Inotropic Agents in Hypoxic Cat Myocardium

DEPRESSION AND POTENTIATION

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ABSTRACT

The effects of several inotropic agents were investigated in papillary muscle preparations from normal cats and cats that had undergone previous total extrinsic cardiac denervation. The papillary muscles were bathed in a modified Krebs-Ringer's solution which was equilibrated with 95% O₂-5% CO₂ for the control drug studies or 5% O₂-5% CO₂-90% N₂ for the hypoxia studies. This degree of hypoxia produced a 32 ± 3% decrease in tension in papillary muscles from normal cats and a 14 ± 4% decrease in catecholamine-depleted papillary muscles. During hypoxia there was depression of the maximum response to ouabain and norepinephrine with otherwise unchanged dose-response curves. Deterioration of the hypoxic muscles occurred after exposure to norepinephrine concentrations of 5 × 10⁻⁷M and greater. The dose-response curve for angiotensin II was unchanged by hypoxia. However, during hypoxia, the dose-response curves for two heptapeptide analogues of angiotensin II, 1-des-5-Val and 1-des-5-Ile-angiotensin II, were augmented. The inotropic effects of these analogues under control and hypoxic conditions were the same in the normal and catecholamine-depleted muscles. The potentiation by hypoxia of the inotropic effects of the angiotensin II analogues is unique among the agents studied.

KEY WORDS norepinephrine angiotensin II analogues ouabain oxidative metabolism papillary muscle

The frequent clinical requirements for inotropic agents for treating hypoxic or ischemic heart disease have led to this evaluation of the effects of inotropic agents in isolated hypoxic heart muscle. To circumvent the profound reflex changes in ventricular performance induced by hypoxia (1, 2), the responses to inotropic agents were studied in isolated cat papillary muscles. A level of hypoxia was used which produced reversible reductions in the contractile force but not rapid deterioration of the muscle preparations. The results showed that each of the inotropic agents had different effects during hypoxia. Some analogues of angiotensin II had the unexpected property of potentiation by hypoxia.

Methods

Adult male cats, weighing 2–3.5 kg, were anesthetized with sodium thiamylal (Surital), 30 mg/kg, intrapleurally. The hearts were quickly removed, and right ventricular papillary muscles (0.9–2.2 mm in diameter) were removed after the tendinous end was tied with a 5-0 Mersiliene suture. The muscles were then mounted in a 25-ml flow-through Lucite bath. The cut mural end was inserted in a spring-loaded clip which was attached to a Minneapolis Honeywell 762025 isometric force-displacement transducer. The tendinous end was tied to a fixed post in the bath. Modified Krebs-Ringer's solution (composition, mM: Na⁺ 146, K⁺ 3.6, Ca²⁺ 2.5, Mg²⁺ 1.2, H₂PO₄⁻ 1.2, Cl⁻ 128, SO₄²⁻ 1.2, HCO₃⁻ 25, and glucose 5.6) was equilibrated at 25°C with 95% O₂-5% CO₂ in a remote column. The pH of the solution was 7.4. The solution was circulated to the muscle bath from the column by gravity and a return pump. The muscles were stimulated by two fine platinum electrodes near the base of each
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muscle at 12/min and approximately 5% above threshold voltage. The resting tension of the muscle was the tension at the peak of the length-tension curve. The first derivative of the tension (dT/dt) was obtained on-line by a differentiating circuit (operational amplifier, Nexus SQ-10) with bandwidth of 0.12-340 Hz.

For the hypoxia studies, after control tension measurements were made, the equilibrating gas mixture was changed to 5% O2-5%CO2-90% N2. The Po2 of the Krebs-Ringer's solution (henceforth called Krebs solution) was recorded in the following manner. The bath solution was suctioned, 0.3 ml/min, from a port in the bath near the muscle and directed to a Lucite cell which held a Clark oxygen electrode. The electrode was calibrated before and after each experiment with known gas mixtures. The Po2 was continuously recorded on an oscillograph.

Drugs used were: dl-norepinephrine bitartrate monohydrate, ouabain, angiotensin II, and two analogues of angiotensin II (1-des-5-Val-angiotensin II and 1-des-5-Ile-angiotensin II).1 Drugs were made up in Krebs solution, rinsed through the bath in a volume which was four times the volume of the bath, and then recirculated.

Two muscle preparations were studied simultaneously in identical muscle baths. Each muscle was exposed to only one concentration of one drug at either high or low oxygen tension. The protocol of the experiment was as follows. The muscle was first bathed in Krebs solution under high oxygen tension. Resting tension and developed tension at maximum length (Lmax) were determined. The muscle was then allowed to stay at the high oxygen tension for 1 hour, or the equilibrating gas mixture was changed to the low oxygen one. Following the hour of equilibration either at the high or low oxygen tension, the muscle was exposed to one concentration of one drug. Each point on the concentration-response curve represents the average of the responses of four muscles. After addition of the drug, 3-5 minutes were required to reach the final drug concentration in the bath. The responses were observed for the next 15 minutes. The maximum response during this period was measured. Previous experiments with this apparatus have demonstrated that maximum responses to each of these agents occur within 10 minutes. At the end of each experiment, the length of the muscle between the clip and the tied end was measured at Lmax and the muscle was then weighed. Using these measurements, the radius and the surface area were calculated assuming a cylindrical model.

Tension, dT/dt, and Po2 were continuously recorded on a Sanborn 350 direct-writing oscillograph. Tension development was expressed as percent change from base line or as absolute tension in grams; dT/dt was also expressed as percent change from base line or as absolute rate of tension development in g/sec. Responses were expressed as mean values ± se. Group means were compared with Student's t-test. Gas tensions were expressed as mm Hg at standard temperature.

Extrinsic cardiac denervation was performed on nine cats by the technique of mediastinal ablation (3). The procedure was accomplished through a right lateral thoracotomy under halothane anesthesia. The cats were killed 3-6 weeks after the operation, and the papillary muscles were studied. Two muscles were obtained from each of the nine cats. Samples of left and right ventricular myocardium revealed norepinephrine contents less than 0.01 µg/g tissue by the trihydroxyindole method (4).

Results

Effects of Hypoxia.—The Po2 of the bath solution was 440 ± 8 mm Hg when equilibrated with the solution with high O2 tension and 122 ± 6 mm Hg when equilibrated with the solution with low O2 tension. The equilibration of the Krebs solution in the remote column necessitated circulation through plastic tubes which were permeable to oxygen. Furthermore the horizontal baths allowed oxygen transfer. Therefore the resultant Po2 of the bath solutions was a function of the equilibrating gases and the oxygen exchange (loss for high Po2 and gain for low Po2 solutions) with the air. The Pco2 remained 35 ± 4 mm Hg. The Po2 of the bath fell continuously over a 60-minute period after the equilibrating gas mixture was changed to that with low O2 tension, as shown in Figure 1. After reaching the stable low Po2, the mean reduction of tension was 32% in papillary muscles of normal animals (3.8 ± 0.7 to 2.6 ± 0.6 g/mm²) and 14% in catecholamine-depleted papillary muscles (3.6 ± 0.5 to 3.1 ± 0.5 g/mm²). The differences of these two groups were statistically significant (P<
The average isometric force for the groups of muscles exposed to each agent under control or hypoxic conditions appears in the legends of the concentration-response curves. There was no statistical difference in the average force for any group of muscles. In six control muscle preparations, the equilibrating gas was maintained at 95% O\textsubscript{2}-5% CO\textsubscript{2} and the contractile force did not change over a 180-minute period. During the following 60-minute period, the PO\textsubscript{2} was maintained at 122 ± 6 mm Hg. There was a variable reduction of tension during this period which never exceeded 10% of the initial hypoxic tension if no drug intervention was made. It was during the first 15 minutes of this period that drug interventions were made. After 60 minutes of hypoxia, control tensions could be restored if the equilibrating gas mixture was changed to 95% O\textsubscript{2}-5% CO\textsubscript{2}. In six control preparations, 100% of control tension was achieved after this reoxygenation. Time to peak tension shortened after hypoxia in papillary muscles from normal animals (control 0.48 ± 0.03 seconds, hypoxia 0.39 ± 0.02 seconds, P < 0.01) but did not shorten significantly in the muscles from chronically denervated animals (control 0.43 ± 0.02 seconds, hypoxia 0.42 ± 0.03 seconds, P > 0.1).
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Responses to Norepinephrine.—Figure 2 depicts the dose-response curves for norepinephrine under control conditions and during hypoxia. Not only was the dose-response curve shifted to the right, but at concentrations of $5 \times 10^{-7}$ M and greater the developed tension of the muscle preparations began to deteriorate after the maximum response to norepinephrine even if the drug was washed out (Fig. 3). Fifteen minutes after exposure to norepinephrine ($5 \times 10^{-7}$ and $10^{-6}$ M) during hypoxia, the developed tension had fallen to $30 \pm 15\%$ of the control tension during hypoxia. There was a significant difference ($P < 0.005$) in the maximum response under hypoxic conditions at doses of $5 \times 10^{-7}$ M and larger. Time to peak tension shortened under both control and hypoxic conditions following the administration of norepinephrine.

Responses to Ouabain.—The dose-response curves for ouabain under control and hypoxic conditions are shown in Figure 4. A significant reduction of the response occurred at doses of $2 \times 10^{-6}$ M and greater during hypoxia. Although the depression of the responses was similar to that caused by norepinephrine during hypoxia, no deterioration of the muscle preparation was observed after exposure to ouabain. During hypoxia, the developed tension 15 minutes after exposure to ouabain ($2 \times 10^{-6}$ and $10^{-5}$ M) was $130 \pm 20\%$ of the control tension during hypoxia. Time to peak tension was not shortened by ouabain in either the control or hypoxic conditions.

Responses to Angiotensin II.—The dose-response curve for angiotensin II was not altered by hypoxia. Figure 5 shows the control dose-response curve previously reported (5).
Angiotensin II concentration-response curves obtained under control and lowered O2 tensions. Responses obtained under both conditions were not statistically different. Isometric force before drug addition: hypoxia: 2.4 ± 0.8 g/mm².

Responses to Angiotensin II Analogues.—The dose-response curves for two heptapeptide analogues of angiotensin II, 1-des-5-Val- and 1-des-5-Ile-angiotensin II, are shown in Figures 6 and 7, respectively. The inotropic responses to both of these agents at any given concentration were greater during hypoxia than under control conditions. Not only was there a shift to the left of the dose-response curves, but greater maximum responses were obtained. The maximum rate of tension development (dT/dt) also increased in a dose-dependent manner in response to both of these agents. Figure 6 shows dT/dt for 1-des-5-Val-angiotensin II. The time to peak tension and total contraction time were unchanged after these agents under control and hypoxic conditions. 1-des-5-Val-angiotensin II was the more potent inotropic agent of the two analogues.

Catecholamine-Depleted Papillary Muscles.—1-des-5-Val-angiotensin II was tested in 18 catecholamine-depleted muscles. The responses of these muscles were essentially the same as the responses obtained in normal muscles and are included in the averages shown in Figure 6. Neither the average responses nor the standard errors were changed by excluding the responses of the denervated muscles. The responses in Figure 6
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Controlled hypoxic conditions were established in isolated cat papillary muscles to evaluate the myocardial responses to hypoxia and alterations in the effects of inotropic agents. A reduction in maximum developed tension and dT/dt occurred as the P0₂ was decreased. Isolated papillary muscles bathed in Krebs solution depend on the diffusion of oxygen from the solution for oxidative metabolism. Creatine phosphate has been found to be adequate in this preparation when the bathing solution is equilibrated with 95% O₂ (6). Certainly as the oxygen tension in the Krebs solution is decreased, the oxygen available to the muscles is lowered. This decrease in available oxygen undoubtedly compromises oxidative metabolism, although there is no direct evidence for this. Although high oxygen tensions have been reported to

FIGURE 6
Concentration-response curves for 1-des-5-Val-angiotensin II. Both isometric developed tension and dT/dt were significantly higher at all doses under hypoxic conditions. The responses of the catecholamine-depleted papillary muscles are included in the responses here. No difference in the mean or SE was observed between normal and catecholamine-depleted muscles. Isometric force before drug addition: control 3.4 ± 0.5 g/mm², hypoxia 2.5 ± 0.4 g/mm².

FIGURE 7
Concentration-response curves for 1-des-5-Ile-angiotensin II. The isometric developed tension was significantly greater during hypoxia at concentrations of 5 x 10⁻⁷M and greater. Isometric force before drug addition: control 3.8 ± 0.8 g/mm², hypoxia 2.4 ± 0.7 g/mm².

Discussion

at each concentration under control and hypoxic conditions include at least one response of a catecholamine-depleted muscle. Figure 8 shows the characteristic response to 1-des-5-Val-angiotensin II in a catecholamine-depleted papillary muscle.
TENSION gra|ms/sec

\[ \text{Tension} \quad \text{grams/sec} \]

\[ \text{CONTROL} \]

\[ 1.1 \times 10^{-7} \text{ M} \]

\[ 20' \text{ AFTER WASH} \]

**FIGURE 8**

Response to 1-des-5-Val-angiotensin II in a catecholamine-depleted papillary muscle. The responses to this agent under control and hypoxic conditions were identical to the responses of normal muscles, i.e., the responses were potentiated during hypoxia.

depressed myocardial contractility in intact hearts (7), this was not observed in our experiments. If the control tensions were depressed by the high PO₂, one would have expected a transient increase in contractile force as the PO₂ was lowered. This did not occur (Fig. 1). The reduction in contractile force caused by hypoxia was reversible after 1 hour. Furthermore, the preparation was quite stable during the period of time required for the evaluation of the responses to the inotropic agents.

The developed tension and dT/dt of catecholamine-depleted papillary muscles decreased less during hypoxia than did in papillary muscles of normal cats. The reason for this difference is not clear. Tyberg et al. (8) have subjected isolated cat papillary muscles to a more profound degree of hypoxia than that used in the present study. Time to peak tension and total tension fell similarly in muscles from both control and reserpine-treated cats (8). However Scheuer and Stezoski (9) reported that pretreatment with reserpine 24 hours in advance prevented mechanical deterioration and creatine phosphate depletion when the rat hearts were made hypoxic. The authors felt that this protective effect of reserpine pretreatment was due to increased cardiac glycogen rather than to diminished catecholamine stores. Cardiac glycogen stores in denervated cat hearts are not known, although increased glycogen stores in autotransplanted canine hearts have been documented (10).

The augmented oxygen consumption of papillary muscles in response to norepinephrine and ouabain is related to the increased contractility (11, 12). In the present study, norepinephrine produced greater augmentation of contractility than ouabain. Although the maximum responses to both of these agents were depressed during hypoxia, the deterioration of the muscles following the maximum responses to norepinephrine could be related to the increased contractility which was not achieved by the maximum concentrations of ouabain. On the other hand, angiotensin II and its analogues produced increases in contractility which were as large or larger than those produced by norepinephrine without evidence of deterioration during hypoxia.

The degree of depression of contractility induced by hypoxia can influence the response to inotropic interventions. Tyberg et al. (8) demonstrated a larger percent response in isometric force to paired stimulation when developed force was decreased 69% by hypoxia. The absolute increase in force, however, was less during hypoxia. In the present study, isometric tension was decreased 32% by...
hypoxia. The absolute responses to the inotropic agents, therefore, were 68% of the responses depicted in Figures 2, 4–7. Thus there were larger separations of the absolute responses to norepinephrine and ouabain and an insignificant difference in the responses to angiotensin II. The absolute responses to 1-des-5-Val-angiotensin II remained significantly augmented at all concentrations. The absolute responses to 1-des-5-Ile-angiotensin II were significantly greater at concentrations of 5 × 10^-7M and larger. Furthermore, during hypoxia significantly greater absolute tensions were achieved at the peak of the concentration-response curves. Therefore, the potentiation of the effects produced by these agents was not simply a function of previously depressed contractility.

The analogues of angiotensin II have direct myocardial inotropic effects which are independent of catecholamine stores. This direct myocardial effect has been demonstrated previously for angiotensin II (5, 13). Furthermore, these analogues possess the unique characteristic among the inotropic agents tested in this study of producing augmented inotropic responses in hypoxic myocardium when compared to the responses in myocardium under higher oxygen tensions. This characteristic may be related to the effects of angiotensins on mitochondria (14). Some polypeptides of the angiotensin series increase the rate of oxidative phosphorylation and the respiratory control index of mitochondria from bovine heart. Tritiated angiotensin localizes over myocardial mitochondria and nuclei within 30 seconds after coronary infusion (P. Khairallah, personal communication). If these results apply to the experiments reported here, they suggest that angiotensin benefits the hypoxic myocardium partly by increasing the number of high-energy phosphate compounds synthesized per oxygen molecule consumed. When oxygen supplies are reduced, the high-energy phosphate compounds may limit myocardial function. In that situation, mitochondrial efficiency would be a potential site of cardiotonic effects.

The coronary vasoconstrictor effects of angiotensin II could be one of the factors which inhibit the direct inotropic effects in controlled intact heart preparations (15, 16). The vasoconstrictor potency of 1-des-5-Val-angiotensin II is 22% of that of angiotensin II (17). Further investigations will be necessary to determine the relative vasoconstrictor and inotropic effects and the net myocardial properties of angiotensin II analogues in the intact, perfused heart.

Thus, in hypoxic cat papillary muscles, norepinephrine, ouabain, angiotensin II, and analogues of angiotensin II produce different types of inotropic effects. Two analogues of angiotensin II have been found which exert a greater inotropic effect under hypoxic conditions. This peculiar inotropism may be related to the ability of these same substances to stimulate mitochondrial oxidative metabolism selectively under hypoxic conditions.

Acknowledgment

The authors gratefully acknowledge the assistance of Mrs. Hope Cook for the norepinephrine determinations and that of Mr. James Hopkins and Mr. Everett Hodge for technical assistance with the operative procedures.

References


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Circ Res. 1972;30:196-204
doi: 10.1161/01.RES.30.2.196

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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