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Isolated Cardiac Muscle Performance during Fluorocarbon Immersion and Effects of Metabolic Blockade[1] (40246)

OSCAR H. L. BING[2] AND WESLEY W. BROOKS

Department of Medicine and Thorndike Laboratory, Harvard Medical School and Beth Israel Hospital, Boston, Massachusetts 02215

It has recently been shown that when perfused with fluorocarbon solutions, tissues will maintain their integrity for relatively prolonged periods of time (1, 2). Fluorocarbon[3] is a biologically and chemically inert liquid in which carbon dioxide and oxygen are highly diffusible. In the present studies, we have compared the mechanical performance of isolated rat left ventricular muscle while immersed in Krebs-Henseleit solution and fluorocarbon under oxygenated conditions and during metabolic blockade. Since fluorocarbon permits free diffusion of gases while entrance and egress of substrates and metabolites are limited, immersion of isolated muscle preparations during exposure to varying O_2 and CO_2 mixtures may represent a new approach for evaluating factors which affect myocardial function during ischemia.

Methods. Preparation of muscle specimens. Male albino Charles River rats were sacrificed by decapitation. The hearts were quickly removed and placed in oxygenated Krebs-Henseleit solution (3) at a temperature of 28–30°. Left ventricular papillary or trabeculae carneae muscles were excised and mounted between two spring clips and suspended in a jacketed muscle chamber containing oxygenated Krebs-Henseleit solution with 5.5 m*M* glucose. Temperature was maintained at 28° by a Lauda K-2 constant-temperature circulating pump. The upper clip was attached by a thin gold chain to a rigid magnesium lever arm above which a micrometer stop was mounted so that the resting length of the muscle could be adjusted. The lower spring clip was connected to a tungsten wire (diameter: 15/1000 inch), which passed through a mercury seal at the bottom of the chamber.

Solutions. The composition of the Krebs-Henseleit solution, in millimoles per liter, is as follows: 118.5 NaCl, 4.69 KCl, 2.52 CaCl, 1.16 $MgSO_4$, 1.18 KH_2PO_4, 5.50 glucose, and 25.88 $NaHCO_3$. The PO_2 of the perfusate was maintained between 550 and 600 mmHg by passing 95% O_2 and 5% CO_2 through a scintered-glass disk located at the bottom of the muscle chamber. The PO_2 was brought to less than 10 mmHg within 5 min by changing the gas mixture to 95% N_2 and 5% CO_2. This gas mixture maintained a pH of 7.4. In other experiments the Krebs-Henseleit solution was replaced with fluorochemical Liquid Mediflor brand (FC-47).

Mechanical recordings. The muscles were stimulated 12 times per minute by a Grass model S-88 stimulator delivering 10-msec square-wave pulses through parallel platinum electrodes at voltages which were 10% above the minimum required to produce a maximal mechanical response. When the muscles were bathed in fluorocarbon it was necessary to carefully adjust the electrode position so that they were very close to the preparations in order to stimulate them. Following a 30-min equilibration period, the muscles were stretched to the apices of their length-tension curves in Krebs-Henseleit solution. After a 15-min period of stable isometric contractions, the experimental protocol was begun. Muscles the mechanical performance of which was not stable prior to hypoxia were discarded. The *in vitro* length of each preparation was measured at the apex of its length-tension curve with a Gaertner Scientific cath-

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[3] Mediflor™ Brand Inert Fluorocarbon M-6014 (FC-47) (Medical products division of Minnesota, Mining and Manufacturing Co., St. Paul, MN 55101).

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etometer–telescope combination. At the end of each experiment, the portion of the muscle between the spring clips was blotted and weighed. Developed tension (T) and maximum rate of tension development (dT/dt) were normalized for muscle cross-sectional area, assuming a specific gravity of 1.000. Values for isometric tension (T), time-to-peak tension (TPT) and dT/dt were calculated, and statistical differences were evaluated from the normalized data using Student's *t* test. Data were then recalculated and plotted as a percentage of control values.

Muscle preparations were divided into four groups. Group I: Mechanical activity was recorded in oxygenated Krebs-Henseleit solution for 60 min. Performance was then followed during 60 min of hypoxia (95% N_2, 5% CO_2). Group 2: Iodoacetic acid 10^{-4} *M* was added to the muscle baths which contained oxygenated Krebs-Henseleit solution and mechanical activity was recorded for 60 min. Performance during combined hypoxia and glycolytic blockade was then recorded over a subsequent 60-min period. Group 3: After equilibration in Krebs-Henseleit solution, the muscle bath was replaced by fluorocarbon bubbled through with 95% O_2 and 5% CO_2 and mechanical activity was recorded for a 60-min period. The gas mixture was then changed to 95% N_2 and 5% CO_2 and performance was recorded for a subsequent 60-min period. Group 4: Fifteen minutes prior to fluorocarbon addition, iodoacetic acid 10^{-4} *M* was added to the muscle baths. Mechanical activity was then recorded in oxygenated fluorocarbon solution for 60 min. After this period, the gas mixture was changed to 95% N_2 and 5% CO_2.

Results. Baseline data. Baseline mechanical performance of the four groups of muscle preparations is presented in Table I. No significant differences in muscle cross sectional area or mechanical performance was present prior to interventions.

Mechanical activity in Krebs-Henseleit and fluorocarbon solutions. A. *Performance in oxygenated solutions.* There was no significant change in developed tension of preparations in Krebs-Henseleit or fluorocarbon solution for 60 min (Fig. 1). In Krebs-Henseleit and fluorocarbon solutions respectively, TPT decreased to values of 88 ± 2% ($P < 0.01$) and 73 ± 6% ($P < 0.05$), respectively, of those at the beginning of the 60-min period. DT/dt on the other hand, rose significantly to 114 ± 4% ($P < 0.05$) in KH solution and also in FC solution to 118 ± 9%, although the latter change did not achieve statistical significance.

B. *Performance in oxygenated solutions during glycolytic blockade (Iodoacetate 10^{-4} M).* While muscle preparations demonstrated stable tension development under oxygenated conditions in both Krebs-Henseleit and fluorocarbon solutions, relatively small changes were also seen in the presence of glycolytic blockade (Fig. 2). Muscle preparations in Krebs-Henseleit solution demonstrated a gradual but progressive fall in tension development at 60 min to 80 ± 4% ($P < 0.01$) of that at the beginning of the experimental period. In fluorocarbon solution, a slight but significant increase in developed tension was seen at 15 and 30 minutes to a maximum value of 110 ± 2% ($P < 0.01$) above values prior to fluorocarbon addition. Developed tension subsequently fell gradually so that by 60 min tension was not significantly different from that of the beginning of the experimental period.

C. *Performance during hypoxia.* During hypoxia, developed tension fell quickly and, after 15 min, was 26 ± 2% and 13 ± 2% of prehypoxia control values in Krebs-Henseleit

TABLE I. BASELINE DATA ON THE FOUR GROUPS OF MUSCLE PREPARATIONS IN KREBS-HENSELEIT SOLUTION PRIOR TO SOLUTION CHANGE.

Solution to be introduced	(n)	Muscle cross sectional area	Developed tension	Maximum rate of tension development	Time to peak tension
		(mm^2)	(g/mm^2)	(g/mm^2/sec)	(msec)
Krebs Henseleit (KH)	(8)	0.71 ± .10*	5.20 ± .30	54 ± 8	162 ± 6
Fluorocarbon (FC)	(6)	0.76 ± .13	6.21 ± .26	69 ± 8	182 ± 10
KH + iodoacetate 10^{-4} *M* (IAA)	(6)	0.69 ± .12	7.38 ± .97	90 ± 16	178 ± 8
FC + IAA	(6)	0.75 ± .09	6.71 ± .53	78 ± 5	173 ± 7

* Means ± SE of the mean.

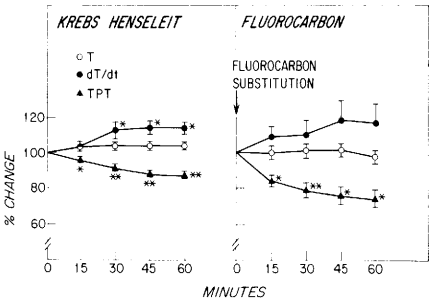

FIG. 1. The effect of oxygenated Krebs-Henseleit and fluorocarbon solution on isometric mechanical parameters of isolated rat left ventricular muscle preparations. Changes in developed tension (T), its maximum rate of rise (dT/dt) and time to peak tension (TPT) are demonstrated in Krebs-Henseleit solution (left panel) and fluorocarbon (right panel), bubbled through with 95% O_2 and 5% CO_2 for 60 min. Values are expressed means as ± SE of the mean. $*P < 0.05$, $**P < 0.01$ vs. value at beginning of experimental period.

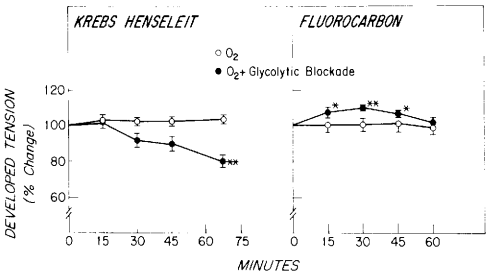

FIG. 2. The effect of glycolytic blockade (Iodoacetate 10^{-4} *M*) on developed tension in oxygenated Krebs-Henseleit and fluorocarbon solution. Changes in developed tension are expressed as a percent of control values. $*P < 0.05$, $**P < 0.01$ vs. value at beginning of experimental period.

with 5.5 m*M* glucose and fluorocarbon solutions respectively (Fig. 3A). By 30 min tension had fallen to almost zero in fluorocarbon solution; however, low levels of isometric tension persisted until 60 minutes. In Krebs-Henseleit solution, on the other hand, tension fell more gradually. At 30 and 60 min, isometric tension was 18 ± 1% and 10 ± 2% of prehypoxia control values respectively. Significant differences between the responses of muscle preparations in Krebs-Henseleit and fluorocarbon solutions at the $P < 0.01$ level or greater were seen at 15, 30, 45 and 60 min. Eight additional muscle preparations were subjected to hypoxia in zero glucose containing Krebs-Henseleit solution (Fig. 3B). De-

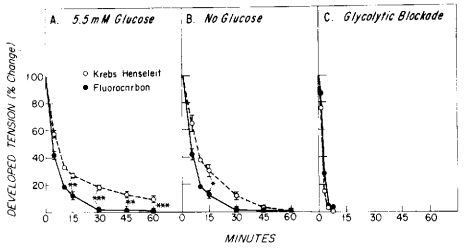

FIG. 3. The effects of 60 min of hypoxia on developed tension in Krebs-Henseleit and fluorocarbon solutions. A) 5.5 m*M* glucose containing Krebs-Henseleit solution, B) zero glucose containing Krebs-Henseleit solution replacement 30 min prior to hypoxia. C) Iodoacetate 10^{-4} *M* added to 5.5 m*M* glucose containing Krebs-Henseleit solution 30 min prior to hypoxia. In experiments where fluorocarbon was added, oxygenated fluorocarbon solution replacement was carried out 15 min prior to hypoxia, ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

veloped tension fell at a rate which was similar to 5.5 m*M* glucose until 30 min when tension fell more quickly. At 15 min tension was significantly greater than the fluorocarbon group ($P < 0.05$). At 30, 45 and 60 min, no significant differences between the zero glucose Krebs-Henseleit and fluorocarbon group was present.

D. *Performance during combined hypoxia and glycolytic blockade.* Preparations were exposed to iodoacetic 10^{-4} for a 15-min period while in Krebs-Henseleit solution prior to hypoxia or fluorocarbon addition and subsequent hypoxia. Developed tension fell to zero in less than 10 min in both fluorocarbon and Krebs-Henseleit solutions. No significant difference in the rate of decline of developed tension was seen between preparations in the two solutions (Fig. 3C).

Discussion. This study demonstrates that the isolated cardiac muscle preparation is able to function in a relatively stable fashion for a considerable period of time in a bath largely free of substrate and ions. It is likely that a small envelope of Krebs-Henseleit solution may surround the preparation. In addition, the preparation contains an interstitial space and remnants of a vascular compartment. Nonetheless, the volumes of these spaces are relatively small, and it is surprising that the isolated muscle preparation is able to maintain function in oxygenated fluorocarbon solution with little evidence of deterioration for as long as 1 hr. When glycolysis is blocked with IAA under oxygenated condi-

tions in Krebs-Henseleit solution, a significant depression in developed tension is seen after 60 min. A decline in muscle performance is not seen following glycolytic blockade in muscle preparations exposed to fluorocarbon. The relatively stable performance of cardiac muscle preparations in the presence of glycolytic blockade under oxygenated conditions is consistent with the well established observation that carbohydrates are normally not the major aerobic substrate for cardiac muscle.

While the performance of preparations in oxygenated fluorocarbon appears to be relatively unchanged despite glycolytic blockade, an abrupt decline in mechanical activity is seen when the PO_2 of the solution is reduced. In the absence of oxygen, the energy to support the mechanical performance of the muscle preparation must be provided through glycolysia. When both aerobic and anaerobic sources of energy production are blocked by hypoxia and iodoacetate 10^{-4} *M*, the mechanical performance of cardiac muscle preparations declines promptly (4). In both Krebs-Henseleit and fluorocarbon containing solutions, developed tension declined to zero in less than 10 min with complete metabolic blockade. On the other hand, during hypoxia alone, mechanical performance appears significantly improved by Krebs-Henseleit solution in comparison to fluorocarbon.

The reason for the additional depression of function seen during hypoxia in fluorocarbon solution is not clear, but may be due to the retention of metabolites of anaerobic metabolism which are free to leave the tissue in Krebs-Henseleit solution. Another possible reason for the greater decline in performance during hypoxia in fluorocarbon solution may be the reduced quantities of substrate available for contraction. Preparations in fluorocarbon must rely on glycogen stores alone, while those in Krebs-Henseleit solution may also utilize the glucose which is present in the bath solution. Our studies with zero glucose containing Krebs-Henseleit solution indicate that while the late effects of fluorocarbon on tension development might be attributable to the absence of glucose, the early rapid decline in mechanical activity cannot. Late depression of performance might also be due to the factor(s) which resulted in early depression. While the reason for the early rapid decline in mechanical activity during ischemia is unclear, the role of metabolite accumulation has been implicated (5). Although the solubilities of many metabolites in fluorocarbon are not known, evidence indicates that biological material is relatively insoluble in this very nonpolar substance ((6), personal communication R. P. Geyer). Like the isolated muscle preparation immersed in hypoxic fluorocarbon solution, the ischemic myocardium is subjected to the effects of substrate deficiency and metabolite accumulation in addition to being deprived of oxygen. Experiments utilizing fluorocarbon in combination with metabolic blockade may be helpful in further elucidating the mechanism(s) for altered myocardial performance during ischemia.

Summary. The mechanical performance of isolated trabecular muscle preparations from the left ventricle of rats was evaluated while immersed in Krebs-Henseleit and fluorocarbon solution under oxygenated conditions and during hypoxia and total metabolic blockade with combined hypoxia and iodoacetic acid 10^{-4} *M*. Under oxygenated conditions, mechanical performance was relatively stable in both Krebs-Henseleit and fluorocarbon solutions for 60 minutes. With glycolytic blockade, performance in both groups was relatively stable, although it was significantly increased in fluorocarbon solution in comparison to Krebs-Henseleit solution at 60 min ($P < 0.01$). With total metabolic blockade, mechanical performance fell to zero within 10 min in both Krebs-Henseleit and fluorocarbon solution. With hypoxia alone, mechanical performance declined more promptly and remained at lower levels in fluorocarbon solution than in Krebs-Henseleit solution ($P < 0.001$).

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