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Correlation of M235T DNA Polymorphism With Cardiovascular and Endocrine Responses During Physical Exercise in Healthy Subjects

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Summary

In our experiments, we evaluated the possible effect of M235T molecular variant of the angiotensinogen gene on the response to a physical workload. A group of volunteers was composed of healthy male subjects, approximately of the same weight and height, same age and not actively trained. None of these subjects was under any medication. Blood sampling was carried out *via* an indwelling catheter. Besides blood pressure and heart rate, angiotensin I, angiotensin II, epinephrine and norepinephrine concentrations were measured in the blood. Our results suggest that only the response of diastolic blood pressure during submaximal exercise corresponded to the presence of M235T molecular variant. In all other parameters we found no significant correlation of the response with the M235T molecular variant.

Key words
Blood pressure – M235T molecular variant – Physical exercise – Hypertension

Introduction

Angiotensinogen is the precursor of angiotensin II which plays an important role in water and electrolyte balance as well as in the control of blood pressure. The M235T molecular variant of angiotensinogen gene has been shown to be associated with hypertension in several Caucasian populations (Jeunemaitre *et al.* 1992, Caulfield *et al.* 1994, Križanová *et al.* 1997). However, the real impact of M235T angiotensinogen molecular variant on the development of hypertension is difficult to establish, because of the polygenic and multifactorial origin of hypertension. Several laboratories attempted to correlate the M235T polymorphism with a variety of endocrine parameters, which might be affected by this molecular variant. It was found that the common M235T mutation of human angiotensinogen gene was associated with a 10–20 % increase in plasma angiotensinogen level (Cohen *et al.* 1996). Increased angiotensinogen concentrations also significantly correlated with increased blood pressure (Watt *et al.* 1992). In addition, it has been recognized that plasma angiotensinogen levels are close to the Km for cleavage by renin, with the consequence that alteration of angiotensinogen levels also affects the formation of angiotensin II (Lifton 1995).

Neuroendocrine, cardiovascular and metabolic responses to exercise depend on the type of the workload (dynamic or static) and its degree and duration (Christensen and Galbo 1983, Kjaer *et al.* 1987). Two different mechanisms of neuroendocrine activation during exercise were proposed: feed-forward regulation with central nervous stimulation (central command) from activated motor centre in the brain, and feedback regulation with peripheral signals generated from peripheral thermo-, osmo- or baroreceptors, as well as from changes in the concentration of energy sources and their metabolites.

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It was found that submaximal dynamic exercise of short duration stimulated the release of catecholamines and other hormones mainly by a central command mechanism (Kjaer *et al.* 1987). We therefore tried to correlate the activation of the sympatho-adrenal and renin-angiotensin system and the cardiovascular response during submaximal dynamic exercise with the presence of the molecular variant M235T on the angiotensinogen gene, which is associated with the onset of essential hypertension.

Material and Methods

Subjects

Fourteen healthy men 22.0±0.5 years-old, weighing 70.5±2.2 kg and 11 middle-aged men (age 45.4±2.3 years) gave their informed written consent to participate in the study, which was approved by the Ethical Committee of the Institute of Experimental Endocrinology, Slovak Academy of Sciences. None of these probands were under any medication. The investigations started at 07:30 h after an overnight fast. An indwelling catheter was inserted into the cubital vein for blood sampling and the first blood sample was withdrawn 30 min after inserting the catheter. Simultaneously with the blood sampling, blood pressure and heart rate were measured using an automated oscillometric method (DINAMAP).

Physical exercise

Probands performed work on a bicycle ergometer. The exercise test consisted of three periods with workloads increasing from 1.0 to 1.5 and 2.0 W/kg. Each period lasted 6 min with one minute rest in between. Blood sampling, blood pressure and heart rate measurements were carried out up to 60 min after the workload had been terminated.

Preparation of the genomic DNA

Genomic DNA was extracted from 500 μl of the whole blood (in EDTA) by the phenol/chloroform procedure. Briefly, 1 μl of lysing buffer (0.32 mol/l sucrose, 1 % Triton X-100, 5 mmol/l $MgCl_2$ and 12 mmol/l Tris, pH 7.5) were added to 500 μl of peripheral blood (in 0.5 mol/l EDTA pH 8.0). The mixture was vortexed properly and centrifuged at 10 000 x *g* for 3 min. The pellet was washed with 500 μl of redistilled water, centrifuged at 10 000 x *g* for 2 min and the sediment was resuspended in 100 μl of 0.375 mol/l NaCl, 0.12 mol/l EDTA, pH 8.0 with 200 μl H_2O and 2 μl of Proteinase K, mixed thoroughly and 10 μl of 10 % SDS were then added. Afterwards, 100 μl of 5.0 mol/l NaCl and 400 μl phenol (pH 8.0)/chloroform mixture were added, the sample was mixed and centrifuged for 3 min at 10 000 x *g*. To the water phase 96 % ethanol was added in twofold excess. The precipitate was centrifuged at 10 000 x *g* for 3 min and the final DNA sediment was washed with 70 % ethanol and suspended in 200 μl of redistilled water.

Polymerase chain reaction (PCR)

Genotypes for AGT codon 235 were enzymatically amplified in the thermal cycler (Techne UK) with Taq polymerase (Pharmacia Biotech). The primers were designed according to Russ *et al.* (1993) using mismatch primer which, together with the M235T molecular variant creates a restriction site for Tth 111 I. The upstream primer used was 5'-CCGTTTGTGCA GGGCCTGGCTCTCT-3' and the downstream primer 5'-CAGGGTGCTGTCCACACTGGACCCC-3'. PCR products were digested with Tth 111 I restriction endonuclease (Amersham) (5 U per reaction) for four hours at 65 °C and analyzed in 2 % agarose (Pharmacia Biotech) gel.

Determination of the plasma renin activity (PRA) and angiotensin II production

PRA was determined as the amount of angiotensin I cleaved from angiotensinogen by renin. Angiotensin I was estimated by ^{125}I RIA kit for angiotensin I (Institute for Research and Production of Radioisotopes, Prague, Czech Republic) according to manufacturer's protocol. The angiotensin II assay was performed by a RIA kit (Buhlman Laboratories, Switzerland) based on the method of Emanuel *et al.* (1973).

Determination of epinephrine and norepinephrine

Epinephrine and norepinephrine were determined by radioenzymatic assay according to the procedure of Peuler and Johnson (1977).

Results

In our trial, five subjects possessed M/M alleles, six M/T alleles and three were homozygous for the T/T molecular variant. Physical exercise increased blood pressure, heart rate and PRA levels throughout the whole working period (Fig. 1). All parameters analyzed continually increased according to the workload and reached maximum at the end of exercise. The heart rate increased from 62±3 to 165±7 beats/min after the physical workload. Systolic blood pressure rose from 121±3 to 173±7 mm Hg and diastolic blood pressure from 71±2 to 80±5 mm Hg (Table 1). Although the increase in systolic blood pressure did not correlate with the M235T molecular variant and was raised 1.4 times on the average (varying from 1.2 to 1.8 times independently of the presence and/or absence of the M235T molecular variant), some weak correlation with the elevation of diastolic blood pressure appeared (Table 1).

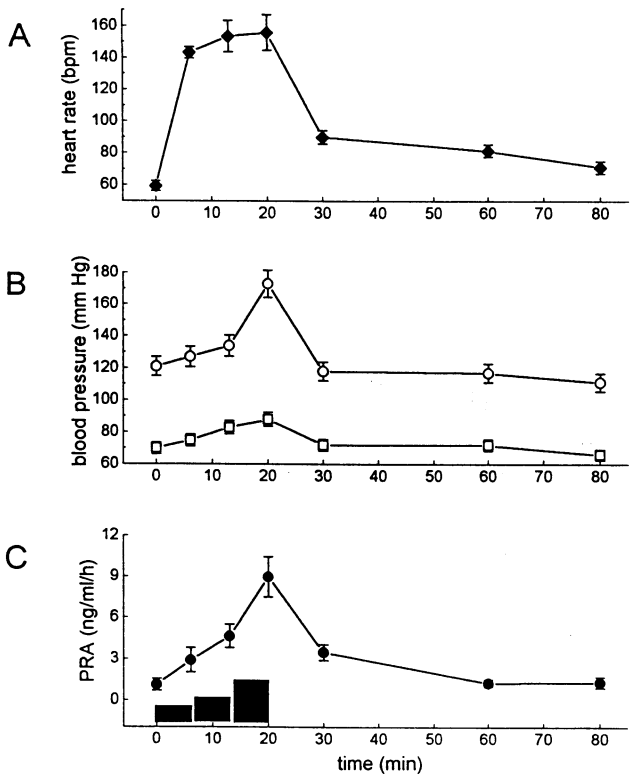

Fig. 1. *The response of the heart rate (A), systolic (open circles) and diastolic (open squares) blood pressure (B) and PRA (C) to the physical workload. Each value represents an average of 14 independent measurements. Filled columns represent three periods of the workload increasing from 1.0 to 1.5 and 2 W/kg. Each period lasted 6 min with one minute rest in between. Values are displayed as mean ± S.E.M.*

The increase in diastolic blood pressure varied according to the allele molecular variant (M/M – 1.32 times, M/T – 1.23 times and T/T – 0.80 times). These results are not conclusive enough, since only three probands exhibited a maintained T/T molecular variant. Therefore, we checked another group of middle-aged probands and focused on the changes in diastolic pressure due to a submaximal workload. In this group, the diastolic blood pressure also responded differently to the workload due to the M235T molecular variant. Results from both sets are shown in Figure 2. Statistical analysis, performed by ANOVA with Bonferroni adjusted t-test revealed a significant difference between the diastolic ratio after and before the physical workload of the group with the M/M compared to the T/T molecular variant. A slight, although insignificant decrease was also seen between M/T and M/M groups.

Table 1. Blood pressure (mm Hg) in individual subjects after the physical load

Patient	M235T molecular variant	BP systolic control	BP systolic max	Ratio	BP diastolic control	BP diastolic max	Ratio	BP mean control	BP mean max	Ratio
1	M/M	110	176	1.6	70	123	1.8	83	141	1.7
2	M/M	127	168	1.3	67	74	1.1	87	105	1.2
3	M/T	114	152	1.3	66	71	1.1	82	98	1.2
4	M/T	116	148	1.3	68	96	1.4	84	113	1.3
5	M/M	112	156	1.4	76	90	1.2	88	112	1.3
6	T/T	108	151	1.4	72	62	0.9	84	92	1.1
7	M/T	124	182	1.5	75	104	1.4	91	130	1.4
8	M/T	119	147	1.2	66	81	1.2	84	103	1.2
9	M/M	115	163	1.4	64	77	1.2	81	106	1.3
10	M/T	134	220	1.6	87	79	0.9	103	126	1.2
11	T/T	137	237	1.7	82	64	0.8	100	122	1.2
12	T/T	131	180	1.4	61	40	0.7	84	87	1.0
13	M/M	125	200	1.6	80	100	1.3	95	133	1.4
14	M/T	115	210	1.8	70	95	1.4	85	133	1.6
Mean ± S.E.M.		121±3	173±7	1.4	71±2	80±5	1.1	88±2	114±4	1.3

Table 2. Changes in heart rate (bpm) induced by the bicycle exercise

Patient	M235T	HR control	HR max	Ratio
1	M/M	69	89	1.3
2	M/M	45	153	3.4
3	M/T	61	178	2.9
4	M/T	64	174	2.7
5	M/M	69	156	2.3
6	T/T	45	163	3.6
7	M/T	67	178	2.7
8	M/T	57	160	2.8
9	M/M	77	182	2.4
10	M/T	68	183	2.7
11	T/T	60	184	3.1
12	T/T	75	175	2.3
13	M/M	-	-	-
14	M/T	51	168	3.3
Mean ± S.E.M.		62±3	165±7	2.7

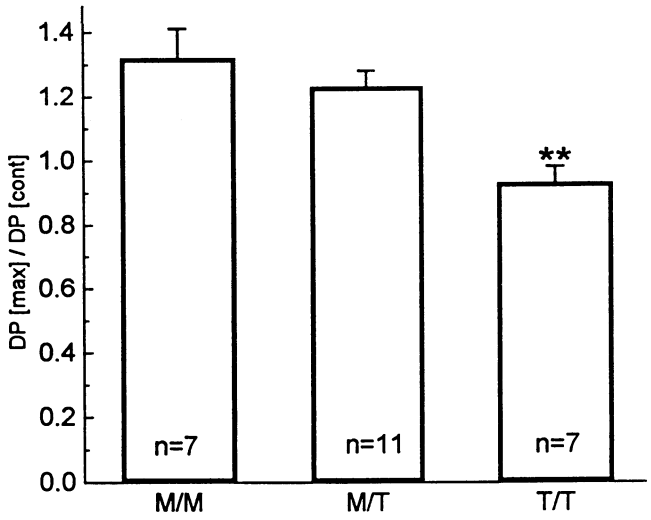

Fig. 2. *The response of diastolic blood pressure to the physical workload compared to the control diastolic blood pressure. Twenty-five individuals (14 young and 11 middle-aged) were subjected to the physical workload and changes in diastolic blood pressure due to M235T polymorphism were compared. Statistical analysis was calculated by ANOVA with Bonferroni adjusted t-test, n gives the number of individuals in each group, **p<0.01.*

Table 3. AI (ng/ml/h) and AII (pg/ml) levels after the physical exercise in relation to M235T polymorphism

Patient	M235T	AI control	AI max	Ratio	AII control	AII max	Ratio
1	M/M	0.4	12.7	31.8	2.0	20.0	10.0
2	M/M	5.1	19.5	3.8	2.0	4.0	2.0
3	M/T	0.4	9.5	23.8	-	-	-
4	M/T	0.4	5.9	14.8	50.0	438.0	8.8
5	M/M	0.3	7.2	24.0	158.01	122.0	7.1
6	T/T	4.0	6.0	1.5	12.5	630.0	50.4
7	M/T	1.4	4.4	3.1	31.6	398.0	12.6
8	M/T	0.2	12.3	61.5	10.0	63.0	6.3
9	M/M	1.0	6.4	6.4	-	-	-
10	M/T	0.2	2.1	13.1	12.6	28.9	2.3
11	T/T	0.6	2.9	5.1	20.5	46.9	2.3
12	T/T	0.3	0.8	2.5	12.0	24.2	2.0
13	M/M	0.6	1.9	3.2	17.8	45.3	2.5
14	M/T	0.2	1.1	4.6	15.2	27.7	1.8
Mean ± S.E.M.		1.2±0.4	7.5±1.4	15.9	31.1±11	277.5±57	10.4

We have not observed any relationship of the molecular variant M235T with either the control values of heart rate, or the values after the highest workload. Furthermore, the elevation in heart rate did not correlate with the type of polymorphism (Table 2) and was on the average 2.7 times greater after the workload of 2 W/kg. In our trial, we did not observe enhanced control values of either AI or AII concentration due to the M235T molecular variant. The AI production increased after the highest physical workload from 1.2±0.4 ng/ml/h to 7.5±1.4 ng/ml/h (15.9 times), while AII production was 10.4 times greater (Table 3). The elevation of plasma epinephrine (9.5 times) and norepinephrine (6.5 times) also did not correlate with M235T polymorphism (Table 4).

Table 4. Changes in epinephrine and norepinephrine concentrations (pg/ml plasma) due to the physical workload

Patient	M235T	EPI control	EPI max	Ratio	NE control	NE max	Ratio
1	M/M	68	346	5.1	532	3182	6.0
2	M/M	42	729	17.4	331	2118	6.4
3	M/T	38	496	13.1	489	4398	9.0
4	M/T	73	249	3.4	511	2818	5.5
5	M/M	49	135	2.8	250	989	4.0
6	T/T	29	113	3.9	311	1106	3.6
7	M/T	33	830	25.2	313	4598	14.7
8	M/T	48	515	10.7	441	3632	8.2
9	M/M	51	167	3.3	484	2063	4.3
10	M/T	19	302	15.9	370	2092	5.7
11	T/T	39	159	4.1	398	1650	4.1
12	T/T	-	-	-	-	-	-
13	M/M	60	327	5.5	337	1047	3.1
14	M/T	-	-	-	-	-	-
Mean ± S.E.M.		46±5	364±68	9.2	397±27	2474±363	6.2

Discussion

Hypertension is a common trait of multifactorial determination associated with an increased risk of myocardial infarction, stroke, and end-stage renal disease. Studies of the angiotensinogen gene have provided compelling evidence for an effect of inherited variants of this gene on blood pressure in humans (for review see Lifton 1995). This finding has motivated a search for molecular variants in the angiotensinogen gene. The M235T molecular variant was found to be significantly more prevalent in hypertensive subjects than in control subjects in Caucasian and Japanese populations (Jeunemaitre *et al.* 1992, Hata *et al.* 1994, Križanová *et al.* 1997). In the Slovak population, frequency of the M235T molecular variant was 0.32 in the control group and 0.42 in the group of hypertensive patients (Križanová *et al.* 1997).

Physical exercise belongs to the physiological stimuli which activate the endocrine system. It is known that both the sympatho-adrenal and renin-angiotensin systems are activated after a dynamic submaximal workload (Sundkvist *et al.* 1990, Guezennec *et al.* 1986). In our trial, we studied the response of 14 healthy young males to physical exercise. To avoid possible side effects, all probands were approximately of the same age and same weight and none of them were under medication. None of the individuals had high blood pressure (Table 1). In our study we focused on the parameters which are known to be involved in the regulation of blood pressure (PRA, AII, catecholamines). All the parameters tested were increased in response to the workload. The most pronounced increase was observed in plasma renin activity (15.9 times).

We found that the physical workload did not evoke an increase in diastolic blood pressure in individuals bearing T/T polymorphism (Fig. 2), while in subjects with M/M or M/T molecular variant we also observed a rise in diastolic blood pressure after the workload. Our observations might point to a dysbalance between the increase in cardiac output and

peripheral resistance. However, under basal conditions no differences were observed between the groups. Up to now, the physiological significance of this observation is not clear. Nevertheless, alterations in total peripheral resistance and arterial compliance were found in some cases of borderline arterial hypertension with normal cardiac output (Julius and Conway 1968).

From our observation, the question arises whether the renin-angiotensin pathway is involved in the diastolic response. Since neither angiotensin I nor angiotensin II production correlates with the occurrence of the M235T molecular variant, we assume that the renin-angiotensin pathway is most probably not involved in this process. Nevertheless, another polymorphism of the angiotensin converting enzyme (insertion/deletion polymorphism) was found to affect the rise in diastolic blood pressure during bicycle ergometry (Friedl *et al.* 1996).

In conclusion, our data indicate that interindividual differences in heart rate, PRA, AII production and the concentration of epinephrine and norepinephrine in the response to physical workload are not due to the molecular variant M235T. Reaction of diastolic blood pressure to the physical workload could be due to the M235T molecular variant.

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