Impaired Cardiac Contractile Response to Isoproterenol in the Spontaneously Hypertensive Rat

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SUMMARY To test the ability of the hypertrophied ventricle to increase its contractility in response to sympathetic stimulation, we compared the chronotropic, inotropic, and relaxation responses to graded infusions of isoproterenol in spontaneously hypertensive rats (SHR) with responses of matched Wistar-Kyoto (WKY) controls. A closed-chested, direct ventricle puncture was used for the study. The SHR required a higher threshold dose (0.04 vs 0.01 μg/kg/min) for a significant chronotropic response, and their maximal response of heart rate was smaller than in WKY (ΔHR = +12.5 ± 5.4 vs +22.8 ± 10.7 beats/min, p < 0.01). Contractility indices did not increase in the SHR after isoproterenol infusion: (ΔdP/dt + 22243 ± 1304.7 mm Hg/sec; ΔdP/dt/P = +5.1 ± 93 sec, p > 0.05) in sharp contrast with the marked increases observed in WKY (ΔdP/dt = +4682.1 ± 435.0 mm Hg/sec, p < 0.01; ΔdP/dt/P + 78.6 ± 8.0 sec, p < 0.001). Left ventricular relaxation rate was markedly diminished by isoproterenol in SHR (Δneg dP/dt = —2598.6 ± 855.0 mm Hg/sec) whereas it was not altered significantly in normotensive rats. Thus, cardiac contractile and chronotropic responses were markedly diminished in SHR, possibly as a result of diminished beta adrenoreceptor mediation; further, the impairment of the relaxation rate induced by isoproterenol in SHR might also interfere with contractile cardiac performance during stress. (Hypertension 3: 380-385, 1981)

KEY WORDS • contractility • left ventricle • isoproterenol • chronotropic response • inotropic response • relaxation response • beta adrenoreceptors • stress • cardiac function • hypertrophy

THE effects of hypertension on myocardial performance are not uniform; investigators have found cardiac function to be increased, decreased, or unchanged in nonfailing hypertrophied hearts. Studies of papillary muscles have demonstrated that hypertension affects differently the determinants of the contractile state according to the specific stimulus for cardiac growth. Extrapolations of these results to the in vivo situation are not always valid, however, since environmental conditions and the overall mechanics of the preparations are very dissimilar.

The effects on cardiac function of the hypertrophy associated with systemic arterial hypertension have been mostly investigated in the spontaneously hypertensive rat (SHR) because of the similarities of this model with essential hypertension. Cardiac pumping ability was found by some to be preserved or even increased and by others to be moderately depressed at early stages of hypertrophy. However, the restriction of these studies to determination of pumping ability as described by cardiac function curves does not allow complete evaluation of the effects of hypertrophy; the function curves obtained describe the total force-generating capacity of the ventricle, which may be independent of the velocity of myocardial fiber shortening. These two indices of contractile state were found to be altered differently in myocardial hypertrophy produced by pressure overload in the isolated right ventricular (RV) muscle. In RV hypertrophy due to pulmonary artery binding, increase in ventricular mass helped maintain total force development in the presence of impaired speed of contraction. Increased velocity of contraction is a major compensatory mechanism allowing the heart to face sudden variations in afterload; the ability to increase this velocity can therefore be regarded as a "contractile reserve." Thus, for better characterization of the functional effects of hypertrophy, descriptions of cardiac pumping ability should be complemented with
descriptions of the capacity of the ventricle to increase its velocity of contraction in response to stress.

Because that response is in large part influenced by the adrenergic system, we have assessed the inotropic response of the intact ventricle in SHR to infusions of isoproterenol (ISU). The ratio of peak left ventricular (LV) pressure first derivative (dP/dt) to the ventricular pressure at which it was attained (dP/dt/P) was used as an index of maximal velocity of fiber shortening (V max).11-13 This index was shown to be most useful in assessing acute inotropic responses, with each subject serving as its own control.14 Alterations in ventricular relaxation rate were determined from neg dP/dt.14 To avoid the disturbances inherent in open-chest preparations and, in addition, secure high-fidelity recordings, a preparation with closed chest and direct left ventricular puncture was devised.

**Material and Methods**

Rats were anesthetized with 60 mg/kg sodium pentobarbital. Trials with a smaller dose (50 mg/kg) led to unstable anesthesia, which would have required additional doses of pentobarbital to complete the test. In a small number of rats (2 WKY and 2 SHR), where the whole test could be performed under a 50 mg/kg dose, results were similar to those obtained with 60 mg/kg. The values reported here consist of the results obtained in tests performed under a single dose of 60 mg/kg without any additional injection of anesthetic during the test. This dose, in our experience and that of others, was more successful in ensuring a steady and long-lasting level of anesthesia.14

Seven male SHR and seven closely matched Wistar-Kyoto rats (WKY) aged 20 to 24 weeks were studied as follows: following anesthesia and subcutaneous atropine sulfate, the rats were placed on a high board, had their tracheas cannulated with a PE-205 (Clay-Adams, New Jersey) tubing and were left to breathe spontaneously. The left carotid artery was cannulated with a 5 cm long plastic catheter (PE-50); the left femoral vein was also cannulated with a 21-G scalp vein needle connected to a plastic catheter (PE-50) for drug infusions.

The skin over the left side of the thorax was then excised from the sternal border to the axillary line and cised from the sternal border to the axillary line and the apex point; the pressure channel was observed for hemothorax was still positioned in the left ventricle. If hemothorax was observed, the experiment was discarded.

Once the ventricular puncture was achieved, the preparation was left to rest for 20 to 30 minutes, and its stability observed. Following a control recording period, isoproterenol hydrochloride (Isuprel, Winthrop Laboratories) was infused through a Harvard pump at sequential rates of 0.01, 0.02, and 0.04 µg/kg/min for 5-minute periods at each dose level. During the last minute of each period, LV pressure, carotid pressure, and LV dP/dt were recorded at paper speeds of 200 mm/sec. After the infusion was stopped, a 5-minute recovery period was allowed, and the same variables were recorded again. Saline was used as diluent for the infusions; the total amount administered did not exceed 0.1 ml. When the experiment was over, the thorax was opened with the needle still positioned in the left ventricle. If hemothorax was observed, the experiment was discarded.

Paired t tests were used to assess the significance of increases in heart rate and contractility within groups, with isoproterenol; because these were predictable responses (at least directionally) to the drug, we used one-tailed p tables to assess significance. However, since the directional changes in relaxation rates within groups were not predictable, two-tailed tables were used to assess the significance of changes in this variable. Comparisons of heart rate, contractility, and relaxation rates between groups were studied by unpaired t tests, using two-tailed p values for significance. Results are expressed as averages ± 1 standard error of the mean (SEM).

**Results**

**Heart Rate (HR)**

Heart rate was increased as expected with ISU in both WKY and SHR, but the pattern and magnitude of the response were quite different in the two groups. In the WKY, the heart rate quickened from the first dose level and continued to increase in dose-dependent fashion (fig. 1). In contrast, the dose response curve was much flatter in SHR; the increment in heart rate
achieved statistical significance only at the dose of 0.04 µg/kg/min; the two lower doses of ISU did not produce a statistically significant acceleration of HR.

Mean Arterial Pressure (MAP)

A dose-dependent decrease in MAP induced by ISU was observed in both groups. In the WKY, MAP dropped from 116.3 ± 9.5 mm Hg (control) to 108.7 ± 9.2 mm Hg (p < 0.01) at the first dose level and to 97.8 ± 7.8 mm Hg (p < 0.01) at the highest infusion rate. In the SHR groups, MAP was reduced from 152.1 ± 13.5 mm Hg during control period to 144.6 ± 15.5 mm Hg by the 0.01 dose of ISU (p < 0.05) and to 140.1 ± 16.6 mm Hg (p < 0.01) at the third level of infusion (fig. 2). The difference in response between the two groups was not statistically significant, whether expressed in absolute values or as percent changes from control (fig. 3). Comparison of the changes in pulse pressure evoked by the three doses of isoproterenol showed no statistically significant differences between groups (3.5 ± 3.3 vs 8.1 ± 2.0 mm Hg, ns; 15.4 ± 5.7 vs 14.3 ± 4.0 mm Hg, ns; and 9.6 ± 2.8 vs 17.4 ± 4.7 mm Hg, ns).

Inotropic Indexes

The response of inotropic indexes to isoproterenol showed major differences between the two groups of rats. In the WKY group, each dose of ISU infused elicited a significant increase in dP/dt so that from a control of 11,437.8 ± 942.6 mm Hg/sec, dP/dt rose to 12,077.1 ± 1,071.2 mm Hg/sec (p < 0.05) at 0.01 µg/kg/min and then to 14,219.0 ± 1,060.0 mm Hg/sec (p < 0.01) at the doses of 0.02 and 0.04 µg/kg/min, respectively. On the other hand, SHR displayed only borderline increments in the velocity of LV pressure rise with ISU; dP/dt increased from 11,425.7 ± 816.3 mm Hg/sec (control) to 12,144.3 ± 706.0 mm Hg/sec (first dose) and to 13,643 ± 1,060.2 mm Hg/sec (second dose) (p < 0.05), and then plateaued at that last level despite doubling the ISU infusion to 0.04 µg/kg/min. During the recovery period, dP/dt remained elevated in the WKY (13,310.0 ± 722.0 mm Hg/sec).

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Increases in heart rate evoked by graded isoproterenol infusions in WKY and SHR (*p < 0.05; **p < 0.01). Significance levels refer to control values.

![Figure 2](http://hyper.ahajournals.org/)

**Figure 2.** Mean arterial pressure (MAP) response to graded infusions of isoproterenol in SHR and WKY.

![Figure 3](http://hyper.ahajournals.org/)

**Figure 3.** Comparison of diminutions in mean arterial pressure (MAP) evoked by graded isoproterenol infusions between WKY and SHR. There was no statistically significant difference between the two groups.
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Hg/sec, \( p < 0.01 \)) whereas it rapidly returned to control values in the SHR (11,132.0 ± 819.2 mm Hg/sec, ns) (fig. 4).

Peak first derivative of LV pressure normalized for developed pressure (\( dP/dt/P \)) showed even more striking differences between the two groups. In the WKY, it increased significantly and in dose-dependent fashion from control (105.9 ± 5.3 mm Hg/sec\(^{-1}\)) to 130.0 ± 5.9, 152.5 ± 6.9, and 186.6 ± 5.3, at the first, second and third dose levels, respectively (\( p < 0.001 \) for all). In contrast, SHR did not show any significant response of \( dP/dt/P \) to ISU throughout the whole dose range of infusion; it averaged 120.4 ± 14.4 mm Hg/sec\(^{-1}\) during control and remained at 124.3 ± 7.0 during the 0.04 \( \mu \)g/kg/min infusion level (\( p, \) ns; fig. 5). During recovery period, \( dP/dt/P \) remained high in the WKY group (126.8 ± 7.7, \( p < 0.01 \)), but was not different from control in SHR (120.2 ± 11.1, ns). Left ventricular end diastolic pressure (LVEDP) did not change significantly (± 1 mm Hg) with ISU infusion in either group of rats and did not exceed 2 mm Hg in any experiment.

**Relaxation Index**

Peak left ventricular negative dP/dt was used as an index of ventricular relaxation rate.\(^{18} \) Since the directional changes in negative dP/dt with ISU were not predictable, significance of the results obtained in this variable were tested using a two-tail probability table. In the WKY group, negative dP/dt decreased during the lowest infusion rate (from 8,650.0 ± 390.6 mm Hg/sec to 8,328.6 ± 227.1, \( p < 0.05 \)), but returned toward control levels with increasing doses (8,668.5 ± 236.8 mm Hg/sec at 0.04 \( \mu \)g/kg/min, ns). In the SHR group, negative dP/dt also decreased slightly (\( p < 0.05 \)) during the first level of infusion (from 8,880.3 ± 1,065.8 to 7,640.0 ± 810.0 mm Hg/sec), but then it continued to decrease with increasing doses of ISU (6,881.4 ± 615.3 mm Hg/sec, \( p < 0.05 \) at 0.04 \( \mu \)g/kg/min). After cessation of infusion, negative dP/dt returned toward control values in WKY, while in SHR, neg dP/dt was reduced still further (6,188.3 ± 650.0 mm Hg/sec, \( p < 0.05 \)) (fig. 6). Thus, there was a gradually increasing difference in response of negative dP/dt between WKY and SHR, which achieved statistical significance during the third level of infusion (+17.1 ± 357.4 vs -1,998.8 ± 827.0 mm Hg/sec, \( p < 0.05 \)) (fig. 6). This difference remained evident throughout the recovery period (+1,005.0 ± 787.7 vs -2,598.6 ± 855.0 mm Hg/sec, \( p < 0.01 \)).

**Discussion**

Although differences in resting cardiac performance were initially demonstrated in SHR as compared to normotensive control rats,\(^{16} \) these differences were not found by the same investigators when the WKY were used as control.\(^{7} \) At the age of 4–5 months, cardiac function estimated by peak cardiac output during volume overload was reported by some\(^{19} \) but not all\(^{20} \) to

**Figure 4.** Changes in left ventricular (LV) inotropic (peak positive dP/dt — upper panel) and relaxation (peak negative dP/dt — lower panel) indices, induced by graded isoproterenol infusion. Significance levels refer to control period values (*\( p < 0.05 \), **\( p < 0.01 \)).

**Figure 5.** Comparison of the contractile response to isoproterenol as assessed by maximal dP/dt/P, in SHR and WKY (**\( p < 0.01 \), ***\( p < 0.001 \)).
be within the same range as WKY in spite of marked cardiac hypertrophy. Studies of cardiac output response to volume overload are not sufficient, however, to fully describe the performance of a pressure-related ventricular hypertrophy. We have attempted, therefore, to define the LV responsiveness to ISU in SHR in order to establish its capability to face hemodynamic stresses. Increase of the velocity of contraction was shown to be a major cardiac compensatory mechanism against sudden variations in afterload, which is influenced to a large extent by adrenergic factors.

Our results show that, despite the SHR having comparable dp/dt/P at rest, they were unable to increase this inotropic index in response to ISU; this was in striking contrast with the marked increase observed during ISU infusion in WKY. This finding is in accordance with the description by Brody, et al. of depressed tension development by SHR atria in response to ISU; the atrial muscle in these experiments was obtained from SHR of the same age at the same stage of cardiac hypertrophy as our experimental animals. The failure of SHR hearts to improve contractility by beta-adrenergic stimulation suggests a lack of contractile "reserve" in this type of hypertrophy, despite a reportedly normal or near normal hemodynamic pump function.

**Possible Causes for Reduced Responsiveness**

A reduced chronotropic response to ISU in SHR had already been reported by Pfeffer, et al. and by Fujiwara, et al. The present study extends these observations to the inotropic response to ISU. This reduced ability of the hypertrophied heart to increase its velocity of pressure rise in response to beta-adrenergic stimulation has potentially important implications. It could be conceivably related to one or more of the following factors: 1) decreased beta-adrenoreceptor mediation, 2) myocardial metabolic imbalances evoked by ISU, or 3) intrinsic alterations in the hypertrophied fiber.

The possibility of impaired beta mediation in the hypertrophied ventricle was studied by assessing the chronotropic responses to ISU. These were found to be decreased in the SHR in studies of both isolated atrial muscle and of open-chested rats and these results were confirmed in our experiments using an atropinized closed-chest preparation. Moreover, we also found a higher threshold dose in SHR for the drug-induced tachycardia (fig. 1). Diminished beta-adrenoreceptor sensitivity has been demonstrated in vitro following continued exposure of myocardial tissue to catecholamines. Since many studies have suggested that an increased sympathetic drive participates in the maintenance of hypertension in the SHR, one could infer that the diminished chronotropic responsiveness demonstrated in this study could be due to diminished sensitivity or decreased number of cardiac beta-adrenoreceptor in this model of hypertension.

Another factor that may help explain the reduced inotropic response to ISU could be that the drug led to an imbalance in the hypertrophied heart between increased myocardial oxygen demands and actual oxygen supply. Myocardial capillary reserve was reported to be reduced because of increased intercapillary distance in cardiac hypertrophy. Recent studies by Marcus et al. have also shown diminished coronary reserve in hypertension. A potential myocardial anoxia relative to increased demands might play a role in reducing contractility; its effects relative to chronotropy are more debatable.

**Relationship to Ventricular Relaxation Rate**

Catecholamines have a relaxing effect on heart muscle; this effect, however, is not easily demonstrable in the whole heart preparation. In fact, Cohn, et al. showed a paradoxical effect of catecholamines delaying the relaxation rate of the dog ventricle as measured by peak negative dp/dt. In these experiments, ISU induced increases in positive dp/dt which were not accompanied by similar increases in relaxation rates. Our results are in agreement with their observation. There was an actual diminution of relaxation rate in both normal and hypertensive ventricles during infusion. A difference emerged, however, with continued ISU administration; while neg dp/dt in WKY tended to return toward control levels, there was a marked deterioration of this index in SHR with increasing doses of ISU. It has been shown that ischemia of myocardium can lead to diminutions in relaxation rates, and this fact might support the idea that ISU could induce an imbalance between oxygen demands and supply, which could in part be responsible for the impairment of their ventricular contractile response. To the extent that a delayed or impaired relaxation can influence contraction, the impairment
in relaxation rate might also explain, in part, the reports of reduced cardiac performance in SHR.  

In conclusion, the myocardium in SHR appeared to have an impaired "contractile reserve," at least as far as responses to beta-adrenergic stimulation are concerned. Further studies are needed to determine whether this abnormality would be common to other types of hypertension and cardiac hypertrophy or whether it is restricted to SHR.

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References

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