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Hemodynamic effects of atrial natriuretic peptide in ischemia-reperfusion injury that occurs after exercise

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Background/aim: Atrial natriuretic peptide (ANP) is known as a protective agent against ischemia-reperfusion injury for cardiomyocytes. We compared the hemodynamic effects of ANP and isatin, which is known as an ANP receptor blocker, in ischemia followed by reperfusion in exercised rat hearts with nonexercised ones.

Materials and methods: Isolated hearts were perfused in 4 exercised (E) groups after a running protocol for 5 days and 4 nonexercised (NE) groups. In the first protocol, ANP was added to the perfusion solution before ischemia in an E and NE group. In the second protocol, different doses of isatin (0.1, 10, 100 μM/L) were added to the perfusion solution before ANP in 3 E and 3 NE groups. Left ventricular developed pressure (LVDP) and maximum and minimum rates of change in left ventricular pressure (dP/dtmax and dP/dtmin) were recorded.

Results: Higher LVDP and dP/dtmin values were observed in the E group than the NE group following addition of ANP before ischemia. Values of dP/dtmax were higher in the E group at the first minute of reperfusion period. Hemodynamic difference was not observed between groups given the same amount of isatin before ANP.

Conclusion: This study indicated that higher ANP concentrations before ischemia were more effective on the left ventricle contractility and relaxation functions in the hearts that were exposed to exercise.

Key words: Atrial natriuretic peptide, ischemia, exercise, cardiac contractility, isatin

1. Introduction

Atrial natriuretic peptide (ANP) is known as a cardiac endocrine hormone that is essential in regulating blood volume through its natriuretic, diuretic, and vasodilator effects. ANP is primarily synthesized and secreted by atrial cardiomyocytes (1). It was demonstrated that an acute mechanical stretch of atrial wall was the most important stimulus for synthesis and secretion of this hormone (2). Exercise is known as a factor that increases mechanical stretch and pressure of the atrium. Additionally, increased plasma ANP concentrations have been observed following exercise in healthy subjects (3–5).

Known as a cardioprotective agent, ANP is reported to be potentially effective on acute myocardial infarction (6). ANP generates inhibitor effects in ischemia-reperfusion injury at the myocardium through the cyclic guanosine monophosphate pathway (cGMP) (7). The ANP receptor/cGMP signaling pathway activates protein kinase G, and thus mitochondrial K-ATP channels open and infarct sizes in rabbit hearts are reduced (8). It has been demonstrated that intravenous administration of ANP ameliorates left ventricle functions in patients with acute myocardial infarction (9). Improved left ventricular function has been reported when ANP is administered during the reperfusion period in isolated perfused rat hearts (10). In addition, it has been shown that administration of ANP in the reperfusion period restricts infarct size in rabbit hearts (8). However, it remains unclear whether there are any differences in cardiac hemodynamics when the heart is exposed to the effect of both exercise and higher ANP concentrations before ischemia.

We hypothesized in this study that ANP administration before ischemia could improve left ventricle contractility function in rat hearts that were exposed to exercise

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compared with hearts that were not exposed to exercise. Thus, the aim of this study was to compare the hemodynamic effects of ANP and isatin, which is known as an ANP receptor blocker, in ischemia followed by reperfusion in exercised rat hearts with nonexercised hearts.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (n = 47; weighing 300–450 g and 4–6 months old) were used in this study. All animals were housed with free access to a standard diet and tap water, with a 12/12-h light/dark cycle, a mean temperature of 21 ± 2 °C, and humidity of 55 ± 2%. Experimental protocols were reviewed and approved by the local ethics committee of Trakya University and the Animal Care and Use Committee of the Trakya University Medical Faculty.

Animals were divided into exercised (E1, E2, E3, E4) and nonexercised (NE1, NE2, NE3, NE4) groups. Global ischemia (35 min) and reperfusion (30 min) were applied in all groups (Figure 1). Two separate protocols were used in exercised and nonexercised groups. In protocol A, ischemia and reperfusion were applied and ANP (0.1 μM/L) was added to the perfusion solution for 15 min before ischemia in an exercise (E1) group (n = 6) and a nonexercise (NE1) group (n = 6). ANP concentration was applied as 0.1 μM/L, since improved cardiac output had been reported when this concentration in reperfusion solution was administered in a previous study (10). After perfusion of the heart with Krebs–Henseleit solution for 15 min, basal hemodynamic measurements (K) were obtained. Measurements were repeated following perfusion of the heart with Krebs–Henseleit solution including ANP at the 5th minute (ANP1) and 15th minute (ANP2), and at the 1st (R1), 15th (R2), and 30th (R3) minute of the reperfusion period (Figure 1). As hemodynamic measurements, left ventricular developed pressure (LVDP), maximum rates of change in left ventricular pressure (dP/dt_{max}), minimum rates of change in left ventricular pressure (dP/dt_{min}), and heart rate (HR) were continuously monitored in this study. Therefore, the hemodynamic effects of ANP before ischemia in exercised and nonexercised hearts were compared. Protocol B was conducted in the second part of the study. Before the administration of ANP, different doses of isatin (0.1, 10, 100 μM/L) were given in the perfusion solution of 3 exercised groups (E2, E3, E4; n = 6 in each group) and 3 nonexercised groups (NE2, NE3, NE4; n = 6, n = 6, and n = 5, respectively). Thus, we aimed to find out whether there were any differences among the hemodynamic effects of ANP when applying low, modest, or high doses of isatin before ANP. Hemodynamic values after perfusion of K measurements following the perfusion with Krebs–Henseleit solution including different doses of isatin (I), ANP1, ANP2, R1, R2, and R3 measurements, were recorded (Figure 1).

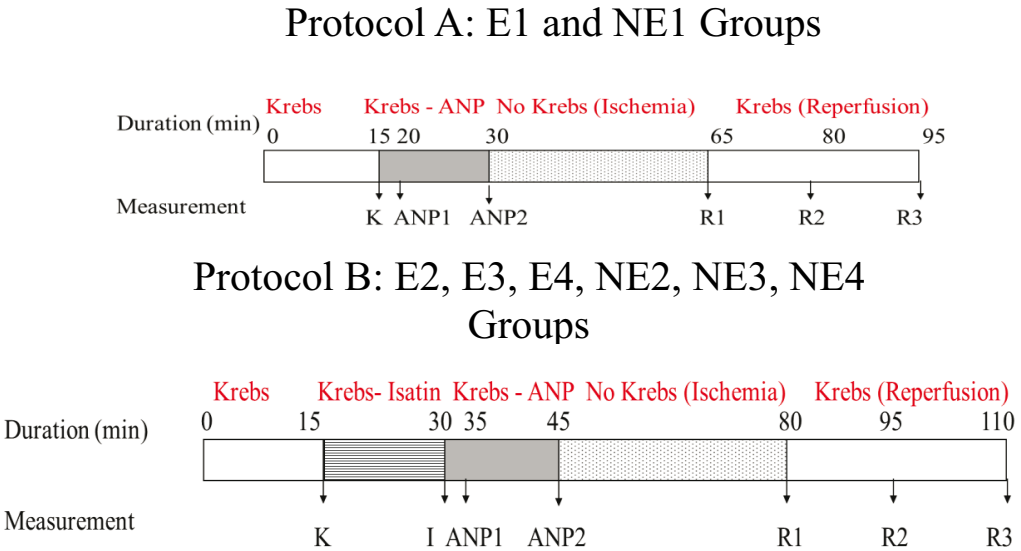

Figure 1. Study groups are shown. E1 = Exercised group, NE1 = nonexercised group, E2 = exercised group with 0.1 μM/L isatin administered before ANP administration, E3 = exercised group with 10 μM/L isatin administered before ANP administration, E4 = exercised group with 100 μM/L isatin administered before ANP administration, NE2 = nonexercised group with 0.1 μM/L isatin administered before ANP administration, NE3 = nonexercised group with 10 μM/L isatin administered before ANP administration, NE4 = nonexercised group with 100 μM/L isatin administered before ANP administration. K, baseline; ANP1, 5 min of atrial natriuretic peptide infusion; ANP2, 15 min of atrial natriuretic peptide infusion; R1, 1 min of reperfusion; R2, 15 min of reperfusion; R3, 30 min of reperfusion; I, measurement made after 1 min of isatin infusion.

2.2. Exercise

A standard exercise protocol was applied in the exercised rats similar to another study (11). Exercise groups were exposed to a running protocol for 5 days according to this protocol. Animals were exercised during the first 4 consecutive days on a treadmill. The duration of daily exercise was 15 min at a speed of 15 m/min in the first 4 days. This period was accepted as moderate exercise and also as a training period to prepare the animals for the last day of vigorous exercise. The vigorous exercise of the 5th day lasted 25 min at 25 m/min (11). At the end of the vigorous exercise period, the animals were killed, their hearts were harvested, and isolated hearts were perfused within 1 h after the last bout of exercise.

2.3. Preparation of isolated heart

After the last bout of exercise, the rats were anesthetized with thiopental (100 mg/kg, I.E. Ulagay A.Ş., İstanbul, Turkey). Heparin was injected (500 IU/kg; Nevparin Flakon, Mustafa Nevzat İlaç Sanayi A.Ş., İstanbul, Turkey) prior to anesthesia. Both of the drugs were administered intraperitoneally. After onset of anesthesia, the hearts were rapidly excised, immersed into ice-cold Krebs–Henseleit solution, and attached to the Langendorff apparatus. The hearts were cannulated from the aorta and retrogradely perfused with oxygenated Krebs–Henseleit solution containing NaCl at 118.3 mmol L^{-1}, $NaHCO_3$ 25.0 mmol L^{-1}, KCl 4.7 mmol L^{-1}, KH_2PO_4 1.2 mmol L^{-1}, $MgSO_4$ 1.2 mmol L^{-1}, $CaCl_2$ 2.5 mmol L^{-1}, and glucose 11.1 mmol L^{-1}. The perfusate was equilibrated with 95% O_2 and 5% CO_2, maintained at 37 °C and pH 7.4 for 15 min in each group at a constant pressure of 65–70 mmHg. A water-filled latex balloon was placed in the left ventricle through the mitral valve. LVDP, dP/dt_{max}, dP/dt_{min}, and HR were continuously monitored by a data acquisition system (BIOPAC MP36 System, Inc., USA) during the experiments.

Catheterization to the left ventricle was applied to obtain LVDP, dP/dt_{max}, and dP/dt_{min} levels in this study. LVDP was estimated by subtracting the end-diastolic pressure from the systolic pressure. As the components to generate dP/dt, P shows LV pressure, t shows time, and d shows rate of change. It is accepted that dP/dt_{max} gives valuable knowledge about the inotropic features of the left ventricle; therefore, it is also called the inotropic index (12).

2.4. Statistical analysis

Statistical analyses were carried out using SPSS 20.0 (License No. 10240642). All values were expressed as median (interquartile range). Hemodynamic variables and intragroup changes of hemodynamic variables were compared by Mann–Whitney U test in 2 groups. $P < 0.05$ was considered statistically significant.

3. Results

For protocol A, when the measurements obtained for K, ANP1, ANP2, R1, R2, and R3 were compared between the E1 and NE1 groups, a significant difference was observed in ANP2 for LVDP values ($P = 0.045$, Table 1). Higher LVDP was shown in the E1 group. Values of dP/dt_{max} were compared in the E1 and NE1 groups and higher dP/dt_{max} levels were found in R1 of the E1 group ($P = 0.028$). When dP/dt_{min} values in the two groups were compared, significantly different values were shown in ANP1 and ANP2 between the E1 and NE1 groups (respectively $P = 0.045$ and $P = 0.045$). dP/dt_{min} values in the E1 group were found to be more negative, which showed higher relaxation, than in the NE1 group (Table 1). When the alterations of heart rate data in the E1 and NE1 groups were compared, no difference was found in heart rate values between E1 and NE1 groups (Table 2).

Changes from K to ANP1, ANP2, R1, R2, and R3 for hemodynamic measurements were also compared between the E1 and NE1 groups. It was found that changes in LVDP from K to ANP2 in the E1 group were significantly higher than in the NE1 group ($P = 0.045$, Figure 2). No difference was found when changes from K to ANP1, ANP2, R1, R2, and R3 for dP/dt_{max} values were compared between the E1 and NE1 groups. It was seen that changes from K to ANP1 and ANP2 for dP/dt_{min} values in the E1 group were significantly decreased while they were significantly increased in the NE1 group (Table 3).

For protocol B, hemodynamic effects of the 3 different doses of isatin that were administered before ANP in exercised and nonexercised isolated hearts were investigated. When the LVDP, dP/dt_{max}, and dP/dt_{min} values were compared between the E2 and NE2 groups, which were administered 0.1 μM/L isatin, they were not found to be significantly different in K, I, ANP-1, ANP-2, R1, R2, and R3 measurements (Table 4).

When the values were compared for LVDP, dP/dt_{max}, and dP/dt_{min} between the E3 and NE3 groups, no significant difference was seen in hemodynamic measurements. However, when compared for the E4 and NE4 groups, higher LVDP values were shown in R3 of the NE4 group ($P = 0.014$). Values of dP/dt_{max} and dP/dt_{min} between the E4 and NE4 groups were not significantly different. Heart rate values did not differ between the E2 and NE2 groups, the E3 and NE3 groups, and the E4 and NE4 groups.

Changes from K to I, ANP1, ANP2, R1, R2, and R3 for hemodynamic values were compared between the E2 and NE2, the E3 and NE3, and the E4 and NE4 groups. No difference was seen when the changes were compared for K to I, ANP1, ANP2, R1, R2, and R3 for LVDP, dP/dt_{max}, and dP/dt_{min} values between the E2 and NE2 groups. Similarly, hemodynamic values between the E3 and NE3 groups and the E4 and NE4 groups were not found to be significantly different ($P > 0.05$ for all comparisons).

Table 1. Hemodynamic data of E1 and NE1 groups. Values are expressed as median (interquartile). E1 = Exercised group, NE1 = nonexercised group, K = baseline measurement, ANP1 = measurement made after 5 min of atrial natriuretic peptide administration, ANP2 = measurement made after 15 min of atrial natriuretic peptide administration, R1 = measurement made after 1 min of reperfusion period, R2 = measurement made after 15 min of reperfusion period, R3 = measurement made after 30 min of reperfusion period, LVDP = left ventricular developed pressure, dP/dt_{max} = maximum rate of change in left ventricular pressure, dP/dt_{min} = minimum rate of change in left ventricular pressure.

		E1 (n = 6)	NE1 (n = 6)	P
K	LVDP	69 (34)	68 (21)	0.465
	dP/dt_{max}	1213 (1265)	1250 (551)	0.584
	dP/dt_{min}	−1247 (826)	−1211 (182)	0.584
ANP1	LVDP	83 (54)	62 (25)	0.100
	dP/dt_{max}	1111 (2314)	1012 (914)	0.584
	dP/dt_{min}	−1462 (965)	−1074 (251)	0.045
ANP2	LVDP	84 (73)	52 (46)	0.045
	dP/dt_{max}	1499 (1722)	1068 (1531)	0.144
	dP/dt_{min}	−1356 (1119)	−904 (575)	0.045
R1	LVDP	83 (104)	1.7 (22)	0.273
	dP/dt_{max}	109 (1181)	−452 (409)	0.028
	dP/dt_{min}	−334 (840)	−595 (395)	0.465
R2	LVDP	0.6 (41)	0.2 (12)	0.647
	dP/dt_{max}	34 (896)	−346 (263)	0.100
	dP/dt_{min}	−153 (843)	−443 (241)	0.144
R3	LVDP	2 (40)	0.2 (18)	0.917
	dP/dt_{max}	−52 (1228)	−150 (358)	0.327
	dP/dt_{min}	−236 (776)	−397 (561)	0.602

Table 2. Heart rate values of E1 and NE1 groups. Values are expressed as median (interquartile). E1 = Exercised group, NE1 = nonexercised group, K = baseline measurement, ANP1 = measurement made after 5 min of atrial natriuretic peptide administration, ANP2 = measurement made after 15 min of atrial natriuretic peptide administration, R1 = measurement made after 1 min of reperfusion period, R2 = measurement made after 15 min of reperfusion period, R3 = measurement made after 30 min of reperfusion period.

	E1 (n = 6)	NE1 (n = 6)	P
K	236 (48)	249 (36)	0.927
ANP1	222 (58)	220 (84)	0.465
ANP2	211 (50)	222 (71)	0.584
R1	166 (159)	273 (195)	0.144
R2	248 (165)	225 (85)	0.584
R3	366 (179)	286 (129)	0.347

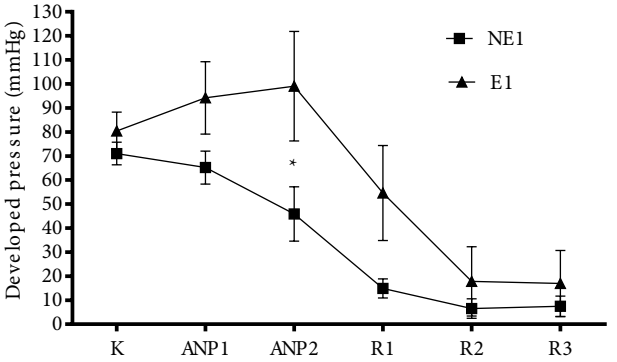

Figure 2. Left ventricular developed pressures in NE1 and E1 groups. *: P < 0.05 when differences of left ventricular developed pressure changes from K to ANP2 are compared between NE1 and E1 groups. E1 = Exercised group, NE1 = nonexercised group, K = baseline measurement, ANP1 = measurement made after 5 min of atrial natriuretic peptide administration, ANP2 = measurement made after 15 min of atrial natriuretic peptide administration, R1 = measurement made after 1 min of reperfusion period, R2 = measurement made after 15 min of reperfusion period, R3 = measurement made after 30 min of reperfusion period.

Table 3. Changes from K to ANP1, ANP2, R1, R2 and R3 of dP/dt_{max} and dP/dt_{max} measurements in E1 and NE1 groups. Values are expressed as median (interquartile). E1 = Exercised group, NE1 = nonexercised group, $\Delta_{K\text{-}ANP1}$ = changes from K to ANP1, $\Delta_{K\text{-}ANP2}$ = changes from K to ANP2, $\Delta_{K\text{-}R1}$ = changes from K to R1, $\Delta_{K\text{-}R2}$ = changes from K to R2, $\Delta_{K\text{-}R3}$ = changes from K to R3.

		E1 (n = 6)	NE1 (n = 6)	P
dP/dt_{max}	$\Delta_{K\text{-}ANP1}$	−79 (−1461)	15 (−724)	0.361
	$\Delta_{K\text{-}ANP2}$	−286 (−804)	128 (−1599)	0.100
	$\Delta_{K\text{-}R1}$	946 (−2293)	1875 (−654)	0.361
	$\Delta_{K\text{-}R2}$	989 (−1697)	1684 (−539)	0.584
	$\Delta_{K\text{-}R3}$	951 (−1360)	1618 (−670)	0.221
dP/dt_{min}	$\Delta_{K\text{-}ANP1}$	215 (−966)	−172 (−251)	0.045
	$\Delta_{K\text{-}ANP2}$	109 (−1119)	−343 (−575)	0.045
	$\Delta_{K\text{-}R1}$	−912 (−840)	−651 (−395)	0.465
	$\Delta_{K\text{-}R2}$	−1093 (−734)	−803 (−240)	0.144
	$\Delta_{K\text{-}R3}$	−1011 (−776)	−849 (−561)	0.602

The body weights of exercised rats (n = 18) were compared before and after exercise in this study. A significant decrease was observed in the body weight after exercise (347 (75) g) when compared before exercise (351 (64) g) in this running model (P = 0.001).

4. Discussion

The results of this study demonstrated that LVDP, which shows the contractility function of the left ventricle, was higher after the addition of ANP into the perfusion solution in isolated hearts that were exposed to exercise compared with the hearts not exposed to exercise. Higher ANP concentrations before global ischemia caused more effective alterations in dP/dt_{min} values, which represent a relaxation function of the left ventricle, in exercised hearts. Additionally, the values of dP/dt_{max} that were measured following global ischemia were found to be higher in exercised hearts than nonexercised hearts in the first minute of the reperfusion period. When the same amount of isatin was administered for blocking of ANP receptors, however, no difference was found in hemodynamic values between groups.

Plasma ANP concentration increases following physical exercise in healthy subjects (3–5). Additionally, higher plasma ANP levels have been observed in athletes compared with healthy controls (13). It was reported in another study that plasma ANP concentrations were found higher in the resting position in archery athletes in comparison to

Table 4. Left ventricular developed pressure, dP/dt_{max}, and dP/dt_{min} values of E2, NE2, E3, NE3, E4, and NE4 groups. Values are expressed as median (interquartile). E2 = Exercises group with 0.1 μM/L isatin administered before ANP administration, E3 = exercised group with 10 μM/L isatin administered before ANP administration, E4 = exercised group with 100 μM/L isatin administered before ANP administration, NE2 = nonexercised group with 0.1 μM/L isatin administered before ANP administration, NE3 = nonexercised group with 10 μM/L isatin administered before ANP administration, NE4 = nonexercised group with 100 μM/L isatin administered before ANP administration, K = baseline measurement, I = measurement made after 1 min of isatin administration, ANP1 = measurement made after 5 min of atrial natriuretic peptide administration, ANP2 = measurement made after 15 min of atrial natriuretic peptide administration, R1 = measurement made after 1 min of reperfusion period, R2 = measurement made after 15 min of reperfusion period, R3 = measurement made after 30 min of reperfusion period, LVDP = left ventricular developed pressure, dP/dt_{max} = maximum rate of change in left ventricular pressure, dP/dt_{min} = minimum rate of change in left ventricular pressure.

		E2 (n = 6)	NE2 (n = 6)	P	E3 (n = 6)	NE3 (n = 6)	P	E4 (n = 6)	NE4 (n = 5)	P
	LVDP	92 (38)	88 (28)	0.873	94 (27)	82 (20)	0.572	85 (43)	85 (57)	0.221
K	dP/dt_{max}	1601 (1321)	1930 (690)	0.715	1991 (1333)	1671 (611)	0.100	1620 (775)	1525 (1625)	0.670
	dP/dt_{min}	−1650 (789)	−1524 (668)	0.273	−1831 (715)	−1330 (1482)	0.078	−1634 (755)	−1187 (632)	0.055
	LVDP	82 (47)	92 (40)	0.470	93 (48)	91 (31)	0.147	83 (45)	62 (104)	0.219
I	dP/dt_{max}	1660 (1645)	2095 (549)	0.175	2209 (2790)	1459 (814)	0.273	1770 (1125)	970 (2118)	0.286
	dP/dt_{min}	−1705 (1209)	−1603 (910)	1.000	−1749 (1098)	−1110 (1258)	0.200	−1667 (609)	−857 (1913)	0.201
	LVDP	82 (59)	81 (37)	0.296	57 (66)	76 (100)	0.575	78 (39)	61 (76)	0.086
ANP1	dP/dt_{max}	1609 (2316)	1725 (407)	0.465	1616 (3127)	1062 (2944)	0.337	1754 (1102)	947 (1992)	0.522
	dP/dt_{min}	−1648 (1598)	−1436 (1078)	0.602	−513 (1705)	−759 (1635)	0.337	−1424 (859)	−808 (1277)	0.201
	LVDP	85 (82)	78 (46)	0.314	40 (72)	53 (94)	0.936	66 (82)	54 (75)	0.624
ANP2	dP/dt_{max}	1425 (2257)	1728 (723)	0.465	954 (2867)	715 (2599)	0.522	1495 (1550)	799 (1994)	0.624
	dP/dt_{min}	−1403 (1424)	−1388 (784)	0.855	−580 (1300)	−740 (1291)	0.262	−883 (1127)	−775 (960)	0.327
	LVDP	16 (18)	7 (25)	0.273	1 (2)	0 (2)	0.748	1 (70)	6 (59)	0.327
R1	dP/dt_{max}	−437 (381)	−187 (887)	0.175	−349 (305)	−366 (1387)	0.749	−198 (794)	−140 (1522)	0.670
	dP/dt_{min}	−668 (444)	−339 (389)	0.201	−389 (335)	−568 (641)	0.522	−396 (921)	−482 (772)	0.831
	LVDP	8 (10)	13 (46)	0.464	1 (3)	0 (3)	0.297	1 (48)	8 (26)	0.176
R2	dP/dt_{max}	−291 (219)	576 (1333)	0.117	−288 (252)	−378 (565)	0.749	−291 (659)	−56 (684)	0.522
	dP/dt_{min}	−484 (381)	−540 (626)	0.584	−442 (390)	−482 (218)	0.749	−345 (679)	−508 (534)	0.670
	LVDP	8 (16)	29 (56)	0.273	1 (5)	1 (9)	0.470	1 (48)	14 (27)	0.014
R3	dP/dt_{max}	−74 (236)	594 (1509)	0.175	−227 (258)	−323 (711)	1.000	−227 (580)	13 (770)	0.394
	dP/dt_{min}	−469 (355)	−765 (936)	0.361	−355 (395)	−417 (167)	0.522	−296 (708)	−569 (567)	0.831

untrained healthy controls (14). It was shown that an increase in plasma ANP concentrations in healthy subjects may eliminate intermittent overhydration and may participate in the regulation of diuresis and natriuresis resulting from postural changes (6). On the other hand, studies demonstrated that plasma ANP concentration increases following myocardial diseases (7). Elevated natriuretic peptides may cause antiischemic effects on the heart (8). It was shown in an isolated heart study that preischemic infusion of a synthetic alpha-human ANP agent exerted cardioprotective effects through mitochondrial K-ATP channel activation (9). Additionally, increase in plasma ANP concentration can play a role in improving left ventricle contractility function via its autocrine/paracrine actions (10), or in affecting cardiocirculatory hemodynamics, including endocrine and renal functions, lipid metabolism, and the pump function of the heart (15). It was shown in a past study that, when administered to male subjects, ANP improved myocardial diastolic function and increased myocardial relaxation depending on the dose used (16). Therefore, an ischemic model was generated in the present study to compare the hemodynamic effects of ANP, which

was applied before ischemia. It was seen in this study that no hemodynamic difference was shown in the exercised (E1) and nonexercised (E2) groups before administration of ANP. However, differences were observed in LVDP, dP/dt_{min}, and dP/dt_{max} values after administration of ANP. These findings demonstrated that higher concentrations of ANP before ischemia may have beneficial effects on the contractility and relaxation function of hearts exposed to exercise.

When considering the effects of ANP on the reperfusion period, significant increases in dP/dt_{max} values were found in the exercised group only in the first minute of reperfusion. Hemodynamic alterations for LVDP or dP/dt_{min} values following ANP administration were not observed in the reperfusion period. This finding may result from the fact that administration of ANP in reperfusion solution did not proceed during the reperfusion period.

The supply of oxygenated perfusion fluid to the myocardium was suspended for 35 min in this global ischemic model. This resulted in obvious hemodynamic changes such as negative dP/dt_{max} values in the reperfusion period in the present study. It has been demonstrated that myocardial ischemia could induce a series of cellular signals, including an increase in Ca^{+2}, intracellular acidosis, accumulation of inorganic phosphates, accumulation of lactate, and stimulation of glycolytic flux (3–5). Inorganic phosphates have been suggested as an important reason for the contractile failure in myocardial ischemia (17). The total ATP content of myocardial tissue drops by 51% and ATP concentration in the cytosol decreases by 36% at 30 min of ischemia (6). The present study demonstrated that the harmful effects of long-term global ischemia on left ventricle contractility were too great to be prevented by the possible antiischemic effects arising from exercise. When considering the different levels of ischemic conditions in humans, the possible effects of ANP in low-flow ischemia, rather than global ischemia, could be investigated in further studies.

Short-term exercise training can produce some changes in factors affecting cell function in skeletal or cardiac muscles. It was shown that short-term exercise induces capillary angiogenesis and muscle hypertrophy in skeletal muscle. Additionally, remarkable effects on contractility function of the heart muscle have been observed. Vascular endothelial growth factor, transforming growth factor, and basic fibroblast growth factor have been found to have increased after 5 days of treadmill exercise in the skeletal muscles of rats (18). Another study demonstrated that even a single bout of exercise induced increases in mRNA levels of some angiogenic growth factors, including vascular endothelial growth factor, basic fibroblast growth factor, and transforming growth factor-beta 1 in skeletal muscle. Looking at the cardiac muscles, it has been indicated that 3 or 5 consecutive days of treadmill exercise improves myocardial contractility via increased myocardial left ventricular heat shock protein 72, glutathione, and manganese superoxide dismutase activities during ischemia and reperfusion (19). Increasing Ca^{+2} sensitivity has also been shown as a part of the mechanism involved in training-induced improvements in the contractility function of the myocardium (20). Coronary vascular resistance has been accepted as the other remarkable factor that regulates cardiac responses to exercise (21). Five consecutive days of an exercise training program that is known as short-term and moderate exercise were applied in this study. The duration time was accepted as adequate to observe the changes in the contractility of the left ventricle in rats. However, no difference was observed in basal hemodynamic measurements between the 2 groups when these duration and intensity levels of exercise were applied.

Considerable difference in dP/dt_{min} values of ANP-1 and ANP-2 were shown in the E1 and NE1 groups in the present study after the addition of ANP to the perfusion solution. The rate of ventricular relaxation was shown as dP/dt_{min}, which is a functional parameter seen in the early phase of diastole (12). Ventricular relaxation is affected by some cellular factors, including phosphorylation of phospholamban for the uptake of calcium into the sarcoplasmic reticulum and viscoelastic features of myocardium. The results of this study revealed that ANP may be effective in exercised hearts in terms of ventricular relaxation in comparison to the nonexercised group.

Isatin is known as an endogenous indole and ANP antagonist (22). The antagonistic effect of isatin leads to the inhibition of guanylate cyclase and, therefore, cGMP is decreased. Isatin has not been reported to result in the alteration of infarct size following ischemia, but it has been demonstrated that isatin could prevent the protective effects of ANP on the heart (23). Interestingly, we did not find any difference in cardiac contractility and relaxation function when different doses of isatin were applied before ANP in exercised and nonexercised hearts. Although isatin has been known to play an antagonist role on natriuretic peptide receptors, no significant change was shown when perfused isatin by itself was administered before ischemia in the present study. Further studies in which ANP is administered together with isatin are needed to understand the role of isatin on ischemia and reperfusion.

This study was unable to determine the infarct size of the rat heart following ischemia. However, it was observed in another study that the infarcted area was approximately 40% of the rat heart following 20 min of global ischemia and 3 h of reperfusion (11). We applied 35 min of global ischemia and 30 min of reperfusion to the isolated rat hearts in this study, similar to other studies (24,25). The duration of ischemia was accepted as sufficient to create a considerable amount of myocardial infarct area.

In summary, this study indicated that increased ANP concentration may cause better contractility and relaxation function of the left ventricle when rast are exposed to short-term and moderate exercise. In the case of exposure to exercise, higher concentrations of ANP before ischemia may generate a remarkable effect of cardiac contractility function in the first minutes of the reperfusion period. However, different doses of isatin did not seem to be effective on hemodynamic parameters in the present study.

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