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Isoproterenol-Induced Cardiac Failure in the Spontaneously Hypertensive Rat (41248)

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Abstract. Thirty-one age-matched, conscious, virgin, male Sprague–Dawley rats and spontaneously hypertensive rats (SHRs) were individually injected with a single subcutaneous dose of 85 mg/kg *dl*-isoproterenol to determine the degree and time course of drug-induced cardiac failure and functional recovery. At 24 hr and 1 week postisoproterenol, rats were anesthetized and prepared for the recording of cardiac output and arterial pressure. Calculated cardiac index was used to determine normal cardiac function. Following that measurement, a 2-min, 15.3 ml/min infusion of Tyrode's solution was performed via a right jugular vein cannula. Volume-loaded peak cardiac outputs and peak stroke volumes were also used as indices of cardiac function. Twenty-four hours after the injection of isoproterenol to the normotensive Sprague–Dawley rats, cardiac failure was evident only during the stress of volume loading. Normal cardiac index was unaffected, but peak cardiac output and peak stroke volume were depressed. By 1 week after isoproterenol, the volume loaded measures of cardiac function had returned to normal. Interestingly, by 1 week postisoproterenol, total peripheral resistance was reduced. This reduced vascular resistance may have aided myocardial repair. At 24 hr postisoproterenol, the volume loaded peak cardiac outputs and peak stroke volumes in the SHRs were reduced to the same degree as in the Sprague–Dawley rats. Here, also, no change in normal cardiac index occurred. In the SHRs, however, total peripheral resistances were elevated at both 24 hr and 1 week. These increases in resistance appeared to impair the myocardial healing process, as both normal cardiac index and volume-loaded peak cardiac output and peak stroke volume were depressed at 1 week postisoproterenol. In normotensive and hypertensive rats, different vascular responses to isoproterenol or its initial cardiac effects may determine the duration and eventual degree of cardiac failure.

In 1959 Rona and Chappel reported that subcutaneous injections of the synthetic beta adrenergic receptor agonist, isoproterenol, produced infarct-like lesions in the myocardium of the rat (1, 2). Subsequent studies have shown that necrogenic doses of isoproterenol increase myocardial Na^+ and Ca^{2+} concentrations and decrease K^+ and Mg^{2+} concentrations (3–5). High doses of the drug also cause a significant release of the myocardial enzymes CPK SGOT, and LDH (6, 7), and decrease myocardial stores of high-energy phosphates (8). Various degrees of cardiac failure subsequently occur (9–11). The degree of cardiac failure has been shown to be dose dependent, as has the extent of necrosis (1, 11).

In arteriosclerotic, mildly hypertensive rats, Wexler *et al.* (12) observed only a moderate elevation of the serum enzymes CPK, SGOT, and LDH and an excellent survival rate during the first few days after a dose regimen of isoproterenol that normally produced very severe effects in normotensive Sprague–Dawley rats. Wexler (13) then used the spontaneously hypertensive rat (SHR, systolic blood pressure = 180 ± 3 mm Hg) to determine whether hypertension, per se, provided protection. The SHRs survived the initial 1–3 days postisoproterenol. Surprisingly, they began to die by 7–10 days. A progressive development of congestive heart failure, as judged by the volume of fluid found in the chest, was postulated to contribute to the mortality of the SHRs. The normotensive rat shows only a transient period of heart failure followed by full recovery (10). Progressive congestive heart failure in the SHRs was theorized to be due to a sustained high arterial pressure postisoproterenol, an appearance of ventricular aneurysms, probably due to the high arte-

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rial pressure, and to cardiac chamber thrombi.

Wexler's finding of chest cavity fluid is an index of isoproterenol-induced congestive heart failure in the SHR. The present study was undertaken to determine whether a different dose of isoproterenol would so affect the SHR, and to give a more complete hemodynamic description of the degree and time course of isoproterenol-induced functional cardiac damage in both the SHR and the normotensive animal (Sprague–Dawley rat). Myocardial function was assessed at 24 hr and 1 week after isoproterenol administration by the examination of both normal cardiac index values and peak cardiac output values attained during an intravenous volume loading infusion of Tyrode's solution.

Methods. Thirty-one virgin, male, Sprague–Dawley rats (417 ± 5 g) and twenty-three virgin, male SHRs (334 ± 4 g) from our inbred colony were used in this experiment. The rats from both strains were 4–5 months old. A single subcutaneous injection of 85 mg/kg *dl*-isoproterenol dissolved in 0.5 ml of distilled H_2O was given to each of 17 conscious Sprague–Dawley rats and 14 conscious SHRs. The normotensive and hypertensive rats were then individually caged, given food and water *ad libitum*, and divided into two groups for assessement of cardiac function: 24 hr and 1 week postisoproterenol. Fourteen Sprague–Dawley rats and nine SHRs were not given isoproterenol and thus served as controls.

Studies of cardiovascular function were performed on sodium pentobarbital (40 mg/kg)-anesthetized rats. The procedures used have previously been described (11). Immediately following the induction of anesthesia, the right femoral artery of each animal was cannulated for arterial pressure measurement. This cannula was connected to a Statham P23db pressure transducer. Heart rate was determined, by a tachometer, from pulsatile arterial pressure, and a mean arterial pressure was recorded on a Beckman R612 recorder. The trachea was then isolated and cannulated, and each rat was ventilated at a minute volume of approximately 160 ml/min. A midclavicular-to-midclavicular thoracotomy was made at the level of the third intercostal space. Bleeding from the internal mammary arteries was prevented by cross-clamping the sternum prior to opening the thoracic cavity. The ascending limb of the aortic arch was exposed and cleared from its origin to the origin of the right innominate artery. A Carolina Medical Electronics electromagnetic flow probe, 2.55 mm in diameter, was placed around the vessel. The flow probe and Carolina Medical Electronics flowmeter were precalibrated within a range of hematocrits from 20 to 60%. The hematocrit of each rat was determined from a blood sample taken from the eye orbit. Pulsatile aortic flow was internally averaged by the CME flowmeter, and the resulting estimate of cardiac output was recorded on the Beckman R612 recorder. Cardiac index was obtained by dividing cardiac output by body weight. Total peripheral resistance index was calculated by the division of mean arterial pressure by cardiac index. Three measurements, 5 min apart, of mean arterial pressure, heart rate, cardiac index, and total peripheral resistance index were made and averaged to yield one value for each parameter.

The right jugular vein of each rat was cannulated, with the cannula advanced to the right atrium. This position was confirmed at autopsy. Through this cannula, volume loading of the heart was accomplished by a 15.3 ml/min infusion of Tyrode's solution (37°). The infusion proceeded for 2 min. It normally took between 60 and 90 sec to attain an elevated plateau on the cardiac output record. The infusion was continued for another 30–60 sec to make sure that cardiac output would not increase further. Stroke volume was calculated using the heart rate measured during the plateau phase of the cardiac output record. At the conclusion of the Tyrode's solution infusion, the aorta was tied distal to the electromagnetic flow probe to check for zero flow. The heart was then removed from the chest, the atria and right ventricular wall were cut away, and the left ventricle plus septum was weighed. Peak cardiac

output and peak stroke volume were expressed per gram of left ventricle. The prevolume loaded hemodynamic data (Tables I and II), control versus 24 hr and control versus 1 week, were analyzed by the Student *t* test. The volume-loaded peak cardiac output and peak stroke volume data (Figs. 1 and 2) were analyzed by analysis of variance and Duncan's multiple-range test. Results are expressed as mean ± standard error of the mean.

Results. Table I lists the prevolume loaded hemodynamics data for the control, the 24-hr postisoproterenol, and the 1-week postisoproterenol normotensive Sprague–Dawley rats. Mean arterial blood pressure was decreased and heart rate was increased at 24 hr after a single subcutaneous injection of 85 mg/kg isoproterenol. The decrease in mean arterial pressure was due to a decrease in cardiac index and in total peripheral resistance index, although neither parameter, by itself, decreased significantly. By 1 week after isoproterenol, mean arterial pressure returned to control levels. Cardiac index was slightly increased, while total peripheral resistance index was reduced.

In Table II are the prevolume loaded hemodynamics data for the control, the 24-hr postisoproterenol, and the 1-week postisoproterenol hypertensive rats. A significant increase in total peripheral resistance index was evident at 24 hr postisoproterenol. This elevated peripheral resistance increased mean arterial pressure in spite of a modestly reduced cardiac index. By 1 week after isoproterenol, mean arterial pressure had returned to control values. Cardiac index was significantly reduced, as total peripheral resistance index remained elevated.

Opening the chest of the SHRs caused a dramatic decrease in mean arterial pressure. For instance, mean arterial pressure in the control SHRs fell from 163 mm Hg, prethoracotomy, to 95 mm Hg postthoracotomy. We have no explanation for this occurrence, but it has been seen before in this strain of SHR (Colfer and Iams, unpublished data). These falls in arterial pressure occur in other laboratories; Spech *et al.* (14) have presented post-thoracotomy cardiac output and peripheral resistance data from the SHR that indicate a significant fall in mean arterial pressures from prethoracotomy values.

Figure 1 shows the volume-loaded peak cardiac outputs of control normotensive and hypertensive rats and of normotensive and hypertensive rats at 24 hr and 1 week after isoproterenol administration. In the normotensive rats, the peak cardiac output attained during the Tyrode's solution infusion was severely depressed at 24 hr after isoproterenol, but returned to control levels by 1 week. In the hypertensive rats, peak cardiac output was reduced at both 24 hr and 1 week postisoproterenol. Left ventricular wet weights increased in the Sprague–Dawley rats by 19 and 7% at 24 hr and 1 week postisoproterenol, respectively. Left ventricular wet weights also increased in the SHRs by 17% at 24 hr and 3% at 1

TABLE I. HEMODYNAMICS IN THE NORMOTENSIVE RAT FOLLOWING ISOPROTERENOL INJECTION[a]

	Mean arterial pressure (mm Hg)	Heart rate (beats/min)	Cardiac index (ml/min/g)	Total peripheral resistance index (mm Hg/ml·min^{-1}/kg)
Control $n = 14$	106 ± 5	422 ± 11	0.164 ± 0.010	0.671 ± 0.038
24 hr $n = 8$	85 ± 6*	505 ± 19*	0.153 ± 0.009	0.563 ± 0.053
1 week $n = 9$	100 ± 4	420 ± 9	0.197 ± 0.013	0.524 ± 0.033*

[a] Measurements made on open-chest, sodium pentobarbital-anesthetized rats.
* $P < 0.05$, significant difference from control.

TABLE II. HEMODYNAMICS IN THE SHR FOLLOWING ISOPROTERENOL INJECTION[a]

	Mean arterial pressure (mm Hg)	Heart rate (beats/min)	Cardiac index (ml/min/g)	Total peripheral resistance index (mm Hg/ml·min^{-1}/kg)
Control $n = 9$	95 ± 5	436 ± 5	0.163 ± 0.013	0.618 ± 0.063
24 hr $n = 6$	125 ± 8*	510 ± 10*	0.124 ± 0.010	1.053 ± 0.128*
1 week $n = 8$	105 ± 8	407 ± 11*	0.103 ± 0.005*	1.020 ± 0.053*

[a] Measurements made on open-chest, sodium pentobarbital-anesthetized rats.
* $P < 0.05$, significant difference from control.

week. These increases in ventricular weights were not accountable for the observed changes in peak cardiac output (ml/min/g of left ventricle). Control peak cardiac output values for the normotensive and hypertensive rats were statistically equal. This equality also existed at 24 hr after isoproterenol. The normotensive rats had a higher peak cardiac output at 1 week.

Figure 2 illustrates the effects of time on the peak stroke volume attained during a volume loading of the hearts of normotensive and hypertensive rats injected with isoproterenol. The normotensive rats showed a limited stroke volume only at 24 hr postisoproterenol. The peak stroke volume of the hypertensive rats was significantly reduced at 24 hr and continued to be so for 1 week. However, the peak stroke volume at 1 week was improved from that at 24 hr. The peak stroke volumes of the normotensive and hypertensive rats were different only at 1 week postisoproterenol.

Discussion. *dl*-Isoproterenol, 85 mg/kg, was used in this study because Rona *et al.* (15) have shown that it produces myocardial structural damage in the rat. We chose this single dose of isoproterenol because we wanted to determine whether a smaller quantity of isoproterenol than that used by Wexler (13), 500 mg/kg body wt twice within a 24-hr period, would produce signs of cardiac failure in the SHR. We examined

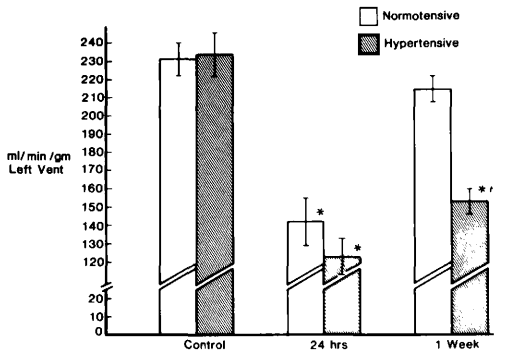

FIG. 1. Peak cardiac outputs from normotensive and hypertensive rats following a single subcutaneous injection of 85 mg/kg isoproterenol. The values shown here were generated by a 15.3 ml/min volume-loading infusion of Tyrode's solution. *$P < 0.05$, significant difference from control. †$P < 0.05$, significant difference between SHRs and Sprague–Dawley rats.

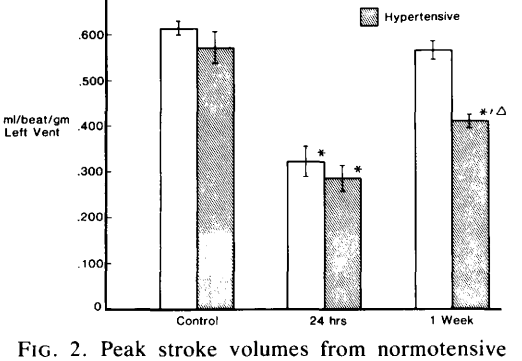

FIG. 2. Peak stroke volumes from normotensive and hypertensive rats following a single subcutaneous injection of 85 mg/kg isoproterenol. The values shown here were generated by a 15.3 ml/min volume-loading infusion of Tyrode's solution. *$P < 0.05$, significant difference from control. †$P < 0.05$ significant difference between SHRs and Sprague–Dawley rats. $^{\Delta}P < 0.05$ significant difference between 24 hr and 1 week.

cardiac function at 24 hr and 1 week post-isoproterenol because Wexler (13) noted chest cavity fluid accumulation in Sprague–Dawley rats and SHRs at 1–3 days and 7–10 days postisoproterenol, respectively. We did not extend our study past 1 week postisoproterenol because chest cavity fluid in the SHR disappears by 12–14 days post-isoproterenol (Wexler, personal communication).

Peak cardiac outputs achieved by volume loading are a highly reproducible index of pump performance (16). Rapid volume loading of the rat heart to achieve an elevated plateau of cardiac output has been used before to assess cardiac function. Beznak (9) and Beznak and Hacker (10) infused polyvinylpyrrolidone into the right side of the hearts of isoproterenol-treated rats. Their results at 1–3 days and 1 week postisoproterenol were similar to those obtained in our normotensive, Sprague–Dawley rats (Fig. 1). Pfeffer *et al.* (17–19) have infused Tyrode's solution into the femoral vein of young, mature, and old Wistar–Kyoto rats and SHRs. In the young and mature SHRs and normotensive rats, peak cardiac outputs and peak stroke volumes per gram of left ventricle were not different (17). We saw no differences between our 4- to 5-month-old Sprague–Dawley rats and the SHRs (Fig. 1 and 2). A comparison of peak cardiac outputs between rats having different arterial blood pressures is valid, for Herndon and Sagawa (20) have shown that peak aortic flow remains constant within a mean arterial blood pressure range of 50–180 mm Hg. Thus afterloads, within that range, do not effect preloaded cardiac outputs. Peak stroke volumes were calculated to eliminate the possibility that residual isoproterenol at 24 hr or even 1 week might mask poor cardiac function by its positive inotropic properties. Using vagotomized and timolol-treated rats, Pfeffer *et al.* (18) found that peak cardiac output was limited by heart rate; peak stroke volume remained constant before and after surgical and pharmacological denervation.

Isoproterenol, 85 mg/kg, did not compromise normal cardiac function in normotensive rats (Table I). It did, however, initially decrease the peak cardiac output and peak stroke volume attained during a volume loading stress (Figs. 1 and 2). This indicates that some degree of derangement of the myocardial contractile apparatus occurred; the muscle fibers could not respond to an increase in length with an appropriate increase in tension production. Within 1 week functional recovery was complete. Beznak (9, 10) also demonstrated this using a different isoproterenol dose regimen.

Isoproterenol, 85 mg/kg, did not initially compromise normal cardiac function in the hypertensive rats (Table II). It did, however, eventually bring about a significant reduction in cardiac index. Isoproterenol produced myocardial myofibrillar damage. This is shown by the reduced values of volume-loaded peak cardiac output and peak stroke volume (Figs. 1 and 2). The myocardial repair process was slow. This is demonstrated by the fact that although peak stroke volume at 1 week postisoproterenol was better than that at 24 hr, it was not completely back to preisoproterenol values.

We believe that in the SHR the slow recovery of volume-loaded cardiac function implies a delayed repair of the myocardium. We also believe that the delayed myocardial repair and the limited cardiac index at 1 week postisoproterenol were due to the vascular response following isoproterenol administration. Total peripheral resistance index was increased during the week following isoproterenol injection. It is not surprising that this increase in vascular resistance occurred. The normal response to cardiac failure is an increase in total peripheral resistance (21). This was probably so evidently manifested in the SHRs because they respond to periods of stress with significant elevations of plasma catecholamine levels (22–24). Also, Wexler (13) has shown that glucocorticoid levels are greatly increased in SHRs after isoproterenol. Thus by 24 hr postisoproterenol, and for possibly a longer period of time thereafter, high plasma levels of these vasopressor agents probably served to increase arterial pressure. An increased left ventricular afterload could then have increased

wall tension and myocardial oxygen demand (25), putting a metabolic burden on both damaged and normal myocardial fibers, and as a result delayed myocardial repair. By 1 week postisoproterenol, prevolume-loaded cardiac index was most probably reduced because of the elevated peripheral resistance and because of an injured left ventricle that could not yet overcome the influence of the high peripheral resistance.

In the normotensive rats, total peripheral resistance index was initially unaffected. By 1 week postisoproterenol, total peripheral resistance was significantly reduced. This has not been demonstrated before and the mechanism is undefined. Nevertheless, we believe that a low left ventricular afterload, caused by a reduced peripheral resistance, decreased the metabolic burden on the injured myocardium and as a result allowed it to repair itself and regain normal pump performance.

The data presented in this study lend support to Wexler's (13) hypothesis that sustained high arterial pressures following isoproterenol administration cause progressive congestive heart failure in hypertensive rats. This study indicates that a significantly increased total peripheral resistance maintains the high arterial pressure and that this elevated peripheral resistance delays myocardial repair. Although the dose of isoproterenol given here was not as high as that given by Wexler (13) and the degree of cardiac failure was probably not as severe, we believe that the mechanisms operating in both studies were similar.

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