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Hemodynamics of the Spontaneously Hypertensive Rat: Effects of Isoproterenol[1] (37946)

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Increased sympathetic neural activity has been implicated in the pathogenesis and maintenance of elevated arterial pressure in several forms of hypertension. Although increased adrenergic participation may alter cardiovascular performance by modifying both vascular smooth and cardiac muscle function, the effect upon the latter has received little attention. Fujiwara, Kuchii, and Shibata have recently reported that atria isolated from spontaneously hypertensive rats (SHR) exhibited lesser increments of cardiac responses from isoproterenol stimulation than normotensive rat atria (NR) (1). In our previous studies, the intact SHR myocardium failed to demonstrate the increases in heart rate and cardiac output which were observed in sex- and age-matched NR during infusions of small doses of exogenously administered norepinephrine (5). These differences of cardiac responses to norepinephrine could indicate possible quantitative or qualitative alterations in the cardiac administered sympathetic neurotransmitter, intrinsic changes in the myocardial contractile machinery, or possible differences in tonic autonomic influences on myocardial function. The present study was undertaken to examine further the responses of intact NR and SHR myocardia to beta-adrenergic stimulation with the specific agonist isoproterenol.

Methods. Studies were performed on the 21st and 22nd filial generation of spontaneously hypertensive rats (3) and on normotensive Wistar rats (West Jersey Biological Supply) ranging from 22 to 27 weeks of age. Commercially obtained normotensive Wistar rats (from the original Wistar strain) were used since Wistar–Kyoto (WKY) rats were not available at the time this study was initiated. In previous studies we have compared the hemodynamic and myocardial functions of these two groups of rats over a wide range (6) and following acute elevation of arterial pressure (5). It was therefore necessary to follow up the mechanisms for these observed differences, and present studies are concerned with the possibility of differences between the NR and WKY rats. Each group of NR and SHR consisted of 5 male (NR 423 ± 57 g, SHR 363 ± 15 g) and 5 female (NR 279 ± 23 g, SHR 224 ± 11 g) rats. They were allowed free access to food (Purina Rat Chow) and tap water until the beginning of the study. Each animal was anesthetized with ether and surgically prepared by a previously described method (4). In brief, the left carotid and right jugular vein were cannulated and connected to pressure transducers (Statham P23Db and P23Bb, respectively). At this time, the systolic and diastolic arterial pressures of the NR were 134 ± 4 and 88 ± 4 mm Hg and the SHR were 192 ± 7 and 137 ± 5 mm Hg, respectively (mean ± 1 SE, $P < 0.001$). The heart rate of the NR was 315 ± 11 beats/min, whereas the SHR was 388 ± 10 beats/min ($P < 0.001$).

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Prethoracotomy central venous pressures of the two groups were both under 1.5 mm Hg and not significantly different. Following recordings of control blood pressures, the trachea was intubated and the lungs ventilated by a constant volume respirator (Harvard Apparatus, Model 680). The chest was entered through an incision extending between the sternoclavicular junction and the fifth costal level. A suture was then placed around the ascending aorta to provide traction for the placement of a 2.0-mm electromagnetic flow probe (Micron Instruments) around the vessel.

Once the animal was prepared, phasic recordings of arterial and venous pressures, aortic blood flow velocity, and the first derivative of aortic flow velocity (flow acceleration obtained by electronic differentiation of aortic blood flow velocity) were recorded continuously on a four-channel polygraph (Hewlett Packard Model 7784A). Every 5 min during a 15-min control observation period, these measurements were recorded at a paper speed of 100 mm/sec to permit more precise analysis of phasic hemodynamic measurements. A summary of the control (pretreatment) hemodynamic data is presented in Table I.

Following these control hemodynamic measurements, isoproterenol hydrochloride (Winthrop Laboratories) was infused into the right femoral vein in progressively increasing doses of 0.16, 0.32, 0.64, and 1.28 μg/kg/min without permitting return to control levels between doses. Before cannulation of the femoral vein, the infusion catheter was filled with a 2 μg/ml solution of isoproterenol and connected to a variable speed infusion pump (Harvard Apparatus, Model 904). Thus, the highest absolute volume rate infused was 0.64 ml/min/kg. The infusion of each dose was maintained for 2 min, and during the terminal 10 sec, a high-speed recording of all hemodynamic measurements was obtained to allow comparison of each dose level of isoproterenol with control (preinfusion) measurements. During the entire study, rectal temperature was monitored and maintained between 35 and 38°C by an electric heating pad previously placed under the animal. Statistical significance of the isoproterenol-induced responses was determined by paired *t*-test comparison of the last (preinfusion) control value with each dose level, using at least $P < 0.05$ to denote significance.

Results. Heart rate. Isoproterenol pro-

TABLE I. Comparison of Hemodynamic Indices of Normotensive (NR) and Spontaneously Hypertensive Rats (SHR) Under Ether Anesthesia and With the Chest Open to Atmospheric Pressure[a]

	NR	SHR
Arterial pressure (mm Hg)		
Systolic	123 ± 2	96 ± 5*
Diastolic	66 ± 3	156 ± 5*
Mean	93 ± 2	121 ± 5*
Heart rate (beats/min)	289 ± 13	374 ± 14*
Cardiac output (ml/min)	77 ± 6	73 ± 5
Cardiac index (ml/min/kg)	225 ± 14	256 ± 18
Stroke volume (ml/beat)	0.27 ± .03	0.19 ± .01**
Peak flow velocity (ml/sec)	4.5 ± 0.1	4.7 ± 0.3
Maximum flow acceleration (ml/sec²)	233 ± 10	258 ± 21
Total peripheral resistance (mm Hg/ml/min)	1.260 ± 0.098	1.803 ± 0.181**

[a] Each value represents the average of recordings obtained after completion of the surgical procedures and then after 5, 10, and 15 min for 10 rats ± SEM.

* $P < 0.001$.

** $P < 0.05$.

gressively and significantly increased heart rate of the NR (Fig. 1). With the 0.64 μg/kg/min dose, heart rate had increased by 61 beats/min to 367 beats/min. But even with the lowest dosage, heart rate increased significantly ($P < 0.05$); this was not observed in the SHR. Higher doses further elevated heart rate only modestly in the NR, and the peak rate of 375 beats/min was attained with the 1.28 μg/kg/min infusion rate. The SHR had a significantly faster heart rate even before isoproterenol infusion (Fig. 1), and the cardioaccelerator response to increasing doses of isoproterenol was significantly less than the NR. Thus, by the 0.64 μg/kg/min dose of isoproterenol, the heart rate of the SHR increased by only 24 beats/min to 402 beats/min ($P < 0.05$). Furthermore, at double this dosage (1.28 μg/kg/min), the heart rate of the SHR failed to increase further (404 beats/min) (Fig. 1).

Cardiac output. Even though the low doses of isoproterenol (0.32 and 0.64 μg/kg/min) increased cardiac output of the NR above preinfusion levels ($P < 0.05$), these same doses failed to significantly increase the cardiac output of the SHR (Fig. 2).

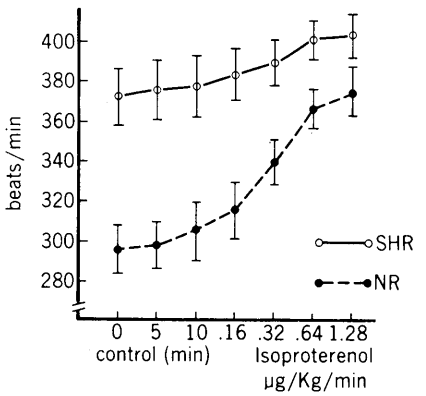

FIG. 1. Heart rate responses of 10 normotensive (NR) and 10 spontaneously hypertensive (SHR) rats to increasing doses of isoproterenol. Presented are mean ± 1 SEM values for each control and dose level measurement. The first three points on the left are control measurements which are followed by successive 2-min infusions of increasing doses of isoproterenol. Time represented on abscissa applies only to the control period.

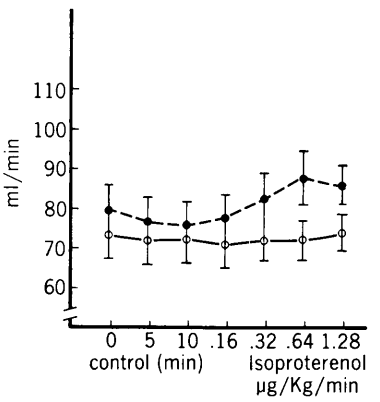

FIG. 2. Cardiac output responses of 10 normotensive (NR) and 10 spontaneously hypertensive (SHR) rats to increasing doses of isoproterenol. Presented are mean ± 1 SEM values for each control and dose level measurement. The first three points on the left are control measurements which are followed by successive 2-min infusions of increasing doses of isoproterenol. Time represented on the abscissa applies only to the control period: ○—○ SHR, ●- - -● NR.

Blood pressures. The fall in arterial pressure observed during isoproterenol infusions was similar in both NR and SHR (Fig. 3). Thus, by the 0.32 μg/kg/min infusion level,

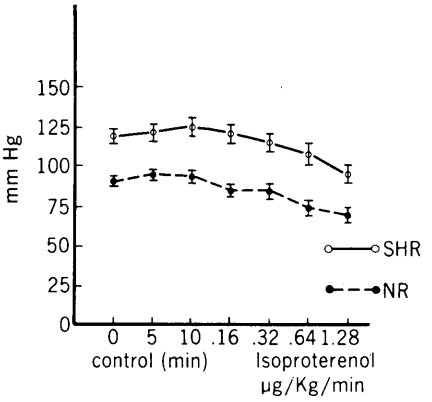

FIG. 3. Mean arterial pressure responses of 10 normotensive (NR) and 10 spontaneously hypertensive (SHR) rats to increasing doses of isoproterenol. Presented are mean ± 1 SEM values for each control and dose level measurement. The first three points on the left are control measurements which are followed by successive 2-min infusions of increasing doses of isoproterenol. Time represented on abscissa applies only to the control period.

mean arterial pressure of the NR and SHR decreased by 9 and 8 mm Hg, respectively. Systolic and diastolic pressures of both groups were reduced by 9 mm Hg from preinfusion levels of 124/67 and 161/99 to 115/58 and 152/90 for the NR and SHR, respectively. By the 0.64 μg/kg/min dose, the arterial pressures of the SHR were reduced further to 147/82 mm Hg; however, a similar decrement in pressure was obtained in NR (112/49 mm Hg). Therefore, even though each dose level of isoproterenol reduced arterial pressure of the SHR, similar degrees of reduction in the NR maintained the significant preinfusion pressure differences between these groups.

Central venous pressure of both NR and SHR groups remained stable throughout the isoproterenol dose range (Fig. 4).

Inotropic indices. Although no differences in either the peak flow velocity or maximum acceleration of aortic flow were observed between SHR and NR during the control (preinfusion) period, responses of these myocardial inotropic indices of NR and SHR to isoproterenol did vary (Fig. 5a and b). Thus, with low doses of isoproterenol, peak flow velocity and maximum acceleration of aortic flow of the NR progressively increased, and the maximum response achieved occurred with the 0.64 μg/kg/min dose level. At this infusion rate, peak flow

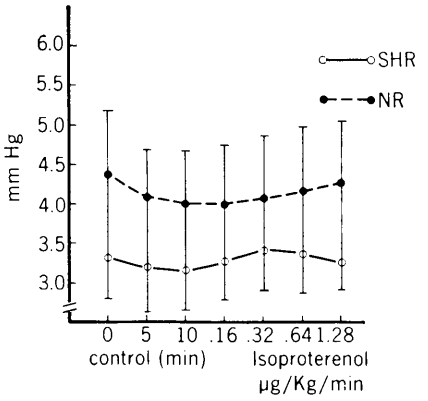

FIG. 4. Central venous pressure responses of 10 normotensive (NR) and 10 spontaneously hypertensive rats (SHR) to increasing doses of isoproterenol. Represented are mean and SEM values for each control and dose measurement.

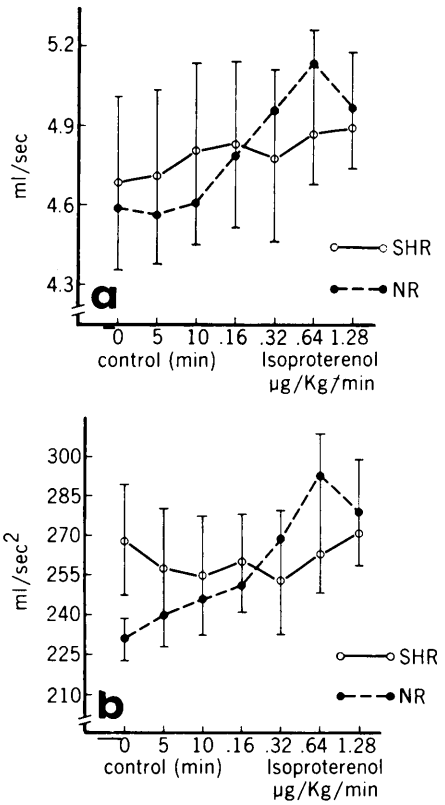

FIG. 5. Inotropic responses of 10 normotensive (NR) and 10 spontaneously hypertensive rats (SHR) to increasing doses of isoproterenol. Figure 5a and b represent the responses of peak aortic flow velocity and maximum acceleration of aortic flow, respectively.

velocity of the NR increased from 4.6 ± 0.2 to 5.1 ± 0.1 ml/sec, and the maximum acceleration of aortic flow from 246 ± 14 to 293 ± 15 ml/sec². The increases in these indices were obtained at a time when stroke volume did not increase (0.26 to 0.24 ml/beat). In contrast, no significant increases of these indices were observed in the SHR at this infusion rate.

Discussion. Under the conditions of the present investigation, low-dose levels of the beta-adrenergic receptor agonist isoproterenol failed to evoke similar increments of cardiac performance in SHR which were observed in NR. These findings in the intact, innervated, SHR myocardium were similar to investigations of isoproterenol responsiveness of isolated SHR atria. Indeed,

Fujiwara, Kuchii, and Shibata (1) have demonstrated that isolated atria from SHR exhibit lesser increases in contraction rate and developed tension from isoproterenol stimulation than atria from NR. And Brody, Heitz, and Greenberg (2) have also shown that SHR atria fail to exhibit as great an increase in tension development as NR to various adrenergic stimuli. However, these investigators also reported that the chronotropic response of isolated SHR atria to adrenergic stimulation was similar to that of NR. It is important to note that the atrial contraction rates of these isolated atria of NR and SHR were the same. But, in contrast, heart rate of the intact, innervated, SHR myocardium is faster than in the NR.

The sluggish responsiveness of the isolated SHR atria (1, 2) and of the intact, innervated, SHR myocardium (the present investigation) to adrenergic stimulation could reflect some alteration in the beta-adrenergic receptor mechanism. Several possibilities for these quantitative differences in cardiac responsiveness to beta-adrenergic stimulation could exist. The SHR myocardium might contain a reduced number of receptor sites. Alternatively, there might be an impaired affinity of these beta-adrenergic receptor sites for the specific agonist. Thus, under these circumstances, the agonist would have to be present in greater concentrations to reach the effective site. It is also possible that the number of myocardial beta-adrenergic receptors is normal, but the mechanism beyond the membrane receptor may be abnormal. All of these possibilities, although highly speculative at this time, stress mechanisms which could produce altered responsiveness in isolated heart muscle as well as in the intact myocardium.

The present investigation concerning responses in intact NR and SHR myocardia to beta-adrenergic stimulation, however, must consider other pertinent explanations for the observed differences. It is possible that the resting *net* adrenergic activity to the SHR heart is already maintained at a higher level; therefore, further increments of cardiac adrenergic activity might require more of the exogenous beta-adrenergic agonist to provide additional stimulation. We have shown previously that although the administration of very low doses of norepinephrine (0.5 and 1.0 μg/kg/min) produced similar increases in arterial pressure (4 and 9 mm Hg, respectively) in NR and SHR, the SHR did not demonstrate the same increase of heart rate and cardiac output as did the NR (5). In addition, the faster resting heart rate of the SHR appeared to result from neurohumoral influences since pharmacological autonomic inhibition abolished these differences (7). Thus, following inhibitors (atropine and sotalol, respectively), the intrinsic heart rate and cardiac indices of the SHR were the same as the NR. The faster heart rate and higher cardiac index in the unblocked SHR may reflect an already increased net adrenergic drive to the SHR myocardium. Therefore, it may well be that higher levels of exogenous beta-adrenergic stimulating agents are required to produce measurable increases in myocardial performance in the SHR because a greater net adrenergic drive already exists.

Lastly, when comparing responses of two groups having differing control (pretreatment) values (i.e., heart rate and mean arterial pressure of NR and SHR), one must consider the "law of initial value" (8). Briefly, this concept emphasizes that the response to a stimulus which produces an increase should be greatest when the initial value is low and least when the control value is high. Thus, one might expect that the faster heart rate of the SHR would demonstrate a lesser increase to the positive chronotropic agent, isoproterenol, than the NR. Conversely, the higher arterial pressure of the SHR should be reduced to a greater extent by the vasodilation of isoproterenol; this, however, was not observed. It appears that the "law of initial value" did not hold for comparisons of normal and hypertensive animals since these groups may have different physical limitations (i.e., maximum attainable levels). In previous studies, we have shown that although the SHR had higher initial levels of arterial pressure, they still responded with a greater pressor rise to alpha-adrenergic stimulation (methoxamine) (5). Thus, the "law of initial value"

could not be invoked to explain these complex physiological responses of the SHR.

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