대한약리학잡지 : 제 8권 제 2호
Korean J. of Pharmacology
Volume 8, Nomber 2, 1972

心臟機能에 미치는 Bicarbonate-Buffer의 重要性 : Buffer 除去에 依한 遊離心房의 收縮性, 膜電位 및 ATP 含量의 變動

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THE IMPORTANCE OF BICARBONATE-BUFFER ON CARDIAC FUNCTION:

Contractility, Membrane Potentials and ATP Content of Isolated Atria in the Absence of External Buffers

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ABSTRACT

The effects of omission of buffers from Krebs-Ringer medium on contractile activity, membrane potentials and ATP content of electrically stimulated isolated rat atria were investigated.

1) Contractile status: A rapid and marked depression of the contractile activity of atria occurred when buffer-free medium was substituted for the normal Krebs-Ringer medium.

2) Electrical status: The omission of buffers from medium did not alter the resting or action potential magnitudes of atria. However, the action potential duration was on initial increase followed by a decrease in the buffer-free medium.

3) ATP concentration: The omission of buffers from medium resulted in a marked decrease in the ATP levels of atria. It has been also found in the present study that bicarbonate buffer plays an important role for the maintenance of the contractility and ATP levels of the heart. The contractile depression by the omission of buffers was not directly associated with electrical alterations in resting or action potentials of the heart. In the absence of bicarbonate-buffer, glucose no longer plays to maintain the contractile activity and the ATP levels of rat atria.

INTRODUCTION

The physiological buffers of bicarbonate-phosphate system are widely used for the study of biological sciences. However, the buffers themselves play an important role on the heart function, besides its action on pH regulation of the physiological medium. Thus, the pharmacologic objective of this investigation is the elucidation of the

factors of buffers themselves on the myocardial function in relation to the drug action, as it has been commonly known that many drugs may be administered to the living body through the medium with various buffer systems. In experiments with isolated cardiac preparations, it would be convenient at times to omit the buffers from the medium. For example, in studies on the influences of calcium on cardiac function, the formation of complexes with the buffers may modify the results. In stuies on the influences of pH, the buffers themselves might modify the cardiac function. The present investigation was undertaken to determine the effects of omission of buffers from the Krebs-Ringer bicarbonate medium on the developed tension, membrane potentials and ATP content of electrically stimulated isolated rat atria.

METHODS

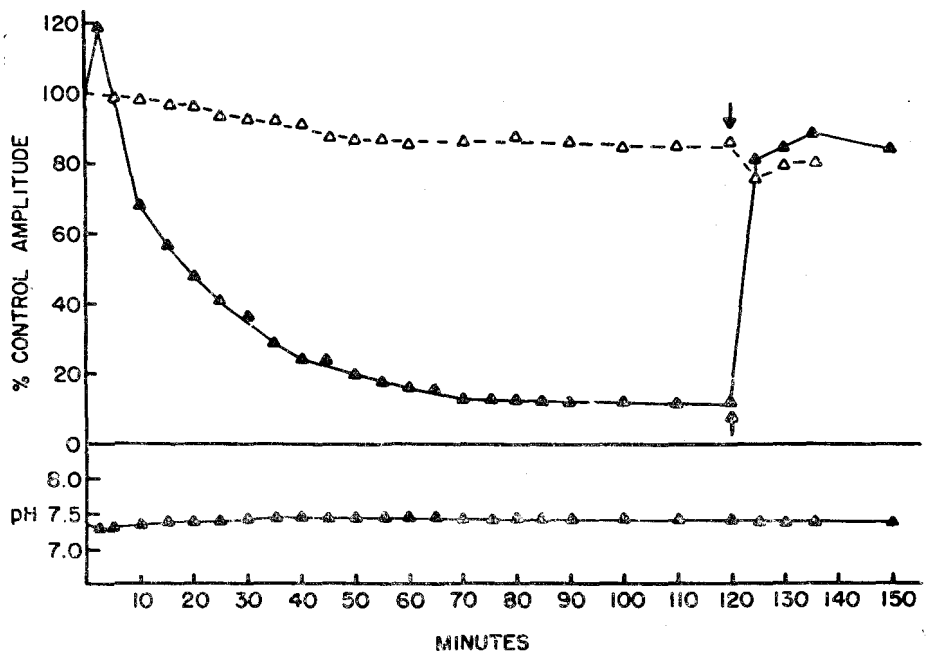
Male Sprague-Dawley rats weighing 180 to 200 g with *ad lib.* access to food and water were used for the study. Atria were removed from decapitated rats and suspended in a modified Krebs-Ringer bicarbonate glucose medium of the following composition (mM)[1] NaCl 120; KCl 4.8; $CaCl_2$ 1.22; $MgSO_4 \cdot 7H_2O$ 1.33; KH_2PO_4 1.2; $NaHCO_3$ 25.3; glucose 5.55. The medium was gassed with 95 per cent O_2—5 per cent CO_2 at pH 7.4 and 30 C. A constant tension of 750 mg was maintained throughout the experiments. The developed tension was recorded with a Statham strain guage, and the atria were electrically stimulated at a rate of 200 pulses/min. An equilibration period of one hour was allowed before readings were taken. The experimental values of contractility (peak tension) were compared with those of control records obtained at zero time (following equilibration) and expressed as per cent change in developed tension. In the experiments with buffer-free medium, the pH was monitored throughout the experimental period and maintained at 7.4 by additions of dilute sodium hydroxide or hydrochloric acid. By this method the pH was maintained within 7.3 to 7.5 throughout the experimental period. The buffer-free medium was prepared by replacing the sodium bicarbonate and potassium phosphate in the Krebs-Ringer medium with equivalent concentrations of sodium and potassium chloride. In this way, the concentrations of sodium and potassium were kept constant. When the bicarbonate was omitted, the medium was gassed with 100 per cent oxygen. Transmembrane potentials were obtained with microelectrodes by methods previously described [2]. ATP was determined by the method of Kornberg[3] and expressed as micromoles/g wet weight.

RESULTS

Effect of omission of buffers from the Krebs-Ringer medium on developed tension of rat atria.

The atria were suspended in normal Krebs-Ringer bicarbonate glucose medium. After a 60 min equilibration period of the tissue in this medium, the medium was replaced with buffer-free medium containing 5.5 mM glucose as the same concentration with the normal medium. It is evident from figure 1. that after a transient initial stimulation, a rapid depression of contractility occurred when buffer-free medium was substituted for the Krebs-Ringer bicarbonate. The contractility was restored to the control level when the buffer-free medium was replaced with Krebs-Ringer bicarbonate. It is also evident from the figure that the pH was maintained within 0.1 pH unit during the experiment.

Effect of omission of buffers from the Krebs-



△—△, Krebs-Ringer bicarbonate control (4 exp.);
▲—▲, buffer-free medium (6 exp);

Fig. 1. Effect of omission of buffers from Krebs-Ringer medium on the developed tension of isolated rat atria electrically-stimulated at a frequency of 200/min. The Krebs-Ringer bicarbonate medium was replaced with buffer-free medium(aerated with 100% O_2) at zero time. At arrows, the medium was replaced with Krebs-Ringer bicarbonate (gassed with 95%O_2−5% CO_2).

Ringer medium on membrane potentials of rat atria.

The results from experiments with buffer-free medium in which simultaneous measurements of membrane potentials and developed tension were made are shown in figure 2. It is evident that omission of the buffers from the medium did not markedly alter the magnitudes of the resting or action potentials. During the two-hour period in the buffer-free medium, the values of the resting action potential magnitudes were within 5 per cent of the control levels. The effect on the action potential duration was biphasic, an initial increase followed by a decrease. After ten minutes incubation of the atria in buffer-free medium, the action potential duration increased approximately 20 per cent. At 30 min the action potential duration was at the control level. This was followed by a progressive decrease in the action potential duration. The effects of buffer-free medium on the action potential duration and developed tension of the atria were reversed when the medium was replaced with Krebs-Ringer bicarbonate.

Effect of omission of buffers from Krebs-Ringer medium on ATP content of rat atria.

The ATP concentrations of atria electrically stimulated in Krebs-Ringer bicarbonate or buffer-free medium are shown in Table 1. It is evident that omission of the buffers from the medium resulted in a marked decrease in the ATP concentration of the atria. The presence of glucose in the buffer-free medium prevented neither the decrement in contractility nor the decrease in the

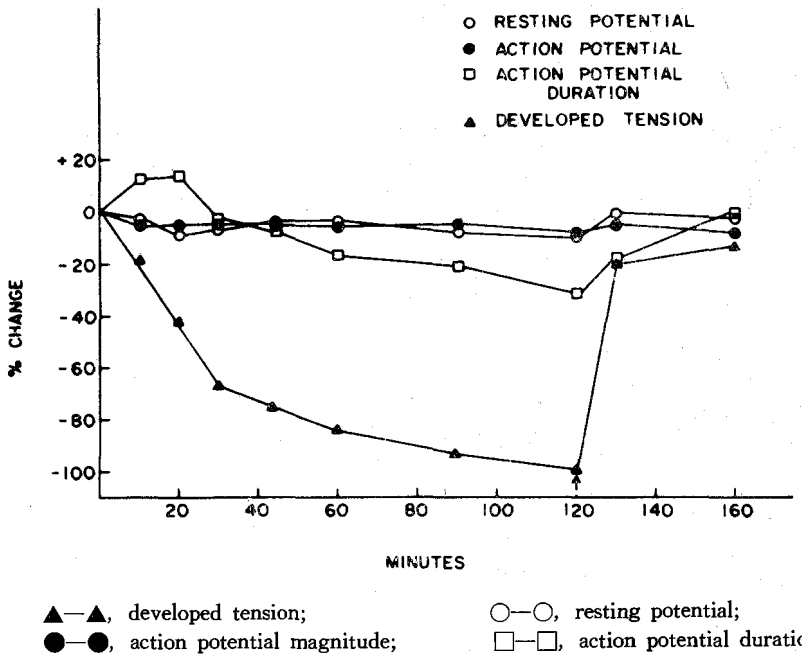


Fig. 2. Effect of omission of buffers from Krebs-Ringer medium on membrane potentials and contractility of isolated rat atria. The Krebs-Ringer bicarbonate medium was replaced with buffer-free medium at zero time. At arrow, the medium was replaced with Krebs-Ringer bicarbonate. The curves are the average of 4 exp.

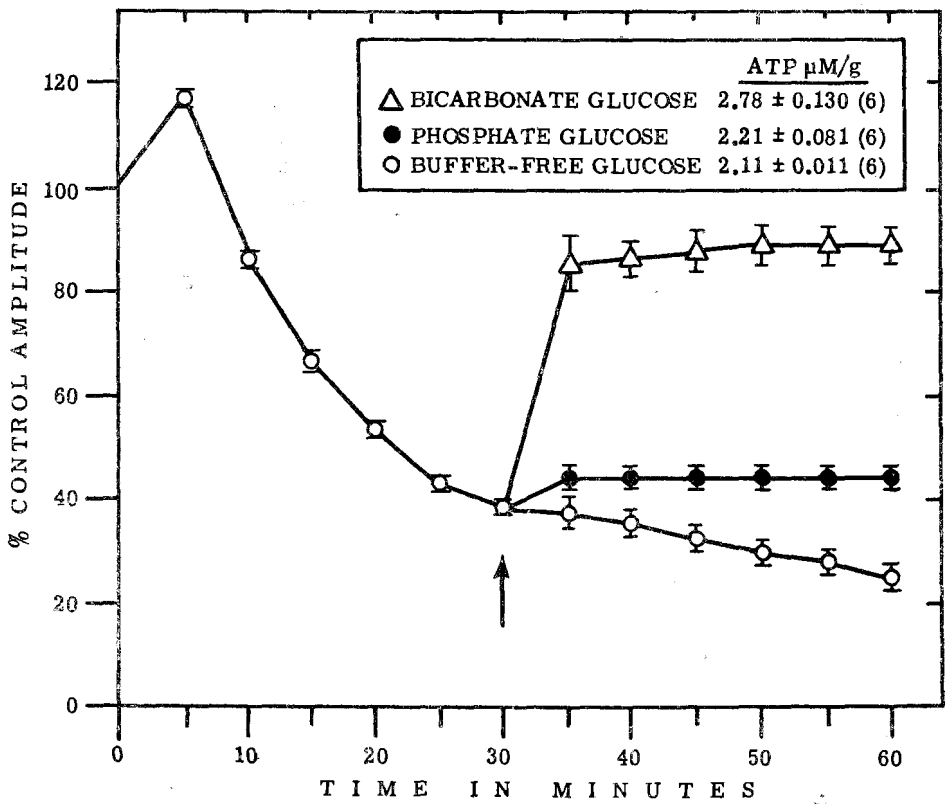
ATP concentration in the atria. Omission of glucose from the Krebs-Ringer medium resulted in a decrease in the ATP concentration to the level observed in the atria suspended in the buffer-free medium; however, the decrement in contractility was less in this medium than in the buffer-free. Thus, the depression of contractility was not directly correlated with the ATP levels, in agreement with the conclusions reached by Furchgott and Lee[4].

Abilities of bicarbonate and phosphate buffers to reverse contractile activity of rat atria depressed in buffer-free medium.

Experiments were performed to determine the ability of phosphate and bicarbonate buffers to reverse the depression of contractility resulting from incubation of atria in buffer-free medium (Fig. 3). After 30 min incubation in buffer-free medium, the medium was replaced with either a medium containing phosphate buffer(aerated with 100 per cent O_2) or bicarbonate buffer (gassed

Table 1. Effect of omission of buffers and glucose from Krebs-Ringer medium on ATP concentration of electrically-stimulated rat atria.

Conditions	No. of exps.	ATP, moles/g
Krebs-Ringer bicarbonate-phosphate, with glucose	6	2.8±0.08
Krebs-Ringer bicarbonate-phosphate, glucose-free	6	2.0±0.05
Buffer-free, with glucose	6	2.0±0.08
Buffer-free, glucose-free	6	2.0±0.05
Krebs-Ringer bicarbonate with glucose	6	2.8±0.05
Krebs-Ringer bicarbonate glucose-free	6	2.2±0.03
Krebs-Ringer phosphate with glucose	6	2.1±0.08
Krebs-Ringer phosphate glucose-free	6	2.1±0.05



△—△, bicarbonate buffered medium with 5.5 mM glucose :
●—●, phosphate buffered medium with 5.5 mM glucose;
○—○, buffer-free medium with 5.5 mM glucose (control);
Each curve is the mean of 6 experiments. Vertical lines represent standard errors of the means.

Fig. 3. Effect of bicarbonate and phosphate buffer on contractility and ATP content of isolated rat atria depressed in buffer-free medium. At arrow, bicarbonate or phosphate were added after 30 min exposure to buffer-free medium.

with 95 per cent O_2—5 per cent CO_2). The buffers were present in the concentrations found in Krebs-Ringer bicarbonate medium and contained 5.5 mM glucose. Incubation of the atria in the bicarbonate-buffered medium restored the developed tension and ATP concentration to the control levels. On the other hand, phosphate buffer only partially restored the developed tension of the atria and had no significant effect on the ATP level.

DISCUSSION and SUMMARY

In the present investigation, it was demonstrated that omission of buffers from Krebs-Ringer medium resulted in significant alterations in the developed tension of isolated rat atria (Fig. 1). The importance of bicarbonate buffer for the maintenance of cardiac contractility has been reported by White and Salter[5]. Berman and Saunders [6] demonstrated that glucose was relatively inffective as a substrate for restoring the contractility of rat ventricle strips made hypodynamic by

prolonged incubation in bicarbonate-free medium. Rice and Berman[7,8] found that the oxidation of glucose by rat ventricle strips incubated in bicarbonate-free medium was slower than the oxidation of pyruvate or acetate. In contrast, they have observed that in medium containing bicarbonate glucose maintains contractile activity of the myocardium[6], and Hood and Saunders have reported that glucose is rapidly oxidized in this medium[9]. The resultes of the contractile depression of rat atria in buffer-free medium are consistent with the biochemical data of Shaw and Stadie on diaphragm which indicate that the Embden-Meyerhof pathway is inhibited at phosphofructokinase step by a bicarbonate-free medium [10,11]. It has been also established tha the enzyme activity of phosphofructokinase is an important regulatory step in glycolysis in the cells[12~14].

Thus, it is possible that the rapid decrement in developed tension of atria that occurs in buffer-free medium may in part be due to a defect in glucose metabolism, and in particular, to the inhibition of phosphofructokinase step. The results of the present study show that the depression of developed tension in atria suspended in buffer-free medium was not directly corrected with alterations in the resting or action potential magnitudes or in the action potential duration (Fig. 2). However, the decrease in the action potential duration after prolonged exposure of atria to buffer-free medium may have contributed to the depression in contractility. The results also show the importance of bicarbonate ion in the maintenance of contractility and ATP levels in the heart In the absence of the bicarbonate, glucose no longer plays a significant role in the maintenance of the contractility and the ATP content of the rat atria.

REFERENCES

1) 高啓昌, 鄭址昌, 韓大燮：中央醫學, *23: No. 1, 33, 1972.*

2) Webb, J.L. and Hollander, P.B.: *Effects of enzyme inhibitors on the contractility and membrane potentials of the rat atrium. Circulation Res., 7:131-137, 1959.*

3) Kornberg, A.: *Methods in Enzymology. New York: Academic Press, 1955.*

4) Furchgott, R.F. and Lee, K.S.: *High energy phosphates and the force of contraction of cardiac muscle. Circulation, 24:416-428, 1961.*

5) White, W.F. and Salter, W.T.: *The response of hypodinamic myocardium to known concentrations of cardiac glycosides. J. Pharmacol. Exp. Therap., 88:1-9, 1946.*

6) Berman, D.A. and Saunders, P.R.: *Energy sources for contration of the rat ventricle in phosphate medium. Circulation Res., 3:559-563, 1955.*

7) Rice, L.I. and Berman, D.A.: *Malonate and fluoride effects on metabolism and concentration of electrically stimulated heart strips. Am. J. Physiol., 200:727-731, 1961.*

8) Rice, L.I. and Berman, D.A.: *Oxidation of glucose-1-C^{14}, glucose-6-C^{14} and pyruvate-2-C^{14} by contracting rat ventricle strips in the presence and absence of arsenate. J. Pharmacol. Exp. Therap., 127:11-14, 1959.*

9) Hood, J.E. and Saunders, P.R.: *Oxidation of C^{14} labeled glucose, pyruvate and succinate by isolated contracting myocardium. Am J. Physiol., 190:525-528, 1957.*

10) Shaw, W.N. and Stadie, W.C.: *Two identical Embden-Meyerhof enzyme systems in normal rat diaphram differing in cytological location response to insulin. J. Biol. Chem., 234:2491-2496, 1959.*

11) Shaw, W.N. and Stadie, W.C.: *Coexistance of insulin-response and insulin-non-responsive glycolytic systems in rat diaphragm. aJ. Biol. Chem., 227:115-134, 1957.*

12) Cori, C. F.: *In "A symposium on Respiratory Enzymes", p. 175, Univ. of Wisconsin Press, Madison, Wisconsin, 1942.*

13) Lyen, F., Hartman, G., Netter, K. F. and Schuegraf, A.: *"Regulation of Cell Metabolism" p. 256(ed. by GEW Wolstenholme) Little Brown, Boston, Mass., 1959.*

14) Newsholme, E. A., Randle, P. J. and Manchester K. L.: *Inhibition of the phosphofructokinase reaction in perfused rat heart by respiration of ketone bodies, fatty acids and pyruvate. Nature 193:270-271, 1962.*