

Potential of the Contractile Effects of Norepinephrine by Hypoxia

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ABSTRACT Hypoxia has been found to depress the concentration response curve of norepinephrine (NE) in isoalted cat papillary muscles. To investigate the effects of hypoxia in intact hearts, a heart-lung preparation was developed and maximum left ventricular dp/dt (max dp/dt) was measured at constant heart rate, preload, and after load. Left main coronary arterial flow (Q_c) was measured with an electromagnetic flow probe. As arterial P_{O_2} decreased from 90 mm Hg (96% saturation) to 20–25 mm Hg (40% saturation) at constant P_{CO_2} and pH, no change in max dp/dt occurred and Q_c increased 298%. In contrast to cat papillary muscles, the contractile responses to NE were augmented in hypoxia. The NE dose-response curves shifted to the left. No deterioration of contractility occurred after exposure to NE. In contrast, the chronotropic response was unaltered in hypoxia. Dose-response curves to isoproterenol also were shifted to the left in hypoxia, but responses to paired pacing were unchanged. The responses to NE under oxygenated conditions were unaltered by mechanically increased coronary flow or by increased coronary flow with nitroglycerin. Although the mechanisms responsible for these effects are unknown, the results suggest that hypoxia may open previously nonfunctioning vascular channels and thereby allow more extensive exposure of beta adrenergic receptors to circulating catecholamines.

INTRODUCTION

The question of catecholamine administration often arises in patients who, for any of several reasons, have low oxygen saturations. Experimentally, however, it has been demonstrated that hypoxia diminishes the responsiveness

of the myocardium to exogenous catecholamines (1, 2), and that deterioration of contractility can occur upon continued exposure to these agents (1). Although these results would argue against the use of catecholamines in the presence of hypoxia, the experimental data referred to above were obtained in isolated cardiac tissues perfused with solutions free of red cells. It is therefore unknown whether similar changes would have occurred if the heart were perfused with blood through an intact coronary circulation. The present study was designed to determine the influence of hypoxia on the responses to catecholamines of blood-perfused hearts. Since the induction of hypoxia elicits profound reflex responses, and since systemic hypoxemia could influence cardiac responses, an areflexic hemodynamically controlled heart-lung preparation was used for the experiments.

METHODS

26 mongrel dogs of either sex, weighing 22–26 kg, were anesthetized with sodium pentobarbital, 30 mg/kg i.v. A transverse thoracotomy was made at the fourth intercostal space with the animal on positive pressure ventilation. The left main coronary artery, descending aorta proximal to the first intercostal artery, left subclavian artery, brachiocephalic artery, and superior and inferior vena cavae were dissected free from the surrounding tissue. The azygous vein was ligated. A wide bore (5 mm) metal cannula was inserted into the apex of the left ventricle. Heparin, 100 U/kg was administered intravenously and the brachiocephalic artery was cannulated with an 18 F aortic perfusion cannula. A wide bore cannula was inserted into the right atrium through the atrial appendage. The aortic and right atrial cannulas were connected to the perfusion circuit as shown in Fig. 1. The blood was directed through tubing ($\frac{3}{8}$ inch I.D.) from the aortic cannula to the systemic and coronary perfusion circuits. The systemic circuit consisted of an electromagnetic flow meter, a plastic 1-liter reservoir placed 137 cm above the animal's heart, a heat exchanger maintained at 37°C by a circulating water bath, a bubble trap, and a resistance clamp. The tubing was filled with buffered saline ($NaHCO_3$, 20 mEq/liter). The priming volume was approximately 200 ml. The systemic perfusion was started in the following

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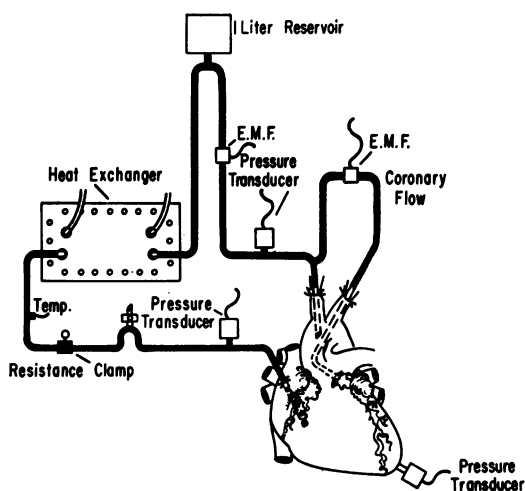


FIGURE 1 Schematic of perfusion circuits for heart-lung preparation. E.M.F., electromagnetic flowmeter. Temp., blood temperature.

manner. Maintaining the animal on positive pressure ventilation, the input gas to the respirator was changed from room air to 21% O_2 , 5% CO_2 , and 74% N_2 . The subclavian artery was ligated and the descending aorta was cross-clamped and tied proximal to the first intercostal artery. When the aorta was occluded, a clamp proximal to the reservoir of the systemic perfusion circuit was opened thereby diverting the entire cardiac output into the reservoir but maintaining adequate coronary perfusion pressure (90 mm Hg). During this 3–5 min period, the systemic perfusion resistance clamp was closed and the vena cavae remained open. 600–750 ml of blood was obtained in the reservoir. Only this autologous blood was used in the experiment. As the reservoir stopped filling, the vena cavae were ligated, the systemic flow was started by slowly opening the resistance clamp until the systemic flow was 1 liter/min. At this point the circulation of the heart and lungs was completely isolated from the remainder of the animal. The blood gas content was regulated by the input gas of the respirator. 10–15 min after this circulatory isolation procedure no neural reflexes (heart rate or contractility responses to aortic pressure or blood gas changes) were observed.

Once systemic perfusion was instituted, a Gregg cannula was inserted into the left subclavian artery, which previously had been ligated distally. The cannula was passed across the aortic arch and into the left main coronary artery where it was tied in place by a 4-0 silk ligature that had been passed around the artery during the earlier dissection. The coronary perfusion circuit consisted of $\frac{1}{8}$ inch tubing, an electromagnetic flowmeter, and the cannula. Both electromagnetic flow meters were standardized by passing canine blood through them with a constant flow pump and timed collection of blood for calibration. This procedure was repeated at monthly intervals with no significant changes observed in the calibration curves.

Blood samples were obtained for arterial saturation, pH, and hematocrit after establishing control conditions and at approximate 30-min intervals thereafter. Blood pH was maintained between 7.36 and 7.44 by the addition of 0.9 M $NaHCO_3$ or 0.1 M HCl .

Aortic pressure was maintained at 85–92 mm Hg (mean)

by the arterial reservoir. Heart rate was maintained by electrically pacing the right atrium. Left ventricular pressure (LVP)¹ was measured with a Statham P23Gb transducer (Statham Instruments, Inc., Los Angeles, Calif.) connected directly to the wide bore left ventricular cannula. LVP was differentiated electronically (dp/dt). The frequency response of the differentiating circuit was linear from 0.1 to 1200 Hz. Aortic and venous perfusion pressures, LVP at two sensitivities (0–200 mm Hg and 0–40 cm H_2O) LV dp/dt , systemic (Q_b) and coronary (Q_c) flows were continuously recorded on an eight channel oscillograph. Lead II of the electrocardiogram was also monitored in the control experiments when no drugs were given. Maximum LV dp/dt at a constant heart rate and afterload and at a preload which changed by less than 5 mm Hg was used as an index of contractility. Agents were administered either as an intravenous injection or intracoronary infusion. After the maximum responses were obtained, dp/dt was allowed to return to and remain at base line for 5 min before additional studies. To induce hypoxic conditions the P_{O_2} of the respirator input gas was reduced from 155 to 22 mm Hg at a constant P_{CO_2} of 37 mm Hg. Dose-response curves to norepinephrine were obtained first under control conditions and then during hypoxia. This was felt justified since we found that when two animals were maintained at 95% oxygen saturation for 90 min, the dose-response curves obtained after 15 min and 75 min were identical.

Drugs used were L-norepinephrine bitartrate monohydrate, DL-isoproterenol-HCl, and nitroglycerin. The electrodes used for paired pacing were sutured to the right ventricle. The delay of the second impulse of the pair was approximately 200 msec.

RESULTS

Cardiac responses to hypoxia. Under control conditions at respirator input P_{O_2} of 155 mm Hg, the arterial saturation was $96 \pm 2\%$ (mean \pm SD). Hypoxic conditions were established at an arterial saturation of $40 \pm 2\%$. Aortic pressure (92 ± 6 mm Hg), systemic blood flow (1050 ± 30 ml/min), and heart rate (120 ± 12 beats/min) were kept constant in this preparation as previously described. In this controlled situation, hypoxia caused no significant changes in left ventricular maximum dp/dt (max dp/dt) or left ventricular end-diastolic pressure (LVEDP). Max dp/dt was 1250 ± 40 mm Hg/sec under control (oxygenated) conditions and 1280 ± 50 mm Hg/sec under hypoxic conditions; LVEDP was 6 ± 3 mm Hg under control conditions and 8 ± 4 mm Hg under hypoxic conditions. When the hearts were unpaced, induction of hypoxia was not associated with any significant changes in heart rates. Coronary flow increased markedly as hypoxia was induced, 92 ± 14 ml/min at an arterial saturation of 95% and 298 ± 36 ml/min at an arterial saturation of 40%. Max dp/dt and LVEDP remained constant for 90 min when no interventions were

¹ Abbreviations used in this paper: LVEDP, left ventricular end-diastolic pressure; LVP, left ventricular pressure; max dp/dt , maximum left ventricular dp/dt ; NE, norepinephrine; Q_b , systemic blood flow; Q_c , left main coronary arterial flow.

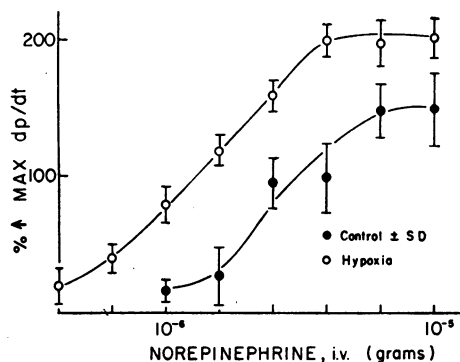


FIGURE 2 Dose-response curve to intravenous norepinephrine. Per cent change in maximum dp/dt is shown as a function of increasing doses of norepinephrine under control (arterial saturation 95%) and hypoxic conditions (arterial saturation 40%). $n = 5$.

made in three animals maintained at an arterial saturation of 95% and in three animals maintained at an arterial saturation of 40%. The hematocrit was $41 \pm 3\%$ in all animals studied and did not change during the experiment. Lead II of the electrocardiogram revealed no

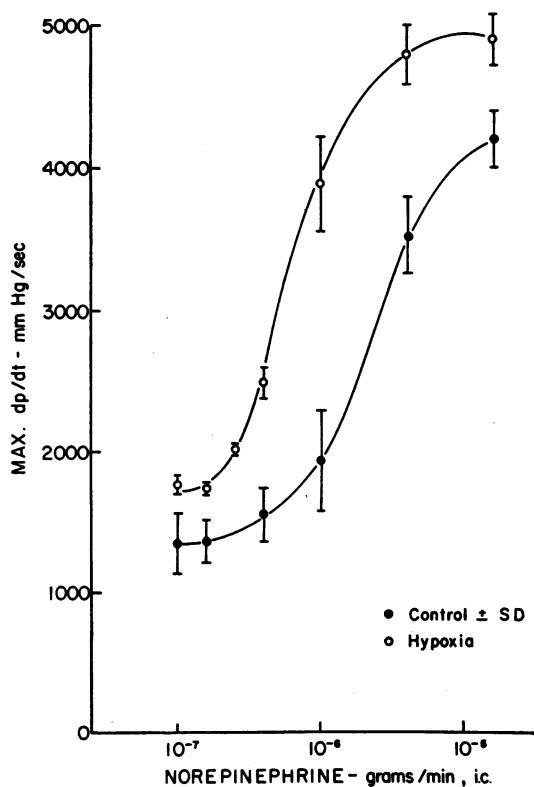


FIGURE 3 Response curve to intracoronary norepinephrine infusions in control and hypoxic conditions. Max dp/dt is shown as a function of norepinephrine infusion rate. At 10^{-7} g/min there was no response under control conditions and a 30% increase in max dp/dt under hypoxic conditions.

rhythm or conduction disturbance and no ST-T wave changes when the hypoxic conditions were established.

Inotropic responses to intravenous norepinephrine. Responses to increasing doses of norepinephrine were obtained in five animals under control and hypoxic conditions. The responses to each dose of norepinephrine were significantly greater in hypoxia (Fig. 2). Furthermore, the threshold doses under hypoxic conditions were lower, and the maximum responses were greater. Heart rates were maintained constant in each animal and averaged 184 beats/min.

Intracoronary infusion of norepinephrine. Since coronary flow increased over threefold in response to hypoxia while systemic flow was unaltered, a greater percentage of the total drug dosage would have reached the coronary circulation during hypoxia and could have influenced the results. Therefore, in four additional experiments norepinephrine (10^{-7} to 10^{-5} g/min) was infused directly into the coronary perfusion circuit. It was found that the responses to intracoronary norepinephrine also were augmented during hypoxia (Fig. 3). The responses of this preparation to hypoxia as well as the responses

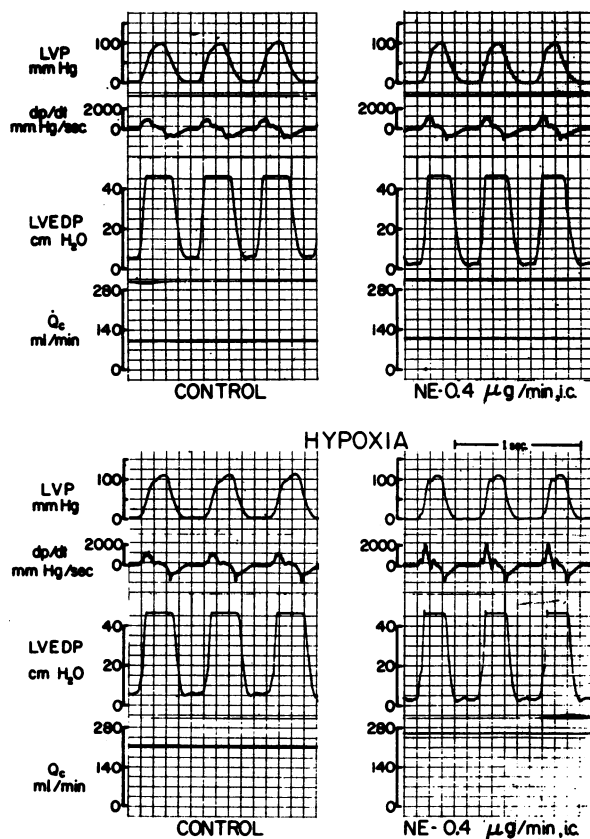


FIGURE 4 Representative example of intracoronary (i.c.) norepinephrine under control (upper panels) and hypoxic conditions (lower panels).

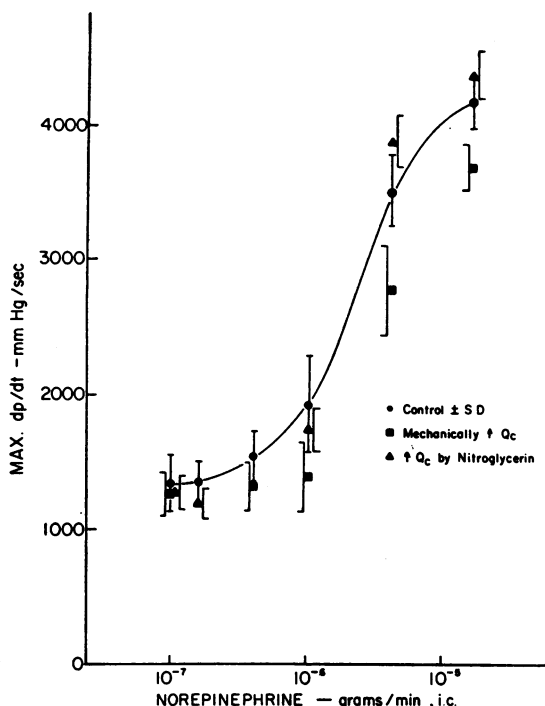


FIGURE 5 Response to intracoronary norepinephrine under control conditions (●), under conditions of increased coronary perfusion rate by a pump in the circuit (■), and under conditions of simultaneously infused nitroglycerin (▲). Arterial saturation was maintained at 95% throughout the experiment.

to norepinephrine under control and hypoxic conditions are depicted in the original tracings of one experiment in Fig. 4. The small effect of norepinephrine on Q_c un-

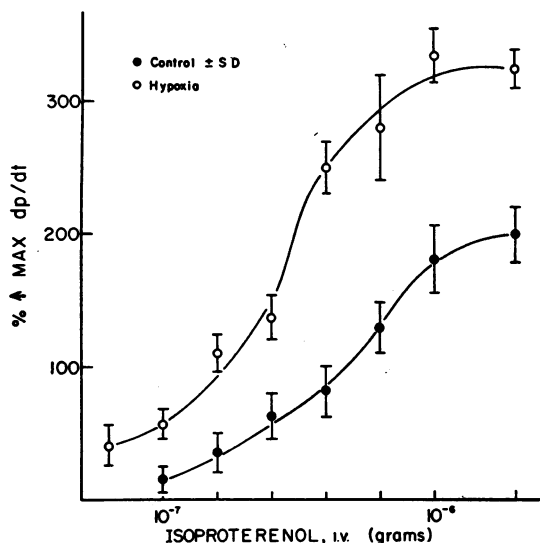


FIGURE 6 Dose-response curve to intravenous isoproterenol under the same conditions depicted in Fig. 2.

der control conditions is contrasted with the larger effect in hypoxia. There were no chronotropic responses to these amounts of norepinephrine infused directly into the coronary perfusion circuit at these infusion rates.

Responses to norepinephrine with mechanically increased coronary flow. After the responses to intracoronary infusions of norepinephrine were obtained under control conditions, coronary flow was increased 300% in three animals by introducing a peristaltic pump (Harvard model 500-1200, Harvard Apparatus Co., Inc., Millis, Mass.) in the coronary perfusion line. Arterial saturation was maintained at 95% as norepinephrine was again administered. There was no change in max dp/dt when coronary flow was increased. LVEDP rose 5-7 mm Hg transiently but then returned to control levels. The mechanically-induced increase in coronary flow did not lead to a potentiation of the responses to norepinephrine (Fig. 5).

Responses to norepinephrine with coronary flow increased by nitroglycerin. In order to achieve a redistribution of coronary flow, nitroglycerin (0.1 mg/min intracoronary) was administered to four animals. After control responses to the intracoronary norepinephrine were obtained, nitroglycerin was infused at a rate that caused coronary flow to increase 30%. The responses to intracoronary norepinephrine were then reassessed; arterial saturation was maintained at 95%. The changes in coronary flow induced by nitroglycerin did not alter the contractile responses to norepinephrine (Fig. 5).

Chronotropic responses to norepinephrine. In four animals in which heart rate was not controlled, norepinephrine was administered intravenously in increasing doses from 10^{-6} to 10^{-5} g under control and hypoxic conditions. The heart rate responses to norepinephrine under control and hypoxic conditions were similar (Table I).

Inotropic responses to isoproterenol. Increases in max dp/dt as a function of increasing doses of isoproterenol administered intravenously in five animals are shown in

TABLE I
Effect of Norepinephrine on Heart Rate under Oxygenated and Hypoxic Conditions

Norepinephrine	Heart rate	
	Control 95% SAT	Hypoxia 40% SAT
μg		
0	124 \pm 6*	118 \pm 9
2	132 \pm 8	126 \pm 3
4	145 \pm 7	150 \pm 6
8	185 \pm 4	182 \pm 6
10	188 \pm 8	186 \pm 4

* Average in beats/min. $n = 4 \pm \text{SD}$.
SAT, saturation.

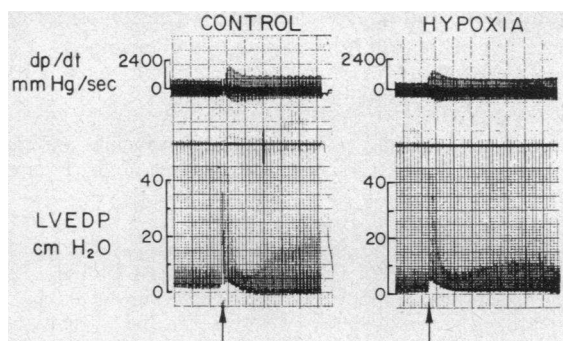


FIGURE 7 Paired pacing in hypoxia. Max dp/dt and LVEDP changes in response to paired pacing were the same under control and hypoxic conditions.

Fig. 6. The responses to isoproterenol were significantly greater in hypoxia at all doses. The threshold doses were lower and the maximum responses were significantly greater.

Responses to paired electrical stimulation. Paired ventricular pacing was obtained under both control and hypoxic conditions in four animals. The responses to paired pacing were increases in max dp/dt and decreases of LVEDP at constant systemic flows. These responses under hypoxic conditions were not significantly different from those measured under control conditions: dp/dt increased $126 \pm 22\%$ under hypoxic conditions and $112 \pm 18\%$ under control conditions. A representative example is shown in Fig. 7. Under control conditions coronary flow increased 22 ± 14 ml/min (24%) during paired pacing. However the coronary flow response to paired pacing was much larger in hypoxia increasing 64 ± 18 ml/min (21%).

DISCUSSION

It previously has been shown that when norepinephrine is administered to isolated myocardial preparations perfused with solutions free of red cells, hypoxia results in decreased or absent myocardial responsiveness to norepinephrine (1, 2). It appears, however, that these results do not apply to the intact heart since hypoxia, at the level employed in the present investigation, *enhanced* the contractile response of the intact blood-perfused heart to catecholamines.

The opposite effects of hypoxia on the responsiveness of the intact versus isolated hearts to norepinephrine would appear to be caused, at least in part, by a limitation in the capacity of diffusion processes, in the absence of an intact coronary circulation, to transport adequate quantities of oxygen to stressed hypoxic myocardium. However, even when an intact coronary arterial tree is perfused with cell-free low-oxygen solutions, the response to inotropic agents is depressed (2). Furthermore, while contractility of the intact blood-perfused heart does not

fall from control values when arterial P_{O_2} is decreased from 87 to as low as 20 mm Hg, contractility falls substantially when P_{O_2} of red cell-free solutions perfusing isolated myocardium is decreased from 440 to 120 mm Hg (1). Thus, it appears that the presence of hemoglobin as well as an intact coronary circulation are necessary for optimal responsiveness of the heart to an hypoxic stress. In addition the responses of hypoxic papillary muscles to paired electrical stimulation is diminished (3) which was not found in the present study.

Another possible explanation for the differences in the responses between the intact and isolated hearts is that the hypoxia-induced potentiation is related to changes in the amount and/or distribution of coronary blood flow. For example, it is possible that the large increases in coronary blood flow that occur after reduction of arterial oxygen content opens vascular channels not previously utilized, thereby exposing a greater quantity of beta receptors and myocardium to circulating norepinephrine. Although we attempted to test this hypothesis by increasing coronary flow mechanically and by infusing nitroglycerin into the coronary bed, neither of these interventions caused significant changes in the responsiveness of the heart to norepinephrine. However, the increase in coronary flow caused by the pump undoubtedly increased "nonnutritive" flow, and even large doses of nitroglycerin, although probably causing a redistribution of flow (4), produced only relatively small increments in total coronary flow compared to those produced by hypoxia. These results therefore cannot be considered to rule out firmly the above hypothesis. On the other hand, the observations that hypoxia did not potentiate the contractile response of the heart to paired electrical pacing suggests that only circulating inotropic stimuli are potentiated by hypoxia. If this distinction is also true of other inotropic stimuli, it would suggest that the mechanism responsible for the enhanced contractile responses to catecholamines during hypoxia is related to an increase or redistribution of coronary flow, an alteration that in turn might increase the total quantity of myocardium exposed to circulating inotropic agents.

One other alternative mechanism that might be responsible for the potentiation of myocardial responses to catecholamines during hypoxia should be considered. Inactivation of norepinephrine is dependent on intact adrenergic nerve endings and any interference with the binding or re-uptake of norepinephrine by the nerves results in an enhanced physiologic response (5). It is possible that an hypoxia-induced defect of this inactivation pathway could be responsible for the potentiated response. This is unlikely, however, since we found that hypoxia also caused potentiation of the myocardial response to isoproterenol, a catecholamine that is inactivated predominantly by a different mechanism.

The responses of intact hearts to hypoxia have been determined previously (6-8). In contrast to the findings of the present investigation in which cardiac function was unimpaired at an arterial saturation of 40% (P_{O_2} of 20 mm Hg), cardiac failure was found to occur: (a) 16 min after reaching an arterial saturation of 40% in intact dogs which had received hexamethonium and propranolol to block the neural reflexes to hypoxia (7); (b) at an arterial saturation of 40% in two of four isolated perfused nonworking canine hearts subjected to hypoxia with constant pressure perfusions of the coronary arteries (6); (c) at a P_{O_2} of 32 mm Hg in autosupported cat hearts subjected to hypoxia after the CNS was excluded (8). The reasons for the relative resistance of the present model to the effects of hypoxia are unclear except that the complicating effects of systemic hypoxemia, homologous blood transfusions, adrenergic blocking agents, and artificial oxygenators were avoided.

In summary, we found that when intact blood-perfused hearts isolated from neurological reflexes were made hypoxic by lowering the arterial P_{O_2} to 20 mm Hg, an increased coronary flow occurred without changes in contractility. Under these conditions the contractile responses to norepinephrine and isoproterenol were enhanced while those to paired electrical stimulation were unchanged. It is postulated that the mechanism responsible for the enhanced responses to catecholamines might be related, at least in part, to the amount and/or distribution of coronary flow and thereby to an increased exposure of beta receptors to these agents. Whether these results are relevant to the administration of catecholamines to patients with congenital heart disease and right to left shunts, to patients with lung disease and arterial

desaturation, and to patients with arterial desaturation due to left heart failure remains to be determined.

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