and Ts. V. Serbenyuk, DAN SSSR, 62, 5, 725 (1948).
2. I. A. Shabanova, Farmakol. i Toksikol., 5, 410 (1959).
3. W. Antopol, S. Glaubach, and D. Glick, Proc. Soc. Exp. Biol. (N. Y.), 42, 280 (1939).
4. K. B. Augustinsson, Acta physiol. scand., 15, Suppl. 52 (1948).
5. K. B. Augustinsson, in the book: The Enzymes, Vol. 1, New York (1950), p. 443.
6. F. Bergmann and R. Segal, Biochim. Biophys. Acta, 16, 513 (1955).
7. S. Briscoe, J. physiol. (Lond.), 126, 623 (1954).
8. E. Bülbring, S. R. Kottegoda, and H. Shelly, J. physiol. (Lond.), 123, 204 (1954).
9. D. K. Chaudhuri, Ann. Biochem., 9, 85 (1949).
10. L. Girardier, F. Baumann, and J. M. Posternak, Helv. physiol. pharmacol. Acta, 17, C24 (1959).
11. L. Girardier, F. Baumann, and J. M. Posternak, Helv. physiol. pharmacol. Acta, 18, 467 (1960).
12. M. G. Ord and R. H. S. Thompson, Biochem. J., 46, 346 (1950).
13. M. G. Ord and R. H. S. Thompson, Biochem. J., 49, 191 (1951).
14. H. W. Robinson and C. G. Hogden, J. Biol. Chem., 135, 707 (1940).
15. P. Torsti, Ann. Med. exp. Fenn., 37, Suppl. 4 (1959).
16. S. Tucek and J. Vlk, Physiol. bohemoslov, 11, 319 (1962).
17. J. Vlk et al., Nature, 189, 923 (1961).
18. J. Vlk and S. Tucek, Physiol. bohemoslov, 11, 46 (1962).

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