Metabolic and hemodynamic consequences of mannitol following myocardial anoxia

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SMITHEN, CHARLES, JAMES CHRISTODOULOU, THOMAS KILLIP, AND NORMAN BRACHFELD. Metabolic and hemodynamic consequences of mannitol following myocardial anoxia. Am. J. Physiol. 229(3): 1847-1852. 1975. The mechanism of action of hyperosmolar mannitol was evaluated by hemodynamic and metabolic studies in 79 isovolumic nonrecirculating paced perfused rat hearts during sequential 15-min periods of aerobic, anoxic, and reoxygenated perfusion. Hyperosmolarity induced by addition of mannitol significantly decreased myocardial water content (wet/dry wt ratio). It improved recovery of hemodynamic function during reoxygenation. With isosmolar perfusion (290 mosmol/kg) left ventricular systolic peak pressure (LVSP) decreased 32% (127 ± 5 to 86 ± 6 mmHg) and maximum dP/dt fell 50% (3,513 ± 328 to 1,758 ± 172 mmHg/s) during the postanoxic recovery period. With hyperosmolar perfusion (350 mosmol/kg), LVSP decreased 23% (132 ± 5 to 102 ± 7 mmHg) and dP/dt fell 21% (3,817 ± 215 to 2,998 ± 234 mmHg/s) (P < .01). Hyperosmolar perfusion did not affect postanoxic total coronary flow, lactate and glucose metabolism, tissue glycogen, creatine phosphate, or adenine nucleotide concentrations. Coronary perfusion with hyperosmolal solution aided recovery, enhanced postanoxic myocardial performance, and minimized tissue swelling. The most tenable explanation for the locus of action of hyperosmolar mannitol during anoxia under our experimental conditions is its direct effect on myocardial water content.

METHODS

Seventy-nine isolated rat hearts were perfused in a modified Langendorff apparatus. Male albino rats of the Sherman strain weighing 250–290 g were used and fed ad libitum on Wayne laboratory chow. Rats were killed by axial fracture and the heart was rapidly removed and mounted on the aortic cannula. The procedure required 20 s from sacrifice to onset of perfusion.

Rat heart apparatus. The apparatus used was a modification of the orthodox rat heart perfusion apparatus (Fig. 1). A tandem system was used to permit rapid exchange of perfusate varied as to osmolality and Po2. Perfusion was pumped from a storage reservoir to a bubbling reservoir for appropriate gas exchange. The perfusion reservoir was fixed to deliver a coronary perfusion pressure of 75 mmHg. Perfusion was controlled by retrograde flow via a temperature control coil. All glassware was jacketed and buffer solutions maintained at 37°C.

A modification of the method of Kadas and Opie (7) was used to assess left ventricular function. Left ventricular isovolumic pressure was recorded after left atrial appendectomy and insertion of a small latex balloon catheter. The balloon was inflated so that diastolic pressure was equal to atmospheric pressure and the recording system sealed. Ventricular pressure was recorded on a Sanborn model 8800 multichannel system using a Statham P23D pressure transducer. A stab wound in the wall of the left ventricle prevented accumulation of thebesian drainage. The left ventricular pressure signal was passed through a resistor-condenser (RC) differentiating circuit for recording of dP/dt. Heart rate was maintained at a constant rate of 300 beats/min during aerobic perfusion by bipolar pacing using an external battery-powered pacemaker. During anoxic perfusion, hearts stopped contracting and threshold for pacing increased so that rate could not be maintained. Coronary flow was measured by collection of fluid ejected
via the pulmonary artery. The error due to loss of thebesian drainage was accepted as insignificant. The venous efflux was assayed as described below. Arterial samples were drawn from the aortic canula.

**Perfusates.** The control perfusate consisted of modified Krebs-Ringer-bicarbonate buffer: NaCl (120 mM), KCl (4.8 mM), CaCl$_2$-2 H$_2$O (1.32 mM), KH$_2$PO$_4$ (1.21 mM), MgSO$_4$-7 H$_2$O (0.61 mM) and NaHCO$_3$ (25.4 mM) containing 5 mM glucose, 0.1 mM Na lactate, and 25 mU of glucagon-free insulin per milliliter solution. Osmolality = 290 mosmol/kg. The experimental perfusate differed by the addition of a 20% mannitol solution sufficient to raise the osmolality of the perfusate to 350 mosmol/kg. Osmolality was measured by a Fiske osmometer utilizing the freezing-point depression method.

During aerobic studies perfusate Po$_2$ was maintained 550 ± 10 mmHg by bubbling with 95% O$_2$ and 5% CO$_2$. For anoxic studies, solutions were bubbled with 95% N$_2$ and 5% CO$_2$ yielding a Pa$_o_2$ of 50 ± 5 mmHg. The pH of both solutions was maintained at 7.4.

**Experimental design.** An initial study compared the effects of the two perfusates during three sequential periods: A) 15 min of paced aerobic perfusion (control), B) 15 min anoxic perfusion (anoxia), C) 15 min of paced aerobic perfusion (recovery or postanoxic period). Thirty hearts were studied, 15 were perfused at isosmolal buffer during control and anoxic periods and with the hyperosmolal solution during the recovery phase.

To determine the effect of hyperosmolality when introduced after anoxia had been established, an additional group of seven hearts were perfused with isosmolal buffer during control and anoxic periods and with the hyperosmolal solution during the recovery phase.

**RESULTS**

**Hemodynamics.** During control aerobic perfusion hyperosmolality did not significantly affect left ventricular systolic peak pressure. Active contraction ceased within 60 s of the onset of anoxia, and neither heart rate nor left ventricular pressures could be recorded. No significant differences existed between the control or treated group with regard to time of cessation of contraction. During the recovery period, neither series of hearts reached preanoxic performance levels. Nevertheless, hearts perfused with the hyperosmolal solution showed a significant improvement over those perfused...
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Glucose consumption was not affected by variations in osmolality. With isosmolar buffer, consumption was $3.39 \pm 0.72 \mu\text{mol/min}$ during control aerobic perfusion and remained at $3.39 \pm 0.94 \mu\text{mol/min}$, despite total cessation of contractile activity and work performance during anoxia. With aerobic recovery consumption fell to $2.0 \pm 1.1 \mu\text{mol/min}$. During hyperosmolar perfusion, glucose consumption was $3.28 \pm 0.61 \mu\text{mol/min}$ in period A, $3.0 \pm 0.72 \mu\text{mol/min}$ in period B, and $2.22 \pm 0.56 \mu\text{mol/min}$ in period C ($P = \text{NS}$).

Tissue glycogen concentration was $103 \pm 11 \mu\text{mol/g dry wt}$ during aerobic perfusion with isosmolar buffer. During anoxic perfusion it fell to $41 \pm 5 \mu\text{mol/g dry wt}$ and remained close to this level during aerobic recovery ($43 \pm 4 \mu\text{mol/g dry wt}$) (Fig. 5). Hyperosmolality per se did not affect glycogen concentration. It was $93 \pm 10 \mu\text{mol/g dry wt}$ during period A, $37 \pm 1 \mu\text{mol/g dry wt}$ during period B, and $47 \pm 7 \mu\text{mol/g dry wt}$ during period C ($P = \text{NS}$).

**Tissue high-energy phosphate stores.** Hyperosmolality did little to alter the depletion of myocardial high-energy phosphate during anoxia or its repletion during recovery. Creatine

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**FIG. 3.** Effect of mannitol on left ventricular maximum dP/dt. Enhanced ventricular performance during reoxygenated recovery period (post = after anoxia) was noted with hyperosmolar perfusion.

Fused with Krebs-Ringer-Henseleit bicarbonate buffer alone. Thus, postanoxic left ventricular systolic pressure fell from a control of $127 \pm 5$ to $66 \pm 6$ mm Hg during isosmolar perfusion. During hyperosmolar perfusion, peak systolic pressure reached $102 \pm 7$ mm Hg from a control of $132 \pm 5$ mm Hg ($P < .01$) (Fig. 2).

Enhanced performance was more evident when the first derivative of the ventricular pressure curve was analyzed (Fig. 3). With KRB buffer alone, the mean recovery value of dP/dt had fallen to $1,758 \pm 172$ mm Hg/s or 50% of aerobic control ($3,513 \pm 398$). Perfusion with hyperosmolar solution enhanced recovery performance: dP/dt reached 79% of aerobic control ($2,998 \pm 234$ vs. $3,817 \pm 215$ mm Hg/s) ($P < .01$). Improved recovery was also observed when hyperosmolality was induced at the termination of the anoxic period. Under these circumstances, systolic pressure reached $12 \pm 4$ mm Hg or 86% of control ($141 \pm 6$ mm Hg, $P < .01$). Mean left ventricular dP/dt was $3,360 \pm 311$ mm Hg/s or 81% of control ($4,165 \pm 402$ mm Hg/s, $P < .01$).

Mean coronary flow during aerobic isosmolar perfusion was $11.9 \pm 0.7$ ml/min, rose markedly at the onset of anoxia to reach $19.2 \pm 2.4$ ml/min, and fell to $8.4 \pm 0.9$ ml/min when contraction stopped during anoxia. The mean recovery level was $7.1 \pm 0.2$ ml/min, a flow rate that did not differ significantly from aerobic control when flow was corrected for heart work. Hyperosmolar perfusion did not significantly change these rates. Coronary flow during hyperosmolar aerobic perfusion was $12.2 \pm 1.0$ ml/min, rose to $20.9 \pm 1.9$ ml/min at the onset of anoxia, and fell to $7.8 \pm 0.5$ ml/min during recovery.

**Carbohydrate metabolism.** Mean lactate production during aerobic isosmolar perfusion was $0.57 \pm 0.16 \mu\text{mol/min}$. Production increased to $3.05 \pm 0.53 \mu\text{mol/min}$ during anoxic perfusion and anaerobic metabolism and returned to approach control during the reoxygenated recovery period ($78 \pm 13 \mu\text{mol/min}$) (Fig. 4). The use of hyperosmolar perfusate did not alter lactate metabolism at any phase of the study. Production was $0.01 \pm 0.12 \mu\text{mol/min}$ in period A, $3.04 \pm 0.26 \mu\text{mol/min}$ in period B, and $1.07 \pm 0.13 \mu\text{mol/min}$ in period C ($P = \text{NS}$).

Glucose consumption was not affected by variations in

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**FIG. 4.** Comparison of total lactate production during 3 perfusion periods. Production increased during anoxia and returned toward aerobic control during reoxygenated recovery. No significant differences between the 2 perfusates were seen.

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**FIG. 5.** Comparison of tissue glycogen levels after perfusion with isosmolar and hyperosmolar solutions. Glycogen concentration fell during anoxia and remained depressed during recovery. No significant differences between the 2 perfusates were seen.
phosphate levels of 28.1 ± 1.4 μmol/g dry wt during isosmolal aerobic perfusion fell to negligible levels (0.3 ± 0.1 μmol/g dry wt) during anoxia and rose to 20.6 ± 3.0 μmol/g dry wt with recovery aerobic perfusion. Concentrations obtained with hyperosmolal buffer were: A) 29.4 ± 6.5, B) 0.2 ± 0.03, and C) 24.6 ± 3.0 μmol/g dry wt (P = NS) (Fig. 6). Tissue ATP concentrations showed a qualitatively similar change. With isosmolal buffer, ATP = A) 20.1 ± 1.3, B) 10.1 ± 1.1, C) 8.5 ± 1.2 μmol/g dry wt. Hyperosmolal perfusion did not significantly improve this balance nor did the presence of hyperosmolal buffer significantly alter ADP or AMP concentrations. The effect of hyperosmolality on tissue glycogen and high-energy phosphate concentrations during recovery is summarized in Fig. 7.

Myocardial tissue water content. The presence of hyperosmolal perfusate significantly affected total tissue water content during all three phases of perfusion (Fig. 8). The tissue wet/dry weight ratio with isosmolal perfusion in period A was 6.68 ± 0.41 and rose to 7.48 ± 0.56 (P < .01) during anoxia. It remained elevated throughout the recovery period (7.55 ± 0.17). Hyperosmolal perfusion reduced the ratio during aerobic perfusion (5.80 ± 0.26). Water content during anoxic perfusion was significantly less than that seen with the isosmolal buffer and remained fixed during recovery (6.77 ± 0.19).

DISCUSSION

Leaf (10, 11) has recently emphasized the physiological significance of intracellular volume regulation. His observation that intracellular edema plays an important role during oxygen deprivation and reversal or prevention of cellular swelling has a beneficial effect on organ function has stimulated much interest. In both brain (2) and kidney (6) infusion of hyperosmolal solutions has been shown to improve function previously depressed by an ischemic insult. The demonstration by Willerson et al. (18) of reversal of depressed ventricular function curves in the postcoronary ligated dog heart extended these observations to the myocardium and suggested a new therapeutic modality for the treatment of acute myocardial infarction. Improved ventricular performance was associated with a reduction in the area of ischemic injury as detected by epicardial ST segment maps and by an increase in both total and collateral coronary blood flow. It was suggested that induced obligatory extracellular hyperosmolality improved perfusion by a reduction of endothelial cell swelling and an increase in patency of arterioles and capillaries or by a direct reduction in coronary arteriolar resistance. Willerson et al. (19) have also shown that both hypertonic mannitol and sucrose will improve the performance of isolated isometrically contracting cat right ventricular papillary muscles during hypoxia, suggesting that augmentation of coronary perfusion is not the only mechanism responsible for improved myocardial function during oxygen deprivation. Other studies by Caulfield et al. (4) have demonstrated marked mitochon-
drial swelling and distortion of cristae following acute hypoxia in cat and dog papillary muscle. These ultrastructural changes were prevented by increasing the osmolality of intravascular fluids. It was postulated that the improved myocardial function during hypoxia resulted from more efficient oxidative phosphorylation and energy production due to the maintenance of optimal mitochondrial conformation.

Our results are in agreement with Willerson et al. (18, 19) and demonstrate improved recovery of hemodynamic function following anoxia in the isolated perfused rat heart when perfusate osmolality was raised by 60 mosmol/kg either before or after the anoxic stress. We were, however, unable to demonstrate an increase in total coronary flow during hyperosmolar perfusate at either high or low Po2. Part of this discrepancy may be the result of different models and experimental design. In the absence of formed blood elements, capillary entrapment is, of course, not possible. Furthermore, a reversal of capillary and arteriolar endothelial wall swelling sufficient to improve flow might well have been masked by the gross nature of the coronary flow measurement utilized or by the marked arteriolar vasodilatation induced by anoxia. Subtle changes in regional myocardial flow would also not be detected in the isolated rat heart model, and these changes in regional perfusion may be of extreme importance. Silicone latex injection studies of the vascular tree, reported elsewhere (5), also failed to demonstrate a gross difference in vessel caliber between these two types of huiller.

If hyperosmolar mannitol enhances regional perfusion or improves oxidative phosphorylation due to maintenance of mitochondrial structure, the adverse effect of anoxia on carbohydrate metabolism and high-energy phosphate production should be improved. In fact, there was no biochemical improvement in response to manipulation of osmolality. ATP and CP stores remained depleted and glucose and glycogen utilization unchanged.

It is impossible to determine whether the decrease in high-energy phosphate concentration was due to selective depression of mitochondrial oxidative phosphorylation or was a manifestation of a generalized uncoupling which persisted from the period of anoxia into the recovery phase. Perfusions were not continued long enough to enable us to determine whether control levels would ultimately be reached during recovery. It is noteworthy, however, that the enhanced hemodynamic performance of the hyperosmolar perfused hearts at similar tissue high-energy phosphate concentration might be interpreted as evidence for a relative increase in total energy production.

An important factor in the mechanism of action of hyperosmolar mannitol appears to be its direct effect on myocardial tissue water content. There was a positive relationship between reduction in wet/dry weight ratios and enhanced mechanical performance during the improved recovery phase (C) when mannitol was present and osmolality raised. This may reflect a relative increase in Ca++ concentration as cell volume is reduced, an improvement in compliance due to a reduction in tissue water content, or a more favorable intracellular milieu resulting from stabilization of membranes and preservation of important intracellular structures and organelles. Conclusions about cell swelling must be made with caution because of the colloid-free media used in our experiments. Freshly sampled rat left ventricle has a wet/dry weight ratio of 4.76. The aerobically perfused tissue had a ratio of 6.68 ± 0.41, indicating that these hearts were swollen even before anoxia and that the mannitol effects were occurring in hearts already edematous. Nevertheless, perfusion with a medium containing erythrocytes and protein might have produced even more significant changes.

Koch-Weser (8) suggested that the inotropic effect of hyperosmolality was related to loss of fiber water with a relative increase in intracellular Ca++ concentration in those areas of the muscle fiber in which Ca++ plays an essential role in excitation-contraction coupling. This passive increase in Ca++ concentration might be particularly important during oxygen deprivation when intracellular Ca++ is diluted and the normal contractile response to this ion, already burdened by competition with hydrogen ion for contractile binding sites, is lessened. This hypothesis is supported by Willerson et al.'s (17) studies of the inotropic effect of hyperosmolality in isolated cat right ventricular papillary muscles during increasing Ca++ concentrations. Prior elevation of Ca++ above a critical level abolished the inotropic action.

Compliance changes have been demonstrated with hypertonic mannitol but usually when control osmolality is exceeded by 100 mosmol/kg. Whether compliance was actually altered by increasing osmolality 60 mosmol/kg under our experimental conditions cannot be ascertained as no measurement of the pressure-volume relationship was made.

Further effects may be mediated by the ability of hyperosmolality to alter both contractile element and series elastic mechanical characteristics (15). If these factors were operative, there should have been an improvement in ventricular function during the aerobic control period when the heart was perfused with a solution of enhanced osmolality. We have been unable to demonstrate such an effect, although both Wildenthal et al. (15, 16) and Koch-Weser (8) showed improved left ventricular contractility in the nonischemic state during hyperosmolality. Regan et al. (13, 14) were also unable to show such an effect. Willerson et al. (18) found no improvement in ventricular function curves in the nonischemic dog heart, but this may be due to the relative insensitivity of function curves in animals with high stroke work and low end-diastolic pressure. The lack of an inotropic action of mannitol during aerobic conditions in the rat heart and the apparent discrepancy in the literature might reflect a species difference. It is of interest that despite a significant reduction in myocardial water content during the aerobic control period, no improvement in function resulted. This suggests that there is an optimum myocardial cell volume at which maximum function is obtained and that this volume is close to that found in the control state. Further reduction in cell size may not necessarily result in additional improvement. At a critical volume, crowding of cell constituents may occur and friction between parallel sliding structures may result.

Further investigation of the mechanism of action of hyperosmolar agents in the improvement of left ventricular function following anoxia or ischemia is required. The clinical applicability of an agent that increases serum osmolality...
and expands extracellular fluid volume during or following an episode of myocardial ischemia remains to be determined.

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