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A COMPARISON OF CARDIAC REACTIVITY AND β-ADRENOCEPTOR NUMBER AND AFFINITY BETWEEN AORTA-COARCTED HYPERTENSIVE AND NORMOTENSIVE RATS

PETER CERVONI, HELEN HERZLINGER, FONG M. LAI & TARAK TANIKELLA

Department of Cardiovascular Biological Research, Medical Research Division, American Cyanamid Company, Lederle Laboratories, Pearl River, New York 10965, U.S.A.

1 The effects of noradrenaline (NA) and isoprenaline on isolated atria from aorta-coarcted hypertensive rats (AHR) at early (6 day) and chronic (28 day) stages of hypertension were studied and compared with time-matched, sham-operated, normotensive rats (SNR). The number and affinity of β-adrenoceptors ((−)-[^{3}H]-dihydroalprenolol binding sites) were also studied in cardiac membranes prepared from these animals.

2 Six and 28 days after complete ligation of the abdominal aorta between the two renal arteries, rats became hypertensive with significantly greater arterial blood pressures than time-matched SNR.

3 At both stages of hypertension, the atrial inotropic or chronotropic effects of NA and isoprenaline from hypertensive rats were similar to time-matched SNR. Moreover, no differences in atrial reactivity were observed between the early and chronic stages of hypertension.

4 Irrespective of the stage of hypertension, cardiac membranes from the AHR contained the same number of β-adrenoceptors as time-matched SNR. In addition, the receptor affinity for the radioligand within each group was equivalent. However, the chronic stage hypertensive rats and their time-matched controls contained fewer β-adrenoceptors and these receptors had greater affinity for the radioligand when compared with cardiac membranes from rats at the early stage of hypertension and their controls.

5 The observed equivalent chronotropic and inotropic responses to NA and isoprenaline between the hypertensive and normotensive rats in both stages of hypertension may be explained in terms of similar receptor number and receptor binding affinity.

6 The reduced number of β-adrenoceptors with greater binding affinity in day 28 normotensive or hypertensive rats may be a compensatory mechanism for these animals to maintain normal cardiac function with increasing age.

Introduction

The responses of aortic smooth muscle from the aorta-coarcted hypertensive rat (AHR) to noradrenaline (NA), 5-hydroxytryptamine, potassium and isoprenaline are hypertension stage-dependent (Lai, Tanikella, Thibault, Chan & Cervoni, 1980). Depending on the stage of hypertension (acute, early, middle or chronic), aortic strips from hypertensive rats developed equivalent or reduced tension to vasoconstrictors and relaxed equally or to a lesser extent to vasodilators than those of the corresponding normotensive controls. It has been demonstrated that changes in vascular reactivity in hypertensive rats are different between arterial and venous vasculature. For example, the contractile responses to NA of aortic strips from spontaneously hypertensive rats (SHR) were depressed (Shibata, Kurahashi & Kuchii, 1973), whereas the response of the portal vein to NA was enhanced (Hallback, Lundgren & Weiss, 1971) when compared to those of the Wistar-Kyoto control rats. It has also been shown that the cardiac reactivity of SHR was significantly lower than that of their time-matched normotensive controls (Fujiwara, Kuchii & Shibata, 1972; Kunos, Robertson, Kan, Preiksaitis & Mucci, 1978) and this corresponds well with the finding of a diminished density of β-adrenoceptors in the myocardial membranes of SHR (Limas & Limas, 1978). A lack of information on the cardiac reactivity of the AHR along with the previous findings of stage-associated changes in vascular reactivity and the variable responses of different vasculatures of hypertensive rats prompted us to carry out the present study.

AHR at two different stages of hypertension, acute and chronic, were used to ascertain changes in cardiac inotropy and chronotropy. Possible changes in the number of β-adrenoceptor binding sites and the

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affinity of the β-adrenoceptor for the radioligand in the cardiac membrane were also studied in order to provide a better understanding of the observed cardiac reactivity. The present results and previous studies of vascular reactivity (Lai *et al.*, 1980) suggest that a systematic analysis of the cardiovascular system is a necessity in any attempt to understand changes in function in hypertension.

Methods

Male Sprague-Dawley rats (300–325 g) purchased from Charles River Breeding, Inc. (Wilmington, MA) were used.

Induction of hypertension

Hypertension was induced by complete ligation of the aorta between the two renal arteries according to the method of Rojo-Ortega & Genest (1968) with a minor modification of the surgical procedure (Lai *et al.*, 1980). In brief, rats anaesthetized with methohexitone sodium (Brevital, Eli Lilly & Co.) were placed on their right sides. A 2–3 cm incision was made in the left lateral abdominal wall and a retractor placed in the incision. The kidney was gently pushed aside to locate the renal artery and the aorta was ligated with No. 000 silk suture. Sham-operated normotensive rats (SNR) went through the same procedure except that the aortae were not ligated with sutures. The abdominal muscle was closed with wound clips. Following surgery, the rats were returned to cages and maintained on Purina laboratory chow and tap water *ad libitum* until use. The experiments were carried out at 6 and 28 days after aorta coarctation. The degree of hypertension for these periods was referred to as the early and chronic stages of hypertension, respectively.

Blood pressure measurement

Arterial blood pressure was measured directly through a PE-50 catheter which was implanted into the ascending aorta through the left carotid artery and exteriorized at the back of the neck. The cannula was filled with heparin at a concentration of 1000 u/ml and heat sealed. The surgical implantation of the catheter was performed under pentobarbitone anaesthesia (60 mg/kg i.p.) 24 h before blood pressure recording. Blood pressure was measured in conscious, unrestrained animals which were moving about freely in the cage.

Chronotropic and inotropic responses of isolated atria

After recording blood pressure, the rats were killed by cervical dislocation and the hearts were quickly removed and placed in petri dishes filled with physiological salt solution of the following composition (g/l): NaCl 6.77, KCl 0.439, NaH_2PO_4 0.115, $MgSO_4 \cdot 7H_2O$ 0.246, $NaHCO_3$ 2.1, $CaCl_2$ 2.71 and dextrose 3.94. The combined atria were then carefully dissected free of connective tissues and the ventricles. The left and right atria were then separated. Surgical silk thread was tied to the apical ends of the atria and the other ends were pinned to the bottom of a T-shaped rod. One arm of the perspex rod contained two platinum electrodes (2 mm apart) for stimulating the left atrium while the other arm was used to support the right atrium. The left atrium was mounted so that the electrodes were in contact with the base of the atrial tissues. After the atria were mounted in position, the perspex rod was transferred to a 50 ml tissue bath maintained at 37°C and bubbled with 95% O_2 and 5% CO_2. The threads that were attached to the apical ends of the atria were connected to force displacement transducers (Grass Model FT03C) and the atrial contractions were recorded on a polygraph (Grass Model 7C).

The right atrium was used for assessing chronotropic effects. A tachometer (Grass Model 7P4F) was used to monitor the atrial rate. The resting tension of the left atrium was adjusted to about half that associated with the maximal tension developed. Electrical stimuli at a rate of 5 Hz were delivered from a Grass Model S44 stimulator through a pair of platinum electrodes (located in the perspex rod) to the tissue. The stimuli were square-wave pulses of 5 ms duration at a voltage slightly above threshold. The atria were allowed to equilibrate for at least 30 min during which time the preparations were washed every 10 min. Drugs were added only after the spontaneous rate of the right atrium changed no more than 5% within a 10 min period of observation. Concentration-response curves to drugs were determined by cumulative addition of the compounds. Phentolamine (1×10^{-6} M) was added to the bathing medium in some experiments to determine whether the agonist responses were solely mediated by β-adrenoceptor stimulation.

β-Adrenoceptor binding assay

Cardiac membranes for identification of β-adrenoceptors were prepared essentially as described by Baker & Potter (1980) in groups of rats different from those used in inotropy/chronotropy experiments. After recording blood pressure, the rats were decapitated and their hearts were removed and rinsed in physiological saline. Each heart was homogenized in 10 ml of ice-cold Tris-HCl buffer (10 mM, pH 8.0) with a Polytron-PT10 blender set at 7 for 30 s. Each homogenate was then diluted with

30 ml of 1 M KCl, left on ice for 10 min and then filtered through four layers of cheese cloth. The filtrate was centrifuged at 48,000 *g* for 10 min. Pellets were gently resuspended in 20 ml of buffer, re-sedimented and finally dispersed again in 3 ml of buffer. Cardiac membranes of 300 to 500 μg protein were incubated in triplicate with 0.5 to 10 mM of (−)-[^{3}H]-dihydroalprenolol in HEPES buffer (50 mM, pH 8.0) containing 4 mM $MgCl_2$ with and without 10 μM of (±)-propranolol in a final volume of 150 μl for 20 min at 25°C. At the end of the incubation period, 4 ml of ice-cold HEPES buffer (25 mM, pH 8.0) containing 4 mM $MgCl_2$ was added to each tube and the fluid was filtered through a Whatman GF/C glass fibre filter. Each tube and filter was then rinsed with two additional 4 ml aliquots of washing buffer. The filter was then placed in the counting vial and 5 ml of Ready-Solv HP (Beckman) was added and counted. Dissociation constants (*Kd*) and maximum number of receptors were determined by the method of Scatchard (1949).

Drugs and radiochemicals

(−)-[^{3}H]-dihydroalprenolol HCl (New England Nuclear, 43 Ci/nmol), (−)-isoprenaline bitartrate, (−)-noradrenaline bitartrate, N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (HEPES) (Sigma), phentolamine mesylate (Ciba-Geigy) and (±)-propranolol HCl (Ayerst) were used.

Results

Animal characteristics

The complete ligation of the abdominal aorta caused a marked increase in arterial blood pressure (Table 1). Six days after the surgery, rats developed significantly higher mean arterial blood pressures (MABP) than the corresponding sham-operated controls. At 28 days after aorta coarctation, the rats still had higher MABP than their time-matched SNR. The body weights of the hypertensive rats at both stages of hypertension were significantly less than their respective controls. Atrial weights of AHR at day 6 and day 28 were not significantly different from their respective time-matched SNR. However, the atria from day 28 rats weighed significantly more than the atria from day 6 rats. On the other hand, the ventricles from hypertensive rats weighed more than those of the control at both stages of hypertension. Therefore, when a comparison was made based on organ weight per g of body weight, the hypertensive rat had greater ratios of atria/body weight and ventricle/body weight indicating that the hypertensive rat at both stages of hypertension had developed an hypertrophied heart.

Chronotropic responses to noradrenaline and isoprenaline

Figure 1 shows the chronotropic responses of right atrial preparations to stimulation by NA and isoprenaline in hypertensive rats and their respective time-matched normotensive controls at 6 and 28 days after coarctation. Atrial reactivity to NA and isoprenaline was the same in AHR and SNR at day 6 and day 28 after coarctation except for a smaller chronotropic response to NA at 3×10^{-7} and 10^{-6} M in atria of AHR at day 6. The latter effect may be due to experimental error since it was not observed at lower or higher NA concentrations. Neither were any differences observed when day 6 AHR and SNR were compared with day 28 animals. Pretreatment with phentolamine (1×10^{-6} M) for 5 min had no effect on either NA or isoprenaline-induced responses (not shown in the figure).

Table 1 Characteristics of hypertensive and normotensive rats at different stages of hypertension after aortic coarctation

Characteristics	*6 Days post-coarctation*		*28 Days post-coarctation*	
	Normotensive	*Hypertensive*	*Normotensive*	*Hypertensive*
MABP (mmHg)	118.60 ± 2.68 (5)	166.40 ±4.39****(5)	107.80 ± 6.15 (4)	175.20 ± 7.60**(5)
Body weight (g)	287.00 ±12.90 (5)	238.00 ±0.43**(5)	442.50 ±12.65 (4)	373.00 ±16.63****(5)
Atrial weight (g)	0.067± 0.004(5)	0.077±0.006(5)	0.092± 0.004(4)[a]	0.104± 0.006(5)[b]
Ventricular weight (g)	0.704± 0.040(5)	0.846±0.028*(5)	0.968± 0.039(4)	1.130± 0.041*(5)
Atria/Body weight ($g/g \times 10^3$)	0.236± 0.016(5)	0.320±0.016***(5)	0.209± 0.006(4)	0.280± 0.008****(5)
Ventricle/Body weight ($g/g \times 10^3$)	2.450± 0.042(5)	3.572±0.015****(5)	2.190± 0.080(4)	3.047± 0.145***(5)

MABP: mean arterial blood pressure which was calculated from the equation of diastolic pressure + 1/3 of pulse pressure. Values are means ± s.e.mean for the number of animals in parentheses. *$P<0.05$; **$P<0.02$; ***$P<0.01$; ****$P<0.001$, indicating significant differences from their respective, time-matched, sham-operated normotensive controls. [a]Significantly different from normotensive rats 6 days post-coarctation ($P<0.01$). [b]Significantly different from hypertensive rats 6 days post-coarctation ($P<0.02$).

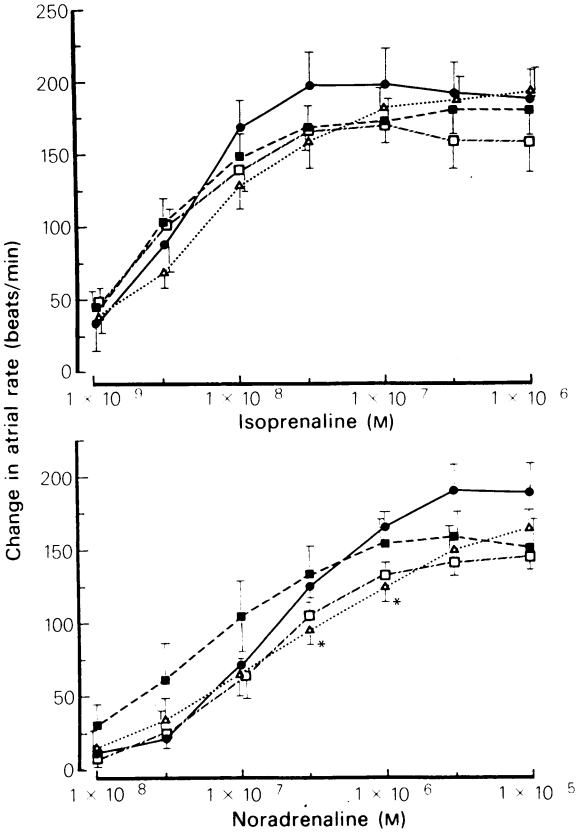

Figure 1 Effects of isoprenaline and noradrenaline on the right atrial rate of normotensive and hypertensive rats at 6 and 28 days after surgery: (●) normotensive and (△) hypertensive on day 6; (■) normotensive and (□) hypertensive on day 28. Each point represents the mean result from 4 to 5 right atria; vertical lines show s.e.mean.
*Significantly different from day 6 sham-operated normotensive rats ($P<0.05$).

Inotropic responses to noradrenaline and isoprenaline

Table 2 summarizes the data observed for the tension developed in left atrial preparations to stimulation by NA and isoprenaline at early and chronic stages of hypertension. Due to the heavier weights of the atrial tissues from day 28 animals (Table 1), the developed tension was calculated as mg tension/g atrial weight. There were no differences in developed tension of left atria from hypertensive and normotensive rats at either stage of hypertension, nor were any differences observed when a comparison was made between animals at day 6 and day 28 after coarctation. Phentolamine (1×10^{-6} M) pretreatment had no effect on the observed responses (not shown in the table).

β-Adrenoceptor binding of cardiac membranes

Myocardial membranes prepared from hypertensive rats at 6 and 28 days after the ligation of abdominal aorta and their respective time-matched, sham-operated controls were analysed for (−)-[^{3}H]-dihydroalprenolol binding sites (Table 3). The number of β-adrenoceptors in cardiac membranes of hypertensive rats in either stage of hypertension was not different from their respective controls. No changes in receptor affinity were observed between hypertensive and normotensive rats in either stage of hypertension. However, as the animals grew older, the number of β-adrenoceptors in the myocardial membrane decreased and the affinity of these receptors increased in both hypertensive and normotensive rats compared to these parameters in younger rats.

Discussion

In the present study, the right atrial rate, in response to stimulation by NA and isoprenaline, of AHR in the early and chronic stages of hypertension were similar to time-matched SNR. Although the resultant hypertension significantly increased the ratios of atria/body weight and ventricle/body weight (Table 1), indicating the development of cardiac hypertrophy, left atrial developed tension (mg/g tissue weight) in response to NA and isoprenaline in hypertensive rats at both stages of hypertension was equivalent to that of their respective controls (Table 2). In addition, no differences in atrial reactivity to NA or isoprenaline were observed between atria obtained from AHR 6 and 28 days after coarctation. Similarly, no differences in atrial reactivity to these agonists were observed between atria obtained from SNR 6 and 28 days after surgery. In those experiments in which cardiac α-adrenoceptors (Nakashima & Hagino, 1972) were blocked by phentolamine, there were no differences in the atrial inotropic or chronotropic responses to the agonists, NA and isoprenaline, suggesting that the responses were solely mediated by β-adrenoceptors.

Our present study demonstrates that the density of cardiac β-adrenoceptors and their affinities in coarctation hypertensive rats is similar to that of their sham-operated, normotensive controls. Alteration of cardiac β-adrenoceptors and receptor affinity has been reported in various models of hypertension. A reduction in the number of cardiac β-adrenoceptors with no changes in their affinities was reported in DOCA-salt hypertension, renal hypertension (Woodcock, Funder & Johnston, 1979) and genetic hypertension (SHR) (Limas & Limas, 1978). However, Giachetti, Clark & Berti (1979) found that

Table 2 Effects of isoprenaline and noradrenaline on rat left atrial contractile force at different stages of hypertension after aortic coarctation

Isoprenaline concentration (M)	*Day-6 normotensive*	*Day-28 normotensive*	*Day-6 hypertensive*	*Day-28 hypertensive*
1×10^{-9}	425.6±145.3(5)	587.6±245.0(4)	356.0± 95.3(5)	479.1±141.3(5)
3×10^{-9}	865.6±292.5(5)	1131.1±289.0(4)	923.5±342.6(5)	993.0±206.6(5)
1×10^{-8}	2123.5±309.0(5)	2373.0±309.0(4)	2308.3±647.1(5)	2560.1±520.5(5)
3×10^{-8}	2144.8±304.9(5)	3506.3±137.0(4)	3304.2±925.3(5)	3525.3±533.5(5)
1×10^{-7}	2830.4±345.7(5)	4044.5±383.5(4)	3671.4±895.8(5)	4079.5±522.8(5)
3×10^{-7}	3391.2±355.5(5)	3979.4±437.5(4)	3472.2±792.4(5)	4301.1±693.2(5)
1×10^{-6}	3380.9±262.1(5)	3674.0±289.0(4)	3351.8±782.2(5)	3968.3±408.7(5)
Noradrenaline concentration (M)				
1×10^{-8}	2.8± 66.2(5)	174.1± 66.0(5)	175.9± 94.4(5)	239.3±128.3(5)
3×10^{-8}	172.7± 24.5(5)	475.2±126.0(5)	296.4±102.4(5)	420.0±143.6(5)
1×10^{-7}	790.0±174.8(5)	1121.3±315.5(5)	898.9±153.4(5)	1144.1±292.4(5)
3×10^{-7}	1244.1±189.1(5)	2243.8±541.0(5)	1927.3±502.7(5)	2343.3±502.7(5)
1×10^{-6}	2963.7±426.6(5)	4037.9±160.5(5)	3171.2±856.0(5)	3394.1±282.6(5)
3×10^{-6}	3330.2±508.0(5)	4863.6±408.0(5)	3812.2±863.6(5)	4170.1±334.1(5)
1×10^{-5}	3976.9±604.6(5)	4961.8±439.0(5)	4059.4±860.4(5)	4393.3±330.0(5)

Values are means ± s.e.mean for the number of tissues indicated in parentheses.
The unit of the values is mg tension/g tissue weight.

cardiac membranes isolated from renal hypertensive rats contained the same number of β-adrenoceptors as normotensive rats but the receptors showed reduced binding affinity for the radioligand. The reason for the discrepancy in the results on receptor characteristics of cardiac membranes of renal hypertensive rats in unknown, although the radioligand used in the studies was different. Our finding of a similar density of cardiac β-adrenoceptors with no change in their affinities in both normotensive and aorta-coarcted hypertensive rats is in contrast to the data obtained by other investigators in other rat models of hypertension. However, the present results suggest that the density of cardiac β-adrenoceptors and/or their binding affinity are not necessarily altered by the induction of hypertension or the duration of hypertension.

The possible mechanism behind the reduction in the number of β-adrenoceptors in DOCA-salt, renal and genetic hypertension has been attributed to either elevated plasma NA levels or to an increased cardiac sympathetic drive (Woodcock, Funder & Johnston, 1978). Giachetti *et al.* (1979) attributed the reduced receptor affinity to functionally hyperactive cardiac nerves of hypertensive rats resulting in a sustained high concentration of NA in the neuroeffector junction, leading to desensitization of the cardiac β-adrenoceptors. We have measured plasma NA levels in AHR 6 and 42 days after surgery and found

Table 3 (−)-[^{3}H]-dihydroalprenolol binding to cardiac membrane from hypertensive and normotensive rats at different states of hypertension

	6 Days post-coarctation		*28 Days post-coarctation*	
	Normotensive	*Hypertensive*	*Normotensive*	*Hypertensive*
MABP	119.50±3.06(4)	166.50±8.02(5)**	120.00±2.44(6)	183.23±3.33(6)***
Number of binding sites (fmol/mg protein)	41.46±1.62(4)	39.21±3.25(5)	25.45±1.12(6)***	29.55±1.95(6)*
K_d (nM)	8.10±1.21(4)	8.23±0.51(5)	1.76±0.11(6)[a]	2.42±0.41(6)[a]

MABP: mean arterial blood pressure which was calculated from the equation of diastolic pressure + 1/3 of pulse pressure.
Values are means ± s.e.mean for the number of animals in parentheses: * $P<0.05$ – significantly different from day 6 aorta-coarcted hypertensive rats; ** $P<0.01$ – significantly different from day 6 time-matched, sham-operated normotensive rats; *** $P<0.001$ – significantly different from day 28 time-matched, sham-operated normotensive rats. *** $P<0.001$ – significantly different from day 6 sham-operated normotensive rats.
[a] Significantly different from both hypertensive and normotensive rats at 6 days after coarctation ($P<0.001$)

no significant differences in plasma NA in hypertensive rats and their respective time-matched, sham-operated controls (Goldstein, Herzlinger, Lai & Cervoni, 1981). Thus, similar plasma NA between AHR and their time-matched normotensive controls could provide a partial explanation for the same density of β-adrenoceptors in these two groups of animals. However, the reason for the lack of change in the binding affinity in the hypertensive rat is unknown at the present time.

The age-dependency of cardiac β-adrenoceptor number and affinity are shown in Table 3. Both hypertensive and normotensive rats 28 days after surgery had fewer cardiac β-adrenoceptor binding sites compared to those rats used 6 days after surgery. However, the binding affinity for the radioligand in the normotensive and hypertensive groups of rats 28 days after surgery was greater than in the 6 day rats. The physiological significance of a diminished number of β-adrenoceptors with increased affinity in the myocardial membrane might be a compensatory mechanism for older animals to maintain normal cardiac function. This hypothesis is supported by the observation of the same degree of atrial response to the agonists between the day 6 and day 28 rats. A decline in the density of β-adrenoceptors in brain tissues of aged rats has been reported (Greenberg & Weiss, 1978). However, the affinity of these receptors did not change with age, suggesting that the properties of central and peripheral β-adrenoceptors may be different.

If one assumes that the number of receptors and/or receptor binding affinities are the limiting factors in determining the maximum response to agonists, then the observation of equivalent cardiac reactivity to NA and isoprenaline between AHR and SNR in both the early and chronic stages of hypertension reported above corresponds well with the density and binding affinity of β-adrenoceptors. Limas & Limas (1978) proposed that the decline of cardiac β-adrenoceptor number in SHR might be responsible for the observed decreased atrial reactivity. The present observation in cardiac tissue of AHR is in contrast to the findings in vascular aortic strips where the maximum developed tension and the relaxant ability to these agonists were impaired in the early stage of hypertension and as hypertension progressed, the tissue regained its ability to contract and relax fully in the chronic stage of hypertension (Lai *et al.*, 1980). The data on cardiac and vascular reactivity of coarctation hypertensive rats suggest that functional changes associated with hypertension are organ-specific and stage-dependent and that a systematic comparison of various parts of the cardiovascular system is important in attempting to evaluate changes in function in hypertension.

In summary, there were no differences in inotropy or chronotropy of atria from AHR and SNR in response to NA and isoprenaline. The duration of hypertension did not affect atrial responsiveness. These physiological results are in agreement with the findings that cardiac membranes from hypertensive rats contained the same number of β-adrenoceptors with equal binding affinity to those from normotensive rats.

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