

PROTECTIVE EFFECTS OF β -ADRENERGIC BLOCKADE IN ISOLATED ISCHEMIC HEARTS*

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The protective effects of the β -adrenergic blocking drugs, propranolol and atenolol, were tested in a model of global ischemia and assessed electron microscopically. Cat isolated hearts were perfused retrogradely with arterial blood drawn from donor cats. After a period of equilibration, isolated hearts were rendered globally ischemic for 1 h and subsequently reperfused for another hour. Hearts were then flushed with physiological salt solution followed by perfusion-fixation with cacodylate-buffered glutaraldehyde, containing ionic lanthanum. Lanthanum was included as a probe of myocardial membrane integrity. Left ventricular subendocardial samples were processed and examined electron microscopically. Nontreated hearts, which underwent normothermic ischemia and reperfusion, displayed extensive ultrastructural damage. Nonischemic and donor cat control myocardial tissue appeared normal in all respects. Hearts that received either propranolol or atenolol maintained their ultrastructural integrity, resembling controls. Ionic lanthanum proved to be reliable as a marker of membrane integrity and permeability, as nontreated hearts displayed intracellular deposition of the marker, indicating that deteriorations of membrane integrity occurred. The results suggest that β -adrenergic blockade may be valuable in preserving myocardium subjected to ischemia and reperfusion.

Isolated cat heart	Electron microscopy	β -Adrenergic blockade	Myocardial ischemia	Ionic lanthanum
Reperfusion	Calcium			

1. Introduction

β -Adrenergic blocking drugs have been shown to be effective in the treatment of certain cardiac arrhythmias, hypertension, and angina pectoris (Frishman and Silverman, 1979; Conolly et al., 1976). However, their efficacy in reducing the extent of irreversible

ischemic injury is presently disputed (Singh, 1978). In the intact experimental animal propranolol has been shown to reduce the amount of infarction after acute coronary occlusion (Maroko et al., 1971; Kloner et al., 1978), but the mechanism of protection is not known. Thus, the evidence for a direct infarct-reducing effect of β -blocking agents remains inconclusive.

The purpose of this study was to determine if β -adrenergic blocking drugs directly protect the ischemic myocardium. An isolated, blood-perfused model of global ischemia, described previously (Vogel et al., 1979) was used in these experiments. Since these isolated hearts undergo total, or global, ischemia and are devoid of reflex hemodynamic influences any protection afforded by intervention can be

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attributed to actions independent of redistribution of coronary blood flow or reflex neural influences.

In order to determine the relative importance of propranolol's intrinsic negative inotropic and chronotropic effects, frequently referred to as 'membrane stabilization' (Frishman and Silverman, 1979), it was compared to atenolol. Atenolol is a 'cardioselective' β -blocker with approximately equal β -adrenergic blocking potency as propranolol, but lacks membrane stabilizing effects (Barret, 1977). Thus, a comparison of these two components of cardioprotection could be made.

In this study ionic lanthanum (La^{3+}) was included in the fixative to serve as a probe for assessing membrane integrity (Burton et al., 1977). In normal myocardium, La^{3+} is confined to the extracellular space, where it displaces calcium from its binding sites on sarcolemmal membranes (Martinez et al., 1973). Lanthanum also localizes intracellularly at calcium-binding sites, e.g., mitochondrial outer membranes, if allowed entry through damaged cell membranes. The presence of La^{3+} deposits intracellularly indicates a breakdown in the integrity of the plasma membrane-basal lamina complex.

A preliminary account of the results of these experiments was reported at the 64th Annual Meeting of the Federation of Societies for Experimental Biology (FASEB) April, 1980.

2. Materials and methods

2.1. Isolated heart model of ischemia

Hearts excised from pentobarbital (30 mg/kg, i.p.)-anesthetized cats (1–2 kg) were perfused via the aorta with arterial blood drawn from blood-donor cats (2.5–3.5 kg) anesthetized with dial urethane (0.7 ml/kg of a solution containing allobarbitol, 100 mg/ml; urethane, 400 mg/ml; and monoethyl urea, 400 mg/ml). Perfusion pressure was maintained between 70–90 mm Hg by changing the

speed of a roller pump delivering blood to the isolated heart. Coronary effluent blood was collected by means of a cannula within the pulmonary artery and returned to the donor cat via a cannula in the jugular vein. Isolated hearts were allowed to equilibrate for 1 h during which time left ventricular pressure pulse and HR were maintained at 150 mm Hg and 150 beats/min, respectively. This was accomplished by electrical pacing and adjusting the volume of a saline-filled balloon inside the left ventricle. After equilibration either d, l-propranolol (1.90 mg/kg [donor]), atenolol (1.65 mg/kg) or saline was infused directly into the aortic cannula of the isolated heart over a 10 min period, at a rate of 0.15 ml/min. Immediately after drug or saline infusion blood flow to isolated hearts was stopped, rendering them globally ischemic (37°C). Hearts which received saline, but were not rendered ischemic, served as nonischemic controls. After 1 h of ischemia, isolated hearts were reperfused for another hour before fixing for electron microscopy.

2.2. Tissue preparation and electron microscopy

After the reperfusion period the isolated heart was perfused with physiological salt solution (bubbled with 95% O_2 – 5% CO_2 immediately prior to perfusion) for 2 min. The volume of the intraventricular balloon was adjusted during this period so that the end diastolic pressure was 10 mm Hg. The physiological salt solution contained (in mM): NaCl 140; KCl 5; MgCl_2 1.2; glucose 10; CaCl_2 1.8; Tris-buffer 5; pH 7.4 at 30°C. Perfusion pressure during washing and fixing was maintained between 65 and 75 mm Hg. After 2 min of reperfusion with physiological salt solution, the isolated heart was perfused with La^{3+} (1% LaCl_3)-containing 2.5% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.4. The duration of perfusion fixation was 3 min for all hearts. The isolated heart was then placed into a beaker containing enough fixative to totally immerse the heart and removed

from the aortic perfusion cannula. The heart was weighed and the left ventricle was dissected from the rest of the heart and cut into smaller pieces in a dissecting dish which was half-filled with fixative in order to keep the tissue immersed at all times. Left ventricular papillary muscles and subendocardium were separated from the remainder of the left ventricle. Samples from these areas were trimmed of connective tissue and sliced into small cubes measuring 1–2 mm on a side. Approximately 25 cubes per region were obtained and allowed to stand in the La^{3+} -containing fixative for 2 hr at room temperature. Samples were transferred to cold (4–5°C) 8% sucrose-0.1 M Na^+ cacodylate buffer, pH 7.4 and kept overnight in this solution at the above temperature.

Samples were post-fixed in cacodylate-buffered 1% osmium tetroxide, dehydrated in ethanol and propylene oxide, and embedded in EPON 812. Blocks were thin-sectioned, placed on grids and stained with uranyl acetate, followed by lead citrate. Sections were examined with a Siemens 101 transmission electron microscope. Preparation and examination of sections were performed without knowledge of the identity of tissue cubes. There were at least seven hearts per group in this study. Two hearts per group were chosen at random for electron microscopy.

2.3. Tissue calcium measurements

Myocardial tissue samples were taken from the interventricular septum of isolated hearts used in another series of experiments, in which hearts were not fixed or perfused with physiological salt solution. Heart tissue samples were dried to constant weight in an oven and dissolved in nitric acid. The dissolved samples were diluted with lanthanum diluent and their calcium concentrations measured by atomic absorption spectrometry.

3. Results

Electron microscopic examination of non-ischemic control hearts revealed normal myocardial ultrastructure (fig. 1), including tightly packed myofibrils, with relaxed and well-aligned sarcomeres. Glycogen granules were abundant in proximity to mitochondria, which were numerous and tightly packed between myofibrils. Mitochondrial morphology in these hearts appeared normal. The mitochondria possessed tightly packed cristae and dense matrices. Several mitochondria were observed to contain dark matrix granules. These have been observed by others, and are presumably normal constituents of mitochondria in this and other species (Fawcett and McNutt, 1969) but should be distinguished from larger amorphous densities which appear in mitochondria of irreversible injured myocardium (Jennings and Ganote, 1976). Sarcolemmal membranes appeared intact in both control groups, remaining in intimate contact with both myofibrils and the basement membrane. Electron micrographs of myocardium from donor cat hearts, were also examined. This group served as an additional control in order to determine if perfusion and handling of isolated hearts adversely affected their morphology. The morphological features of this group (not shown) were virtually identical to nonischemic control isolated hearts.

The myocardium of isolated hearts which received saline instead of drug displayed extensive ultrastructural damage after global ischemia and reperfusion. There was extensive myofibrillar separation and disruption of sarcomeres. Contraction bands were obvious (fig. 2). The damage incurred by mitochondria was a prominent feature of myocardium in this group. Mitochondria were markedly swollen, their matrix spaces clarified and cristae often indistinguishable. Many mitochondria contained large amorphous matrix densities. There was extensive accumulation of La^{3+} tracer around the perimeter of mitochondria indicating that cells in this group of

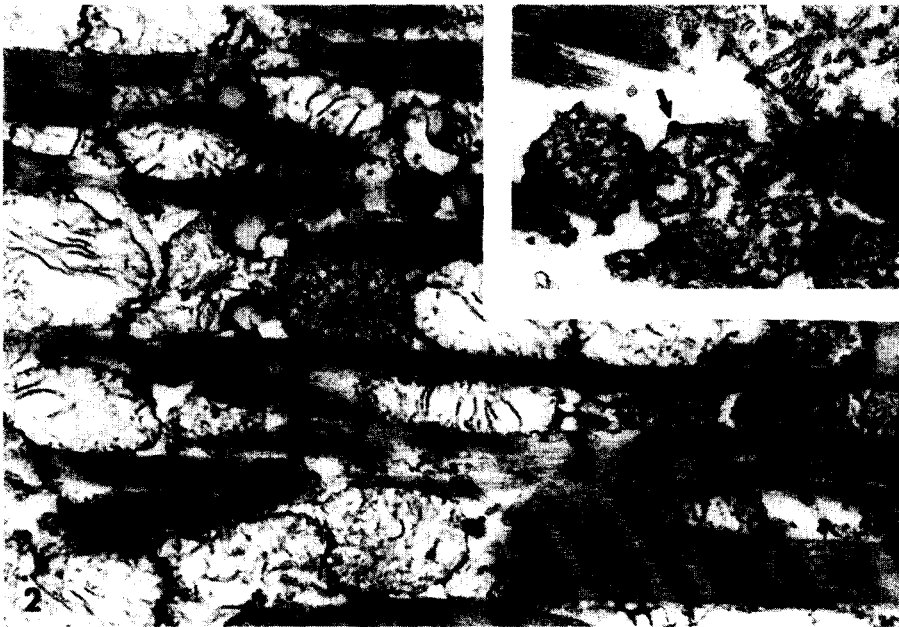


Fig. 1. Left ventricular subendocardium from nonischemic control heart. Two apposing myocytes are shown. Sarcolemmal membranes possess normal ultrastructure; basement membrane (BM) is intact and continuous with the glycocalyx. Mitochondria have normal features: dense matrices, tightly packed cristae, and small matrix granules. Glycogen (G) granules are abundant. Myofibrillar organization appears normal. Mag. 20 000 X.

Fig. 2. Nontreated ischemic-reperfused isolated heart. Extensive myofibrillar damage is apparent. Contraction bands are prominent in this heart. Mitochondria are severely disrupted; the matrices of some show total clearing, while others display irregularly arranged cristae. Mag. 12 450 X. Inset: Lanthanum tracer deposits appear on the outer membranes of mitochondria (arrow). Mag. 20 400 X.

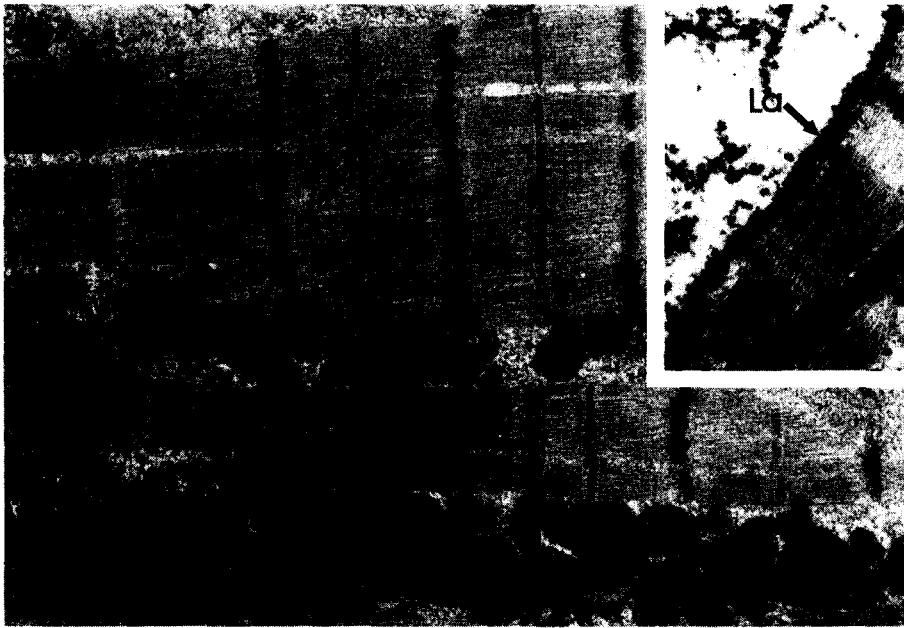


Fig. 3. Left ventricular subendocardium of propranolol-treated ischemic-reperfused heart. Overall myofibrillar organization is normal. Mitochondrial integrity is well preserved, with darkly staining matrices and tightly packed cristae. Mag. 13 500 X.

Fig. 4. Left ventricular subendocardium of atenolol-treated ischemic-reperfused heart. Overall, ultrastructure is normal. Mitochondria appear normal. Several lipid droplets are apparent (*). Mag. 13 800 X. Inset: Exclusion of lanthanum (La) to the extracellular space is apparent. Mag. 21 760 X.

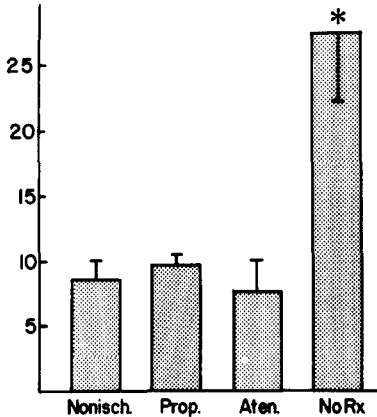


Fig. 5. Myocardial tissue calcium concentrations of samples taken from interventricular septa. Isolated blood perfused cat heart: 60 min ischemic. Isolated hearts from which tissue samples were taken were not included in electron microscopic studies. Values shown represent mean \pm 1 S.E. of at least 5 hearts per group. Asterisk denotes significant difference between nontreated and all other groups ($P < 0.02$). Ordinate: $\mu\text{mol Ca}^{2+}/\text{g dry wt.}$

hearts suffered losses of sarcolemmal integrity. Lipid droplets were also present in the myocardium of these saline-treated hearts.

The myocardium of propranolol-treated hearts appeared well-preserved (fig. 3). Sarcomeres were well-aligned and myofibrils tightly packed. Mitochondria appeared uniformly well-maintained. La^{3+} tracer was confined to the extracellular space and capillary lumens. Glycogen was abundant in the myocardium of this group of hearts. In general, hearts in this group possessed ultrastructural features nearly identical to control hearts.

Atenolol-treated hearts were also well preserved, appearing similar to propranolol-treated hearts and controls (fig. 4). La^{3+} tracer was excluded from the intracellular space in this group and there was no visible evidence of damage to sarcolemmal membranes. Thus, both propranolol and atenolol-treated hearts retained their ultrastructural integrity and appeared almost indistinguishable from the two control groups, while nontreated hearts appeared to suffer extensive damage as a result of 1 h each of normothermic ischemia

and reperfusion.

The results of measurements of myocardial tissue calcium are shown in fig. 5. The myocardium of nontreated isolated hearts contained large accumulations of calcium, while the myocardium of propranolol- and atenolol-treated hearts contained amounts of calcium which were nearly identical to nonischemic controls. These results correspond well with electron microscopic observations.

4. Discussion

Both atenolol and propranolol prevented the ultrastructural deterioration which occurred as a result of global ischemia and reperfusion. Therefore, it appears that β -adrenergic blockade, as opposed to membrane stabilization, was of primary importance in the protection provided by these drugs. Reimer et al. (1976) reported similar findings in which d,l-propranolol but not d-propranolol reduced myocardial ischemic injury. Also, since hearts in these experiments were subjected to total, or global, ischemia, then cardioprotection by β -blockade did not involve alterations of regional myocardial blood flow or hemodynamic reflexes, mechanisms often invoked to explain propranolol's protective effects in models of regional ischemia (Fox et al., 1980; Hillis et al., 1979). A protective effect on microvascular integrity cannot, however, be ruled out as an important influence (Kloner et al., 1977).

There are several mechanisms by which β -blockade may have protected the ischemic myocardium in these experiments. Antagonism of β -receptor activation immediately prior to, and during, ischemia prevented contractile and metabolic stimulation in the face of severely limited energy stores. β -Blocking drugs have been shown to prevent undesirable metabolic responses to ischemia (Abiko et al., 1979). Pretreatment of isolated rat hearts with propranolol has been shown to significantly improve mechanical recovery after anoxia, an effect associated with preservation

of myocardial glycogen stores (Smithen et al., 1975). Franson et al. (1979) recently demonstrated that propranolol competitively inhibits catecholamine-induced increases of sarcolemmal phospholipase and lipoprotein lipase activities, the activation of which would adversely affect the ischemic myocardial cell. Finally, β -blockade has been shown to prevent the reflex stimulation of contractile activity of nonischemic myocardium during regional ischemia (Reimer et al., 1976). Thus, substantial experimental evidence exists to demonstrate preventable β -adrenergic mediated responses to myocardial ischemia.

There are two sources of catecholamines for stimulation of β -adrenoceptors in the isolated hearts during ischemia. Blood-borne catecholamines originating from the blood-donor cat represent one of these. However, donor animals were hemodynamically stable during the experimental procedure and, therefore, probably did not secrete excessive amounts and, of course, during global ischemia of the isolated hearts, delivery of catecholamines from this source was not possible. Therefore, this probably did not contribute substantially to β -receptor stimulation during ischemia. Norepinephrine originating from sympathetic neurons in isolated hearts, most likely, represents a more significant factor. Wollenberger and Shahab (1965) demonstrated that norepinephrine is released from cardiac adrenergic neurons during anoxia and ischemia, most likely due to depolarization by high extracellular potassium concentrations accompanying ischemia (Hill et al., 1980). Additional experimental evidence favoring these conclusions derives from studies in which chronic cardiac denervation in dogs results in smaller infarct size resulting from coronary artery occlusion (Jones et al., 1978).

It has been shown that reperfusion of ischemic myocardium exacerbates myocardial injury (Hearse, 1977; Bush et al., 1980). It is not clear from the present results whether propranolol and atenolol exerted protective effects against reperfusion injury, per se, however, both drugs clearly prevented massive

accumulations of calcium by cardiac tissue (figs. 2 and 5), a process usually associated with reperfusion injury (Jennings and Ganote, 1976). It is possible that blockade of β -receptors prevented increases of intracellular calcium concentrations similar to that which accompanies catecholamine-induced cardiac necrosis (Nirdlinger and Bramante, 1974). A more plausible explanation is that maintenance of myocardial cell membrane integrity during ischemia protected against calcium overload during subsequent reperfusion.

Doses of propranolol and atenolol considerably less than those used in the present study have been shown to adequately antagonize cardiac β -adrenoceptor stimulation in the same species (Lundgren et al., 1979). Also, in a separate series of experiments in the cat isolated heart, the same doses of the two β -blockers were found to competitively alter the dose response relationship of left ventricular developed pressure to isoproterenol (unpublished observations). Furthermore this β -blockade lasted throughout the entire reperfusion period.

Propranolol has been shown to undergo extensive biotransformation (Walle and Gaffney, 1972), the significance of which is probably small in these experiments. It is unlikely that metabolites of propranolol (e.g., 4-OH-propranolol) contributed to cardioprotection during ischemia, since, as indicated in Materials and methods, propranolol and atenolol were infused directly into the aortic inflow cannula, followed immediately by termination of blood circulation. Some metabolism of propranolol probably occurred in the donor cats during the hour of ischemia and pharmacologically active metabolites may have contributed, to a minor extent, to β -blockade by 1 h post-reperfusion. Atenolol undergoes little biotransformation and is eliminated more slowly than propranolol (Frishman, 1979); therefore it can be concluded that β -blockade persisted throughout the duration of these experiments.

In conclusion, the experimental results confirm and help to explain previous obser-

vations of salutary effects of propranolol in the setting of myocardial ischemia (Reimer et al., 1976; Mueller and Ayres, 1976). Although some controversy exists regarding the effects of β -blockade during elective cardiac arrest (Reul et al., 1974; MacGregor et al., 1975; Oka et al., 1980) these agents may prove to be a valuable adjunct to hypothermia in cases of left ventricular hypertrophy. It has been claimed that severely hypertrophied hearts require aggressive treatment to lower myocardial metabolic demands, as these hearts are more likely to develop contracture, or the 'stone heart' syndrome (Hutchins and Silverman, 1979).

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