The Effect of Pharmacologic Agents Upon the Dynamics of Anoxic Perfused Rat Hearts[1] (35787)

JAMES SCHEUER[2] AND S. WILLIAM STEZOSKI

Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213

Knowledge of the response of the hypoxic heart to positive inotropic agents is important in determining modes of therapy that might be beneficial in patients with ischemic heart disease. Shortly after coronary occlusion an ischemic segment of myocardium can respond to positive inotropic agents (1). Hypoxic isolated cat papillary muscles can also respond to positive inotropic interventions (2). In dogs made hypoxic, acetyl strophanthidin has an attenuated effect on overall cardiac function (3). It was recently shown that, during anoxia, hearts with elevated endogenous glycogen stores develop higher rates of anaerobic ATP formation, and that these hearts are partially protected against mechanical deterioration (4). This observation raises the possibility that the glycogen-rich heart might also respond better to positive inotropic agents during hypoxia than hearts with normal glycogen stores.

The present experiments were conducted to study whether anoxic myocardium, with known depressed ATP stores, would be responsive to positive inotropic agents. The responsiveness of hearts from normal rats and reserpinized rats with high glycogen stores were studied.

Methods. Hearts of male Wistar rats, 200–250 g, were perfused retrograde through the aorta at a constant perfusion pressure, using a double perfusion apparatus as described and diagramed previously (5, 6). A needle was inserted into the perfusion line just above the heart so that saline, or a pharmacologic agent could be delivered from a Harvard constant infusion pump. The perfusion medium contained 143 m*M* sodium, 126 m*M* chloride, 25 m*M* bicarbonate, 6 m*M* potassium, 1.2 m*M* magnesium sulfate, 1.2 m*M* phosphate, 1.2 m*M* calcium, 0.4 m*M* sodium EDTA and 5 m*M* glucose. In aerobic experiments, oxygenated perfusion (95% O_2, 5% CO_2 gassing mixture) was conducted for 25 min. In anoxic experiments hearts were perfused for 15 min under aerobic conditions and then an anoxic perfusion medium [95% N_2, 5% CO_2 (pO_2 3–7 mm Hg)] was abruptly switched into the system for 5 min. Oxygenated medium was then switched back for 5 min. Hearts were paced at constant rates (330–340 beats/min) after the first 10 min of aerobic perfusion. In all experiments the infusion of the test agent was begun after 15 min of aerobic perfusion and continued for 7 min. The volume of infusate was 0.1 ml/min, and the agents delivered were 0.9% saline (controls), L-norepinephrine, 4×10^{-10} moles/min, glucagon, 2×10^{-10} moles/min or ouabain 1.4×10^{7} moles/min. Left ventricular pressures, dp/dt and the electrocardiogram was recorded on an Electronics for Medicine photographic recorder. The studies were conducted at 37°.

To study hearts with high glycogen stores, rats were treated with reserpine 5 mg/kg and their hearts were perfused 24 hr later. This regimen has been shown not to produce a direct negative inotropic effect. It results in a doubling of the endogenous cardiac glycogen stores and enhanced anaerobic glycogenolysis and glycolysis (4). Under the conditions used in these studies, anoxia results in a decline in high energy phosphate stores during the first 30 sec (5). During 5 min of

[1] Supported by U. S. Public Health Service Grant HE-09727 from the National Heart and Lung Institute.

[2] Recipient of National Institutes of Health Career Development Award HE 15867.

TABLE I. Dynamic Values After Aerobic Perfusion of Hearts for 15 min.[a]

	Control	Reserpine
Coronary flow (ml/min)	8.1 ± 0.2	9.9 ± 0.4[b]
PLVSP (mm Hg)	47 ± 2	49 ± 2[c]
EDP (mm Hg)	0.7 ± 0.2	0.8 ± 0.2[c]
dp/dt (mm Hg/sec)	2433 ± 245	2308 ± 153[c]

[a] N = 56 for control and for reserpine studies.
[b] $p < 0.001$.
[c] $p > 0.20$.

anoxia, creatine phosphate falls 90%. ATP falls 40% in hearts from normal rats and 25% in hearts of reserpinized rats (4). Reoxygenation effects replenishment of high energy phosphate stores in less than 1 min in hearts from normal rats (5).

Results. Table I shows the values for hearts from normal control rats (CH) and hearts from reserpinized animals (RH) after 15 min of aerobic perfusion, prior to the experimental intervention. Except for the slightly higher coronary flow after 15 min of aerobic perfusion, hearts from control and reserpinized animals act similarly in the isolated state. Figure 1 shows the results from perfusions in which saline was infused. Figure 2 shows the reesults for norepinephrine, Fig. 3 for glucagon, and Fig. 4 for ouabain.

Table II compares the values obtained within any one experimental design. The 20-, 22-, and 25-min values for each heart are compared with its value after 15 min of aerobic perfusion, each heart serving as its own control. Table III compares the effects of anoxia with and without inotropic agents in CH and RH.

Coronary flow. Under aerobic conditions coronary flow declined slightly during experimental perfusion. In general, inotropic agents prevented this fall.

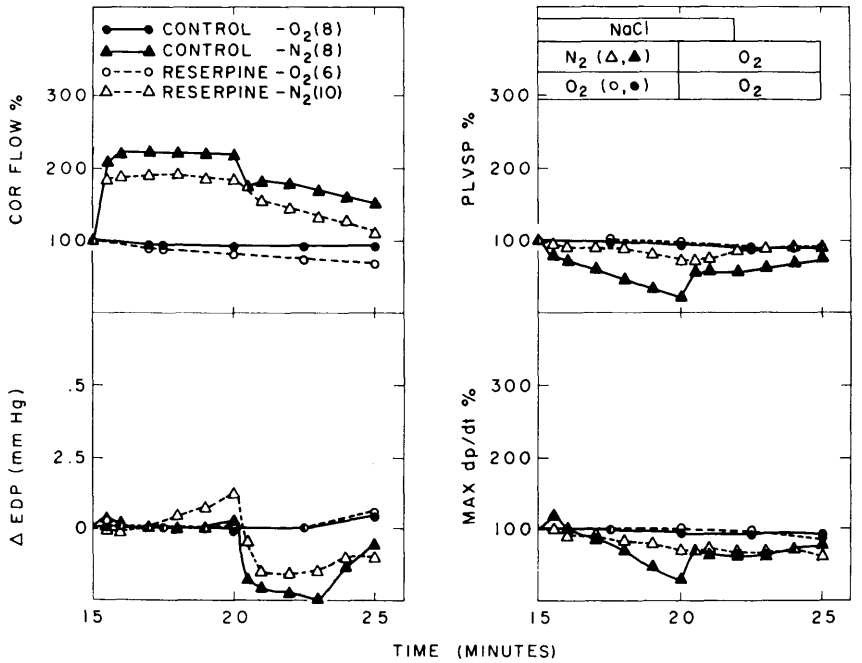

FIG. 1. Performance of CH and RH during aerobic and anaerobic perfusion periods (15–20) and recovery (20–25). Saline was infused from 15–22 min. The numbers of hearts are shown in parentheses. Cor Flow = coronary flow. PLVSP = peak left ventricular systolic pressure; EDP = end-diastolic pressure; Max *dp/dt* = maximum rate of left ventricular pressure rise; N_2 = anoxic perfusion; O_2 = aerobic perfusion. All values except EDP are percentage of the control (15 min) aerobic value. The means for these 15-min values are shown in Table I. EDP is shown as the absolute change from the control value (mm Hg).

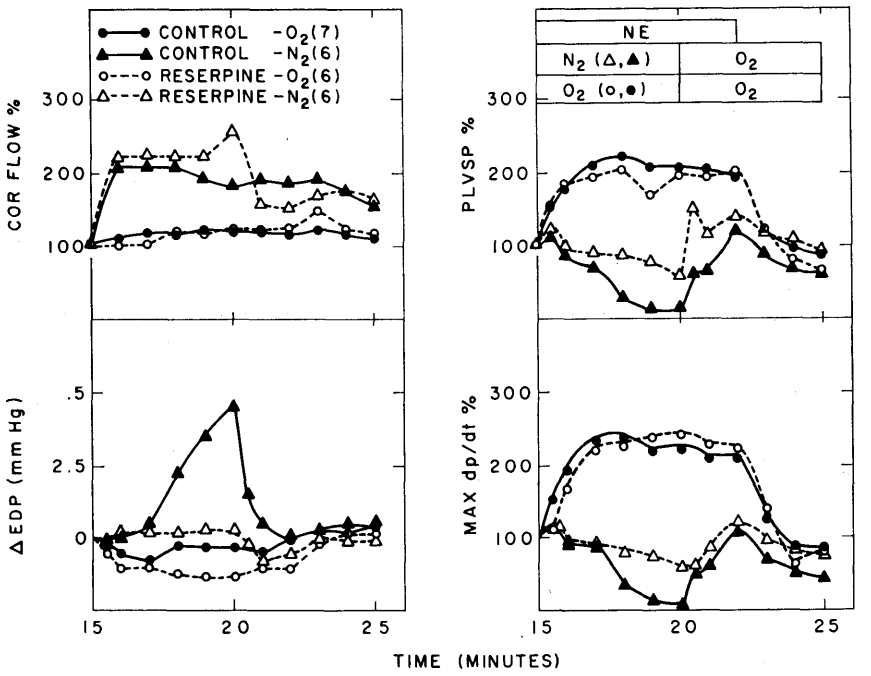

FIG. 2. The effect of L-norepinephrine infusion on the performance of aerobic and anoxic hearts. Format is the same as Fig. 1.

Anoxia increased coronary flow in both CH and RH and this increase persisted throughout the recovery period. The apparent difference in the magnitude of the anoxic response between CH and RH is not significant. The changes during anoxia did not appear to be qualitatively altered by inotropic agents. However, with norepinephrine the return toward the control level during the recovery period was more rapid than with other agents.

After 5 min of anoxia in CH, lower coronary flows were found in the presence of norepinephrine and glucagon than in their absence. These differences were not observed in RH. In anoxic RH, coronary flow during recovery was faster in the presence of these agents.

Peak left ventricular systolic pressure. As shown previously RH had higher peak left ventricular systolic pressure (PLVSP) than CH after 5 min of anoxia. These same relationships were seen whether or not inotropic agents were administered. PLVSP, which is quite constant in CH and RH under aerobic conditions, rose with inotropic intervention in all cases, and returned to the control level when the agents were discontinued. In some cases, PLVSP fell below the control value after cessation of norepinephrine. PLVSP fell with anoxia, and recovery was complete with reoxygenation in CH but not RH. This fall was not significantly prevented by the inotropic agents. During the first 2 min of the recovery period, while infusion of the inotropic agent was continued, PLVSP rose above the control level with glucagon in both RH and CH and with NE in RH. After the inotropic agent was discontinued during the recovery period, PLVSP fell significantly below the control level in CH but not in RH.

The maximal rate of left ventricular pressure rise. As shown previously maximal left ventricular pressure rise (*dP/dt*) fell less during anoxia in RH than in CH. This relationship was not altered by inotropic agents. The findings regarding the *dP/dt* were quite similar to those with PLVSP, except that after anoxia, recovery was incomplete in both CH and RH. During anoxia in CH LV

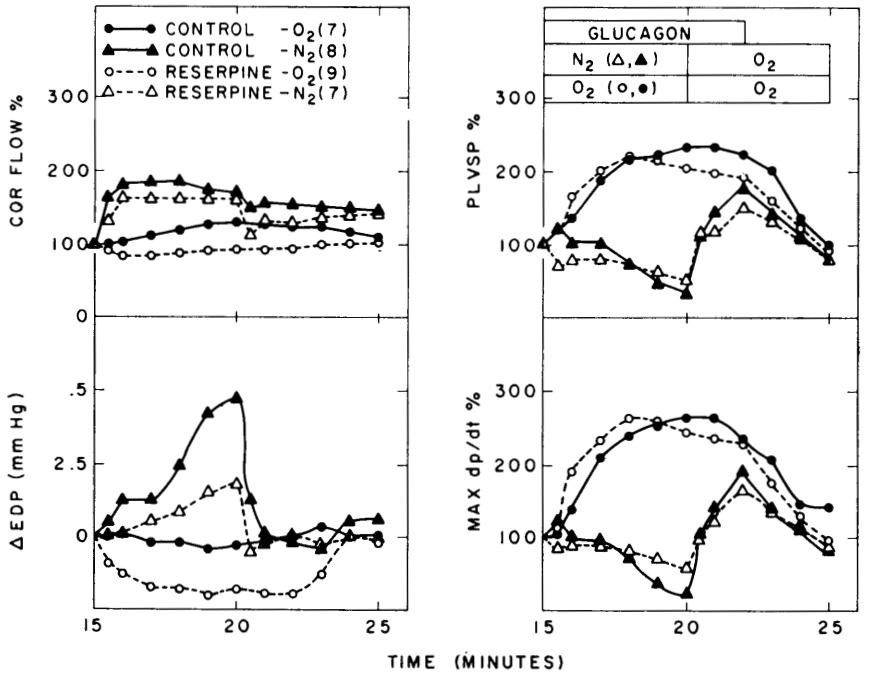

FIG. 3. The effect of glucagon infusion on the performance of aerobic and anoxic hearts. Format is the same as Fig. 1.

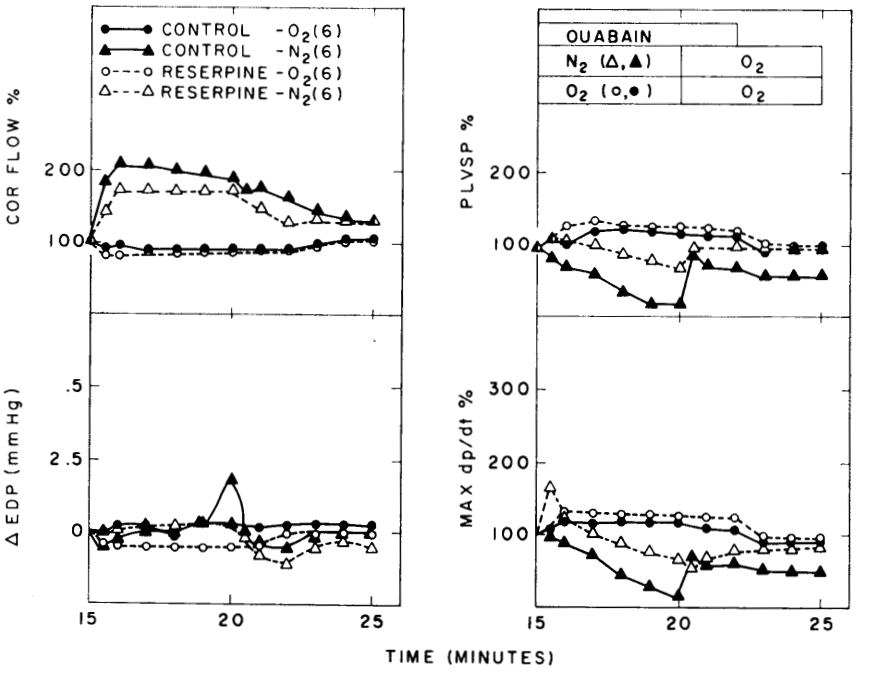

FIG. 4. The effect of ouabain infusion on the performance of aerobic and anoxic perfused hearts. Format is the same as Fig. 1.

TABLE II. The Experimental Values Compared with Control Values for Each Experimental Design.[a] [b]

	No. of hearts	CF 15 vs 20	CF 15 vs 22	CF 15 vs 25	PLVSP 15 vs 20	PLVSP 15 vs 22	PLVSP 15 vs 25	EDP 15 vs 20	EDP 15 vs 22	EDP 15 vs 25	Max LV *dp/dt* 15 vs 20	Max LV *dp/dt* 15 vs 22	Max LV *dp/dt* 15 vs 25
C, O_2	8	↓	↓	—	—	—	—	—	—	—	—	—	—
C, N_2	8	↑	↑	↑	↓	↓	—	—	↓	—	↓	↓	↓
R, O_2	6	↓	↓	↓	—	—	—	—	—	—	—	—	—
R, N_2	10	↑	↑	—	↓	↓	↓	—	↓	↓	↓	↓	↓
L-Norepinephrine													
C, O_2	7	—	—	—	↑	↑	—	—	—	—	↑	↑	—
C, N_2	6	↑	↑	↑	↓	—	↓	—	—	—	↓	—	↓
R, O_2	7	—	—	—	↑	↑	↓	—	—	—	↑	↑	—
R, N_2	6	↑	↑	↑	↓	↑	—	—	—	—	↓	—	—
Glucagon													
C, O_2	7	↑	↑	—	↑	↑	—	—	—	—	↑	↑	—
C, N_2	8	↑	↑	↑	↓	↑	↓	—	—	—	↓	↑	↓
R, O_2	9	—	—	—	↑	↑	—	↓	↓	—	↑	↑	—
R, N_2	6	↑	↑	—	↓	↑	—	—	—	—	↓	—	—
Ouabain													
C, O_2	6	—	—	—	↑	↑	—	—	—	—	↑	—	—
C, N_2	6	↑	↑	—	↓	—	↓	↑	—	—	↓	—	↓
R, O_2	6	↓	↓	—	↑	↑	—	↓	—	—	↑	↑	—
R, N_2	6	↑	↑	—	↓	—	—	—	—	—	↓	—	—

[a] The 20, 22, and 25 min values are compared with paired 15 min values. Each heart serves as its own control. C, nonreserpinized; R, reserpinized; N_2, anoxic (95% N_2, 5% CO_2); and O_2, 95%, 5% CO_2.

[b] ↑ signifies that the mean 20, 22, or 25-min value is greater than its mean 15-min control value, $p < 0.05$; ↓ signifies that the mean 20, 22, or 25-min value is less than its mean 15-min control value, $p < 0.05$; — signifies $p > 0.05$.

dP/dt deteriorated more rapidly in the presence than in the absence of norepinephrine. Prior administration of norepinephrine also appeared to accelerate late deteri-

TABLE III. Comparison Between the Effects of Anoxia on Isolated Hearts With or Without Inotropic Agents.[a] [b]

Conditions compared (min):	CF; time 20	CF; time 22	CF; time 25	PLVSP 20	PLVSP 22	PLVSP 25	EDP 20	EDP 22	EDP 25	Max LV *dp/dt* 20	Max LV *dp/dt* 22	Max LV *dp/dt* 25
C, N_2 vs C, N_2, NE	—	—	—	—	↑	↓	—	↑	—	↓	↑	↓
C, N_2 vs C, N_2, G	↓	—	—	—	↑	—	—	—	—	—	↑	—
C, N_2 vs C, N_2, O	—	—	—	—	—	↓	—	—	—	—	—	↓
R, N_2 vs R, N_2, NE	—	—	↑	—	↑	—	—	—	—	—	↑	—
R, N_2 vs R, N_2, G	—	—	↑	—	—	—	—	—	—	—	↑	—
R, N_2 vs R, N_2, O	—	—	—	—	—	—	—	—	—	—	—	↑

[a] The results compare means expressed as the percentage of the control (15 min) value at 20, 22, and 25 min for hearts made anoxic in the absence or presence of the inotropic agent. EDP results compare the mean changes observed at 20, 22 and 25 minutes from the 15 min value of each heart. C, nonreserpinized; R, reserpinized; N_2, anoxic; NE, L-norepinephrine; G, glucagon; and O, ouabain.

[b] ↑ Signifies the mean value with the inotropic agent is greater than without it, $p < 0.05$; ↓ signifies the mean value with the inotropic agent is less than without it, $p < 0.05$; — signifies $p > 0.05$.

oration in CH. In general the inotropic agents promoted faster recovery from anoxia. This was frequently transient in CH and more sustained in RH.

Left ventricular end-diastolic pressure. Left ventricular end-diastolic pressure (EDP), was constant in aerobic perfusions. With anoxia, EDP tended to rise, and with recovery EDP fell significantly below control level in both CH and RH. EDP rose with aerobic perfusion in RH when glucagon and ouabain were administered. Inotropic agents appeared to have no consistent effect on EDP in anoxic hearts.

Discussion. In the present experiments three positive inotropic agents failed to augment cardiac function during myocardial anoxia. It was realized that rat heart is relatively insensitive to cardiac glycosides (7). Therefore, a very large dose of ouabain was employed. All three agents were delivered in doses found to significantly increase performance under aerobic conditions. It is possible that minor responses in tension development could have gone undetected if alterations in ventricular volumes occurred in these ventricles. However, this isolated heart model is sufficiently sensitive to detect small differences in ventricular performance (4), and almost certainly would detect changes of the magnitude reported by Tyberg *et al.* (2). In the study of Donlon and Yu (3) in which hypoxemia was present, it is probable that partial oxygenation was sufficient to permit generation of the added energy needed for a positive inotropic response. In the studies by Hood *et al.* (1), the period of ischemia was only 2 min and acidosis or depression of high energy phosphate stores may not have been severe enough to abolish a positive inotropic response. Alternatively, it is possible that enough collateral circulation to the region of ischemia was present to permit partial oxygenation of some of the fibers. The excellent responses found by Tyberg *et al.* (2) in cat papillary muscle subjected to anoxic medium are more difficult to explain in the light of the present report. However, there are several important differences in experimental design. In the present study, myocardium was perfused through its vascular system, the temperature was 37°, hearts were beating at physiologic or slightly faster rates (8), and the pO_2 of the "anoxic" solution was less than 7 mm Hg. The studies by Tyberg *et al.* (2) were conducted in papillary muscles bathed in the anoxic solution. The temperature was 29°, the muscles were paced at 12 beats/min and the "anoxic" pO_2 was approximately 15 mm Hg.

Hearts from reserpinized animals consistently performed better during anoxia than hearts from control animals. The failure of hearts from reserpinized animals to respond to positive inotropic agents during anoxia indicates that, although they do have greater glycolytic reserve (4), this reserve is quite limited. Under less severe circumstances such as a brief period of segmental hypoxia, the reserve might be physiologically significant. This suggestion is consistent with the improved response to inotropic agents of RH during reoxygenation.

Hearts perfused under the anoxic conditions used in the present investigation rapidly develop depressed high energy phosphate stores (5). The failure of such hearts to respond to positive inotropic agents is consistent with limitation of energy delivery at the contractile site. Whether this, or other factors such as hydrogen ion accumulation (9) are of primary importance in the depressed contractility of acutely anoxic myocardium is not known.

Although positive inotropic agents appeared to promote improved recovery during reoxygenation, this improvement lasted only as long as the agent was administered. Since the use of such agents during recovery would have a metabolic cost in terms of high energy phosphate utilization, it might ultimately lead to cardiac deterioration. This possibility is supported by the depressed left ventricular pressure formation observed in hearts from control animals during recovery from anoxia after they had been treated with norepinephrine or ouabain.

1. Hood, W. B., Jr., Covelli, V. H., Abelmann, W. H., and Norman, J. C., Cardiovasc. Res. 3, 249 (1969).

2. Tyberg, J. V., Yeatman, L. A., Parmley, W. W., Urschel, C. W., and Sonnenblick, E. H., Amer.

J. Physiol. **218**, 1780 (1970).

3. Donlon, J. V., and Yu, P. N., Amer. Heart J. **78**, 238 (1969).

4. Scheuer, J., and Stezoski, S. W., Circ. Res. **27**, 835 (1970).

5. Scheuer, J., and Stezoski, S. W., Circ. Res. **23**, 519 (1968).

6. Scheuer, J., and Stezoski, S. W., Amer. J. Med. Sci. **257**, 218 (1969).

7. Repke, K., Est, M., and Portius, H. J., Biochem. Pharmacol. **14**, 1785 (1965).

8. Adolph, E. F., Amer. J. Physiol. **212**, 595 (1967).

9. Katz, A. M., and Hecht, H. H., Amer. J. Med. **47**, 497 (1969).

Received Feb. 9, 1971. P.S.E.B.M., 1971, Vol. 137.