Genetic determination of the vascular reactions in humans in response to the diving reflex

Tatiana I. Baranova¹, Dmitrii N. Berlov¹,⁵, Oleg S. Glotov², Ekaterina A. Korf¹, Alexey D. Minigalin¹, Alla V. Mitrofanova³, Ildus I. Ahmetov⁴, Andrey S. Glotov²

¹Department of General Physiology, Saint Petersburg State University, Saint Petersburg, Russia; ²Biobank of the Research Park, Saint Petersburg State University, Saint Petersburg, Russia; ³Katz Drug Discovery Center and Department of Surgery, Miller School of Medicine, University of Miami, Miami, USA; ⁴Ildus I. Ahmetov Sport Technology Research Center, Volga Region State Academy of Physical Culture, Sport and Tourism, Kazan, Russia; ⁵ITMO University, Saint Petersburg, Russia

Correspondence to:
Tatiana I. Baranova, Ph.D.
Department of General Physiology, Saint Petersburg State University,
Universitetskaya Emb., 7/9, Saint Petersburg, Russia, 199034
e-mail: baranovati@gmail.com
tel: +7 921-331-6581

ABSTRACT
The purpose of this study was to investigate the genetic mechanisms of the defense vascular reactions in response to the diving reflex in humans with polymorphisms in the genes ADBR2, ACE, AGTR1, BDKRB2, and REN. We hypothesized that protective vascular reactions, in response to the diving reflex, are genetically determined and are distinguished in humans with gene polymorphisms of the renin-angiotensin and kinin-bradykinin system. A total of 80 subjects (19±1.4 years) participated in the study. The intensity of the vascular response was estimated using photoplethysmogram. The I/D polymorphism (rs4340) of ACE was analyzed by PCR. REN (G/A, rs2368564), AGTR1 (A/C, rs5186), BDKRB2 (T/C, rs1799722), ADBR2 (A/G, rs1042713) polymorphisms were examined using the two-step multiplex PCR followed by carrying allele hybridization on the biochip. Subjects with the BDKRB2 (C/C), ACE (D/D) and ADBR2 (G/G, G/A) genotypes exhibited the strongest peripheral vasoconstriction in response to diving. In subjects with a combination of the BDKRB2 (C/C) plus ACE (D/D) genotypes, we observed the lowest pulse wave amplitude and pulse transit time values and the highest arterial blood pressure during face immersion compared to the heterozygous individuals, suggesting that these subjects are more susceptible to diving hypoxia. This study observed that humans with gene polymorphisms of the renin-angiotensin and kinin-bradykinin systems demonstrate various expressions of protective vascular reactions in response to the diving reflex. The obtained results might be used in estimation of resistance to hypoxia of any origin in human beings or in a medical practice.

Keywords: diving reflex, vasoconstriction, gene polymorphism, renin-angiotensin system, kinin-bradykinin system

Running Head
Genes and Vascular Reactions to Diving

Abbreviations:
ADBR2 – adrenoreceptor beta 2; ACE – angiotensin-converting enzyme; AGTR1 – angiotensin II receptor, type 1; BDKRB2 – receptor 2 to bradykinin; PWA – pulse wave amplitude; PTT – pulse transit time; REN – renin
NEW AND NOTEWORTHY

Our study demonstrates that the vascular reactions in response to the diving reflex are genetically determined and depend on gene polymorphisms of the kinin-bradykinin and the renin-angiotensin systems.

RESEARCH ON OF THE NATURAL ADAPTATION TO A SEMIAQUATIC LIFESTYLE of diving mammals demonstrates that the maintenance of oxygen homeostasis in these species is ensured by a number of defensive physiological, biochemical, immune and neuroendocrine adaptive mechanisms (19, 20, 26, 27, 43, 44). Among these mechanisms, the most important universal form of adaptation to diving is the diving reflex, which is accompanied by reflex apnea, bradycardia, peripheral vasoconstriction and the selective redistribution of blood flow (4, 30). The blood is removed from the organs that can withstand temporary hypoxia and is redistributed to the organs that are most vulnerable to the lack of oxygen, such as the brain and heart (17, 37).

These reactions are qualitatively similar between species, and the diving response elicited in diving mammals is quite similar to that of the humans. However, in animals, these responses have a species determined characteristics (17, 37). In humans, unlike in animals, the diving reaction can be implemented in different ways. In accordance with this, we distinguish four types of chronotropic heart functions in the human diving reflex realization as follows: 1) bradycardia occurs immediately after face immersion in water with a latent time development of less than 9 sec; 2) bradycardia develops slowly with its maximum at the end of apnea time; 3) bradycardia does not appear in response to the diving reflex (in 12-18% of subjects); and 4) tachycardia but not bradycardia emerges following face submersion into water (less than 5% of subjects) (6). According to our observations, vascular constriction during the diving reaction is also different in humans. However, the influence of genetic factors on this variability remains unknown.

It is worth noting that diving mammals are able to maintain their mean arterial pressure, while humans cannot. This ability can be explained by the fact that during diving, the Weddell seal, for instance, not only slows its heart rate but also decreases its cardiac output, thereby decreasing the blood flow, but the pressure is maintained within the normal range. In human beings, during the response to the diving reflex, a cardiac output decrease is not always observed. Additionally, the intensity of the peripheral vasoconstriction is different, and the blood pressure might be greatly increased (6, 7, 14, 15). Importantly, the peripheral resistance magnification due to vasoconstriction can have a significant effect on the blood pressure rise.

The strength, duration and extent of the peripheral vasoconstriction that occurs while diving (or simulation) depends on the ratio of the activity of the neurogenic, humoral and metabolic constrictor and the dilatator factors regulating vascular tone (22, 23, 29, 51, 52). Important factors involved in the regulation of vascular tone belong to the renin-angiotensin and kinin-bradykin systems (5, 50). Vascular tone depends on the sensitivity of receptors of the vascular walls to the products of these systems. At the same time, it is known that the amount of vasoactive substances produced by the renin-angiotensin and kinin-bradykin systems and the number of receptors of the vascular wall and their sensitivity to the vasoactive substances involved in the regulation of vascular tone, is determined by polymorphisms of genes controlling vascular tone (2, 18, 22, 35, 39, 42). However, the individual strategies of adaptation to extreme factors accompanying diving are poorly understood.

Based on these observations, we tested a hypothesis that protective vascular reactions in response to the diving reflex are genetically determined and are distinguished in humans
with gene polymorphisms of the renin-angiotensin and kinin-bradykinin systems. The aim of the study was, therefore, to investigate the genetic mechanisms of the defense vascular reactions and the blood pressure magnification in response to the diving reflex in humans with polymorphisms in the genes ADBR2, ACE, AGTR1, BDKRB2, and REN.

These data might also be used as an estimation of resistance to hypoxia of various origins in humans or in a medical practice, including the risk of developing hypertension (9, 24, 38, 47).

METHODS

Subject Recruitment and Data Collection. Eighty healthy volunteers (19±1.4 years; 26 males, 54 females) without special physical training were included in the study. All of the subjects (students of the Saint Petersburg State University, Russia) participated in the study voluntarily and had no direct benefit from the test (financial rewards or educational requirements or credits). The information about the general results of the research or personal data (such as the genetic variants) was provided to the individuals who were interested. At the time of the experiment, all of the volunteers did not have arteriosclerosis or diabetes and did not take any drugs. A short list of the group characteristics is presented in Table 1. The study was approved by the Saint Petersburg State University Ethics Review Committee for human studies (№40 from 07.03.2012), and all of the volunteers signed an informed agreement.

Experimental Model of the Diving Reflex in Humans. The activation of the diving reflex was performed using face submersion in cold water under laboratory conditions. It is well known (18) that a 10ºC gradient between the air temperature and the water temperature is optimal for the manifestation of the diving reflex, and in our experiments, the water temperature was 12.3±2.3ºC, and the air temperature was 22.3±2.5ºC. Prior to the start of the experiment, all of the subjects stayed in the laboratory for at least 40 min and were adapted to the local temperature. The procedure was performed on a subject who lies in a ventricumbent position on a coach with his or her arms along the body (Figure 1). During an experimental procedure, all of the subjects kept their hands at a heart line, did not change this position and did not move their fingers with a finger sensor. Three face submersions on a normal exhale were performed in cold water. The duration of the first submersion was limited by the feeling of the first discomfort. After the first face submersion, which we considered to be an orienting one, a full recovery of the cardiovascular parameters occurred within 10 minutes. This orienting face submersion was performed to avoid a reaction to stress. As a control values, we used the parameters before the second dive. The duration of the second and third submersions was performed on volitional breath-hold until a maximum time. The pause between submersions was 2 minutes.

Measurements of Physiological Parameters and Photoplethysmography. Before the experimental procedure, an electrocardiogram was recorded and checked for anomalies. During the whole experiment, the heart rate was continuously recorded with the Poly-Spectrum-8/E Superminiatuare 12-channel Digital ECG System (by Neurosoft Company, Russia). An electrocardiogram was registered in 12 leads as follows: limb leads (I, II, III, aVR, aVL, aVF) and precordial leads (V1, V2, V3, V4, V5, V6). The arterial blood pressure was recorded with a sphygmomanometer with the cuff on the right shoulder (AND UA-797, Japan).

The photoplethysmogram method records changes in the tissue optical density, which depends on the blood supply. A relative index of skin capillary blood flow in the left index finger was continuously recorded with a rheograph (RGPA-6/12 “Rean-Poly”, Medicom-
MTD, Russia) by the photoplethysmogram method (the sensitivity of the recorded signals for
the volume rheogram was 1 mm/cm and for the differential rheogram was 20 mm/(s*cm)).

The pulse transit time (PTT, ms) (31), which is typically used as an indirect evaluation of the
elasticity of the arteries and reflects peripheral vascular tone (16, 49), was measured using a
rheograph (RGPA-6/12 “Rean-Poly”, Medicom-MTD, Russia) from the distal phalanx of an
index finger. The pulse wave amplitude (PWA, pm) (31) was calculated based on the
photopletismogram records using “Rean-Poly” software (Elite version). It was previously
reported that PWA indirectly reflects the vascular perfusion of the distal phalanx of the hand
and essentially depends on the sympathetic influences of the autonomic nervous system (3, 21, 45).

The respiratory flow and volume, as well as the expiratory O₂ and CO₂ fractions (pO₂
and pCO₂), for the determination of the alveolar gas exchanges were recorded with a
microprocessor analyzer (MF01, Research and Production Center for Environment and
Health – CEZ, Russia). The current temperature, barometric pressure and humidity were
measured in the laboratory right before each experimental session. The cardiovascular
parameters were recorded from 2 min before the imitation dive, during diving and until 2 min
after the imitation dive. The respiratory parameters were recorded before and after the
imitation dive.

**DNA Isolation and PCR.** Venous blood samples for DNA isolation were collected in 4 ml
EDTA tubes for blood collection. The DNA samples from the blood of all of the patients
were isolated by phenol chloroform extraction as described previously (41). The DNA
concentration was determined using Qubit™ software (Invitrogen, USA) with Qubit™ DNA
HS Assay Kits according to the manufacturer's instructions. An insertion/deletion (I/D)
polymorphism (rs4340) in the ACE gene was analyzed by PCR using the following primers:
F-5’-CTGGAGACCACTCCCATCCTTCT-3’ and R-5’-ATGTGGCCATCACATTCGTCA
GAT-3’. For all of the reactions, the 25 µl PCR mix included deionized water, 1.5 mM
MgCl₂, 0.2 mM of each dNTP (Silex, Russia), 2.5 units of Taq polymerase (Silex, Russia), a
mix of forward and reverse gene-specific primers at 0.2 pM each, 1 µl of DNA template, and
a PCR buffer (6.7 mM Tris-Cl, pH 8.6, 16.6 mM NH₄SO₄, and 0.001% Triton X-100).

After the initial denaturation of the sample at 95°C for 5 min, we carried out 37 cycles as
follows: 94°C for 30 s; 60°C for 30 s; and 72°C for 1 min. A final incubation for 5 min at
72°C was added to complete the DNA synthesis. The **REN** (G/A, rs2368564), **AGTR1** (A/C,
rs5186), **BDKRB2** (T/C, rs1799722), **ABR2** (A/G, rs1042713) polymorphisms were
examined using the two-step multiplex PCR followed by carrying allele hybridization on the
biochip as previously described (18).

**Statistical Analysis.** The values are expressed as the means and SDs. The statistical
analysis was performed using the statistical package Statsoft Statistica 8.0 for Windows. The
results were analyzed using the Wilcoxon and Mann-Whitney nonparametric tests (paired
comparisons). P values < 0.05 were considered to be statistically significant.

**RESULTS**

All of the subjects completed the protocol. In accordance with the instructions, the
subjects did not hyperventilate before the face immersion. Table 1 presents the
anthropometric data of 80 healthy volunteers. We observed no changes in the anthropometric
characteristics of subjects with regard to the **AGTR1**, **BDKRB2** or **ACE** gene polymorphisms.
The diving response, noticeable as a heart rate reduction from the control level, was initiated by apneas and augmented by face immersion (Figure 2). The average duration of apnea in the study group was $T = 31.0 \pm 11.1$ sec. The alveolar $pO_2$ was significantly reduced, while the alveolar $pCO_2$ was significantly increased in the exhaled air after breath holding with the face immersion in water compared to the control level ($p < 0.05$, Wilcoxon test) (Table 2).

Bradycardia, in response to face immersion, is one of the most important characteristics of the diving reflex. In our study group, we found that different subjects have variations in the development of bradycardia in response to imitation diving (6, 7) (Figure 3). For example, in 18 subjects, we observed a well-expressed heart rate (HR) reduction with a latent time of bradycardia development of $\leq 9$ sec (hyper-reactive type). Twenty-five subjects showed well-expressed HR reduction, but the latent time of bradycardia development was $\geq 9$ sec (reactive type). In 22 of the subjects, we found no bradycardia development in response to apnea with face immersion, and 15 subjects were characterized by tachycardia in response to imitation diving.

All of the subjects showed a significant elevation of systolic (SBP) and diastolic (DBP) blood pressure in response to apnea with face immersion (Table 3, 6, 7). The pulse wave amplitude (PWA) was significantly lower in all of the subjects during dimitation diving. The pulse transit time (PTT), which indirectly characterizes vascular tone (16, 48), was also reduced compare to the control level before apnea (Table 3). The average latent time of the PTT reduction was $1.5 \pm 2.3$ sec.

Next, we analyzed the cardiovascular response in the subjects with gene polymorphisms in cardiovascular related genes, such as angiotensin-converting enzyme ($ACE$: I/I, D/D, I/D) (the sizes of the PCR products and sizes of the resulting DNA fragments are presented in Figure 4), adrenoreceptor beta 2 ($ADRB2$: A/A, G/G, A/G), angiotensin II receptor, type 1 ($AGTR1$: A/A, C/C, A/C), receptor 2 to bradykinin ($BDKRB2$: T/T, C/C, T/C), and renin ($REN$: A/A, G/G, A/G).

At the control level, we found that the PWA was significantly lower in the subjects with a G/G genotype in the $REN$ gene when compared to the G/A genotypes (Table 4). Every subject with an A/A genotype had higher PWA value compared with every subject with G/G, but we cannot make a conclusion due to the small size of our group. At the control level, we found that the PTT was higher in the subjects with an A/A genotype for the $AGTR1$ gene when compared to the A/C genotypes (Table 5). Every subject with the C/C genotype had a lower PTT value compared to every subject with an A/A genotype, but we cannot make conclusion due to the small size of our group. During face immersion, we observed a significant ($p<0.05$) reduction in both parameters, PWA and PTT, in the subjects with all of the described genotypes. Although, the subjects with the C/C genotype for the $BDKRB2$ gene demonstrated a lower PTT, and no differences were found in the PWA values during apnea with face immersion. However, at the recovery period, the subjects with a C/C genotype for the $BDKRB2$ gene showed lower PWA values (Table 4). There was a decreasing PWA value in the subjects with a G/A genotype for the $ADRB2$ gene during imitation diving (Table 4) that might be evidence of more sensitivity to the catecholamine-induced desensitization of the receptors.

Interestingly, subjects with the D/D genotype for the $ACE$ gene had no differences in PTT compared to the subjects with other genotypes at the control level. However, the subjects exhibited a more efficient PTT decrease during the face immersion procedure and at the recovery period compared to the subjects with the I/I and I/D genotypes in the second and third face immersions and the recovery periods (Table 5).

The subjects with the combination of the $BDKRB2$ (C/C) plus $ACE$ (D/D) genes demonstrated the highest blood pressure, and during the third face submersion these changes
were significant compared to the other four groups (BDKRB2 (T/C) ACE (I/D); BDKRB2 (C/C) ACE (I/I); BDKRB2 (T/T) ACE (I/I); and BDKRB2 (T/T) ACE (D/D)) (Figure 5, 6).

However, the combination of the BDKRB2 (C/C) plus ACE (D/D) genes was associated with a lower PWA (p<0.05) at the control level compared to the heterozygous subjects (BDKRB2 (T/C) and ACE (I/D)), suggesting a weaker blood supply of the peripheral vessels (Figure 7). No changes were found in the PTT values between the subjects with different combinations of the BDKRB2 and ACE alleles (Figure 8). During face immersion, we observed a significant reduction in the PWA values in all of the subjects (Figure 7, Table 3), indicating a peripheral reduction of blood flow. The subjects with the BDKRB2 (C/C) plus ACE (D/D) gene combination demonstrated a significantly lower PWA (0.273±0.045 pm, p<0.05) during the second face immersion compared to the heterozygous subjects (0.467±0.062 pm) (Figure 7). None of the subjects showed complete recovery during the first two minutes after face immersion. The subjects with a combination of the BDKRB2 (C/C) plus ACE (D/D) genes had significantly (p<0.05) lower PWA (Figure 7) and PTT values (Figure 8) compared to the subjects with the BDKRB2 (T/C) plus ACE (I/I) gene combination. Interestingly, 50% of the subjects with the BDKRB2 (C/C) plus ACE (D/D) gene combination demonstrated a lower PTT during the recovery period compared to the control level.

**DISCUSSION**

We demonstrated that subjects with receptor 2 to bradykinin (BDKRB2) (CC), angiotensin-converting enzyme (ACE) (DD), and adrenoreceptor beta 2 (ADBR2) (GG, GA) genotypes showed the most conspicuous peripheral vasoconstriction in response to diving. Specifically, this group of subjects shows the highest blood pressure changes during the diving reaction, probably due to the increase in peripheral resistance caused by the constriction of the peripheral vessels. Furthermore, we showed that subjects with a combination of the BDKRB2 (C/C) plus ACE (DD) genotype had the lowest pulse wave amplitude and pulse transit time values compared to the heterozygous subjects. Thus, our study identified that gene polymorphisms of the renin-angiotensin and kinin-bradykinin systems in human beings might be a marker of resistance to extreme factors, such as diving.

A complex neural network, integrating the respiratory and cardiovascular systems, controls the diving response. Peripheral vasoconstriction and the redistribution of blood flow to the oxygen-sensitive organs, such as the heart and the brain, are the universal defensive mechanisms (13). Our study also showed that in all of the subjects, vasoconstriction during face submersion, occurred with a small delay of 1-2 sec. Occasionally, vasoconstriction was observed before face immersion and was accompanied by an increasing heart rate, which reflects the increasing stress-adrenergic reactions that might be observed before face immersion in water. However, the reflex nature of the vasoconstriction is caused by the noradrenergic effect of the sympathetic nervous system on the smooth muscle fibers (activation of the $\alpha_1$-receptors) of the blood vessels (36, 40). Additionally, a certain biochemical state of an organism enhances or worsens the diving reflex reactions.

The present study identified significant changes in the PWA and the PTT, which indirectly reflected modifications in the peripheral vasoconstriction between the subjects at the control level (Table 4, Table 5). Our results demonstrated a novel finding that changes in the PWA and PTT levels were associated with polymorphisms in two genes of the renin-angiotensin system – REN (GG, GA, AA) and AGTR1 (CC, AC, AA), which are involved in the regulation of the vascular tone. It was previously shown that renin (the product of the REN gene) is discharged into the blood, which interacts with angiotensin (the product of the AGT gene) and converts it into angiotensin I (33). This peptide, in turn, is a substrate for the
angiotensin-converting enzyme (product of the ACE gene), which converts angiotensin I (AT1) into angiotensin II (AT2). Angiotensin II acts via angiotensin cell receptors and is one of the most powerful vasoconstrictors. By interacting with angiotensin receptors (AT1 – product of the AGTR1; AT2 – product of the AGTR2), angiotensin II causes vasoconstriction (38).

We observed that, at the control level, the subjects with a G/G polymorphism in the REN gene demonstrated the lowest blood supply in the peripheral vessels (the lowest PWA values) (Table 4). However, the highest PWA values were detected in the subjects with an A/A polymorphism in the REN gene. As observed earlier by Ahmad and colleagues (1), the frequency of the A/A genotype is considerably higher in patients with arterial hypertension compared to healthy subjects (34.7% vs 14.0%). Our previous investigations showed that the REN gene polymorphism is involved in the development of the stable form of hypertension in children (18).

The highest PTT (low peripheral vascular tone) values at the control level were found in subjects with the A/A polymorphism of the AGTR1 gene compared to the A/C and C/C genotypes (Table 5). These results are in accordance with the observation showing that changes in the structure of the angiotensin II receptor through its gene polymorphism may result in the changes in the regulation of the vascular tone and the proliferation of the smooth muscle cells of the vascular walls (see (50) for review). Additionally, the presence of the C allele of the AGTR1 gene leads to the synthesis of more molecules of AGTR1 (42). The link between the C/C polymorphism of the AGTR1 gene and arterial hypertension tendency was shown in the study of Henskens and co-authors (25).

We demonstrated that, in simulated apnea diving and at the recovery period, subjects with ADBR2 (G/A, A/A, G/G), BKR2 (C/C, C/T, T/T), and ACE (D/D, I/D, I/I) genes have differences in the parameters of the peripheral vascular tone and the blood filling. Thus, the PWA, the blood filling indicator of the peripheral vessels, was significantly higher in the subjects with the G/G genotype of the ADBR2 48 gene compared to the subjects with the G/A or A/A alleles (Table 4). Therefore, the ADBR2 48 G/A and ADBR2 48 A/A genotypes could be related to the catecholamine-induced desensitization of the β2-adrenoreceptors (11). Moreover, the ADBR2 48 G/G genotype might contribute to a less pronounced reflex noradrenergic peripheral vasoconstriction during simulated apnea diving.

The level of the angiotensin-converting enzyme is associated with allele of the ACE gene. Allele D of the ACE gene is mostly implicated in the elevation of the angiotensin-converting enzyme level, which is the strongest vasoconstrictor. The serum of healthy human beings with the D/D genotype contains a two-fold higher level of the angiotensin-converting enzyme compared to I/I homozygous or I/D heterozygous individuals (8). It was shown that only the tissue-bound form is necessary and sufficient for the regulation of blood pressure (47). The role of soluble ACE in the plasma, in this case, is unclear. It is hypothesized that soluble ACE in the plasma offers a means for delivering the enzyme to the tissues that do not express their own ACE with regional effects on vascular tone (12). Our findings are in concordance with the data from the literature and demonstrate that I/I homozygous individuals have a weaker vasoconstriction (higher PTT level) compared to subjects with the D/D genotype combination, showing a significantly higher vascular tone during the third face submersion and the recovery period (Table 5, Figure 5).

Furthermore, our data showed that subjects with the C/C allele combination of the BDKRB2 gene have significantly lower PTT values (i.e., vascular tone is higher) when compared to subjects with the T/C or T/T allele combination. These findings are completely supported by the previous investigation showing a higher BDKRB2 expression in humans with the T allele (10).
It is a very well known fact that bradykinin is one of the stimuli of the nitric oxide (NO) release by the endothelial cells and is the main relaxation factor of vessels. Angiotensin-converting enzyme degrades bradykinin (28) and inhibits the formation of NO. Therefore, it is of considerable importance to analyze peripheral vascular tone in subjects with different polymorphisms in the ACE (I/I, I/D, D/D) and BDKRB2 (T/T, T/C, C/C) genes. Our data showed that subjects with the C/C allele of the BDKRB2 and D/D allele of the ACE genes have significantly stronger vasoconstriction compared to heterozygous (T/C of the BDKRB2 and I/D of the ACE genes) subjects (Figure 4, Figure 5). Apparently, the C/C and D/D genotypes are associated with an elevated level of angiotensin II and a reduced level of endothelial NO synthase, which results in a more significant potentiation of the reflex noradrenergic vasoconstriction during simulated apnea diving and a delayed recovery of vascular tone after the stimuli is over.

The main function of the renin-angiotensin system is blood pressure regulation. Hyperactivity of this system leads to hypertension. For instance, Kovács and co-authors (32) showed a link between the activation of renin-angiotensin-aldosterone system and heart diastolic dysfunction due to increased tinin-based stiffness in mRen2 rats with hypertension. Based on these data, the reason the highest blood pressure was observed in the subjects with the BDKRB2 (C/C) plus ACE (D/D) gene combination in response to diving is because one of the most important factors of the blood pressure elevation is vessel constriction. This might be related to increased blood viscosity due to the release of formed elements caused by hypoxia during diving (42). Our data indicate that hypoxia challenge in individuals with a combination of the BDKRB2 (C/C) plus ACE (D/D) gene polymorphisms is associated with the most conspicuous hypertensive response compared to the individuals with other allele combination.

Thus, our data, for the first time, demonstrates a link between the level of the vasoconstriction during simulated diving and the renin-angiotensin system. In other words, the most conspicuous constriction of the peripheral vessels is expected in subjects homozygous for the BDKRB2 (C/C), ACE (D/D), and ADRB2 (G/G) genes. Future studies are needed to further describe the mechanism of the gene polymorphism implication in the realization of the diving reflex in humans.

Limitations of research. Some limitations have to be taken into account when interpreting the results of these analyses. Given the relatively small number of subjects included in the analyses and that fact that no correction was made in the p-values for multiple comparisons, we consider the results as representing a present state of knowledge, and the conclusions should be considered as preliminary. Nominally significant differences should be taken as preliminary and require validation and should be developed in future research studies.

CONCLUSIONS

Peripheral vasoconstriction and the redistribution of the blood flow to the organs and systems that are most sensitive to the lack of oxygen (brain and heart) is a universal defense reaction of the organism to hypoxia of any origin. In our investigations, during simulated diving, peripheral vasoconstriction was observed in all of the subjects, but the intensity of this reaction exhibited a significant interpersonal difference. A genetic analysis of the polymorphisms of renin-angiotensin and kinin-bradykinin genes as well as of the gene β2-adrenoreceptor (ADRB2 48) showed that the changes in the vascular tone and blood pressure during the diving response differed in the subjects with various combinations of alleles of the studies genes, and hence are dependent on the genotype. A genetic determination of the
strong peripheral vasoconstriction under hypoxia will contribute to the protection of the
organs most sensitive to the lack of oxygen, while it will also reduce blood supply to the
skeletal muscles, and hence, will limit the physical efficiency. This should be taken into
account when evaluating the resistance of the organism to the extreme effects that accompany
diving and when selecting specialists whose activity will be connected with diving without
equipment (rescuers; synchronous swimmers, etc.). The obtained results can be used for the
evaluation of an organism’s resistance to hypoxia of any genesis as well as in the medical
practice (including an assessment of the development of a hypertension risk in hypoxic
conditions) where the diving reflex enhances the efficiency of the relevant treatment.

GRANTS

The study was supported by the grant of the Russian Science Foundation (project
No14-50-00069), Saint-Petersburg State University.

DISCLOSURES

The authors have no conflicts of interest to declare.

ACKNOWLEDGMENT

We thank Tatiana Podgorskaya for the technical help with the manuscript.

AUTHORS’ CONTRIBUTIONS

Author contributions: T.B., A.G. conception and design of the research; E.K., A.D.M.,
O.G. performed experiments; T.B., O.G. data analysis; T.B., O.G., A.G., and I.A.
interpretation of the experimental results; A.D.M., D.B. figure preparation; T.B. drafted the
manuscript; T.B., A.G., D.B., I.A. and A.V.M. edited and revised the manuscript; T.B., A.G.,
and A.V.M. approved the final version of the manuscript.

REFERENCES

1. Ahmad U, Saleheed D, Bokhari A, Frossard PM. Strong association of a renin
4. Andersson JPA, Linér MH, Fredsted A, Schagatay EKA. Cardiovascular and
respiratory responses to apneas with and without face immersion in exercising
5. Auch-Schwelk W, Kuchenbuch C, Claus M, Walther B, Bossaler C, Friedel N,
Graf K, Gräfe M, Fleck E. Local regulation of vascular tone by bradykinin and


Complete figures legend

**Figure 1.** Scheme of the experiment.

**Figure 2.** Electrocardiogram (ECG) and photoplethysmogram (PPG) of the subjects in response to imitation diving. ↓ – initiation of apnea with face immersion; ↑ – end of apnea with face immersion. PPG_d – differential photoplethysmogram.

**Figure 3.** Electrocardiogram of the subjects with different types of bradycardia development in response to imitation diving. (a) Hyper-reactive type (latent time of bradycardia development ≤9 sec); (b) reactive type (latent time of bradycardia development ≥9 sec); (c) non-reactive type (bradycardia does not develop in response to imitation diving); (d) paradox type (tachycardia development in response to imitation diving). ↓ – initiation of apnea with face immersion; ↑ – end of apnea with face immersion.

**Figure 4.** Gel electrophoresis picture of the PCR products of the I and D alleles of the angiotensin-converting enzyme. 1, 3, 5 – examples of the heterozygous alleles (I/D); 2, 6 – examples of the homozygous alleles (D), 4 – example of the homozygous allele (I).

**Figure 5.** Systolic arterial pressure in the subjects with a polymorphism in the ACE and BDKRB2 genes. The BDKRB2 (C/C) plus ACE (D/D) homozygous subjects show a significantly higher systolic arterial pressure during dive 3 compared with subjects with other combinations of the alleles (BDKRB2 (T/C) plus ACE (I/D); BDKRB2 (C/C) plus ACE (I/I)).

**Figure 6.** Diastolic arterial pressure in the subjects with a polymorphism in the ACE and BDKRB2 genes. The BDKRB2 (C/C) plus ACE (D/D) homozygous subjects show a significantly higher diastolic arterial pressure during dive 3 compared with subjects with other combinations of alleles (BDKRB2 (T/T) plus ACE (D/D); BDKRB2 (T/T) plus ACE (I/I); BDKRB2 (T/C) plus ACE (I/D); BDKRB2 (C/C) plus ACE (I/I)).

**Figure 7.** Pulse wave amplitude in the subjects with a polymorphism in the ACE and BDKRB2 genes. The BDKRB2 (C/C) plus ACE (D/D) homozygous subjects show a significantly lower pulse wave amplitude at the control level (*p<0.05), during face immersion (dive 3; *p<0.05) and at the recovery period (**p<0.01), suggesting that this gene combination is less effective at generating a proper vascular response to the diving reflex compare to the BDKRB2 (T/C) plus ACE (I/D) heterozygous subjects.
Figure 8. Pulse transit time in the subjects with a polymorphism in the ACE and BDKRB2 genes. The BDKRB2 (C/C) plus ACE (D/D) homozygous subjects show a significantly (**p<0.01) lower pulse transit time at the recovery period, suggesting that this gene combination provides a non-adequate vascular reaction in response to the diving imitation compare to the BDKRB2 (T/C) plus ACE (I/D) heterozygous subjects.
Table 1. Anthropometric parameters of the studied group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Males (n=26)</th>
<th>Females (n=54)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>71.0±7.9</td>
<td>59.0±8.5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>179.0±4.6</td>
<td>167.0±5.1</td>
</tr>
<tr>
<td>BMI</td>
<td>23.4±2.2</td>
<td>21.2±2.9</td>
</tr>
<tr>
<td>Age, years</td>
<td>19 ±1.5</td>
<td>19 ±1.3</td>
</tr>
<tr>
<td>The number of smokers, n</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Longevity of smoking, years</td>
<td>2.3±0.3</td>
<td>2.1±0.2</td>
</tr>
</tbody>
</table>

All of the data are the group means ± SD.
Table 2. The alveolar pO2 and pCO2 levels before apneas and at end of apneas

<table>
<thead>
<tr>
<th>Parameter</th>
<th>In ambient air</th>
<th>Before apnea</th>
<th>At end of diving imitation #2</th>
<th>At end of diving imitation #3</th>
</tr>
</thead>
<tbody>
<tr>
<td>pO2, mm Hg</td>
<td>158.0</td>
<td>122.0±12.5</td>
<td>98.8±11.8*</td>
<td>97.6±9.8*</td>
</tr>
<tr>
<td>pCO2, mm Hg</td>
<td>0.26</td>
<td>38.6±6.5</td>
<td>49.1±7.3*</td>
<td>49.7±6.7*</td>
</tr>
</tbody>
</table>

All of the data are the group means ± SD. *p<0.05 when compared to the control level before apnea (Wilcoxon test).
Table 3. Cardiovascular system parameters in the studied group (n=80) at the control level and during apnea with face immersion

<table>
<thead>
<tr>
<th>Phase</th>
<th>HR, beats/min</th>
<th>SBP, mm Hg</th>
<th>DBP, mm Hg</th>
<th>PWA, ppm</th>
<th>PTT, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control level</td>
<td>64.75 ± 10.9</td>
<td>113.8 ± 10.7</td>
<td>71.0 ± 6.1</td>
<td>1.67 ± 1.4</td>
<td>217.0 ± 18.8</td>
</tr>
<tr>
<td>Face immersion 2</td>
<td>59.5 ± 17.5</td>
<td>130 ± 17.8*</td>
<td>84.0 ± 12.0*</td>
<td>0.35 ± 0.3**</td>
<td>199.0 ± 35.8*</td>
</tr>
<tr>
<td>Recovery 2</td>
<td>63.5 ± 11.5</td>
<td>121.1 ± 13.3</td>
<td>76.5 ± 9.8</td>
<td>0.36 ± 0.9**</td>
<td>195.8 ± 26.6*</td>
</tr>
<tr>
<td>Face immersion 3</td>
<td>58.3 ± 16.1</td>
<td>128 ± 20.1*</td>
<td>83.0 ± 17.8</td>
<td>0.35 ± 0.27**</td>
<td>200.0 ± 26.7*</td>
</tr>
<tr>
<td>Recovery 3</td>
<td>67.3 ± 11.7</td>
<td>119.3 ± 11.7</td>
<td>74.2 ± 8.0</td>
<td>0.97 ± 0.94*</td>
<td>191.4 ± 27.7*</td>
</tr>
</tbody>
</table>

All of the data are the group means ± SD. HR – heart rate; SBP – systolic blood pressure; DBP – diastolic blood pressure; PWA – pulse wave amplitude; PTT – pulse transit time. ppm – per mille from calibrated signal. *p<0.05, **p<0.01 when compared to the control level (Wilcoxon test).
**Table 4.** Pulse wave amplitude characteristics in the subjects with a polymorphism in the *REN*, *ADBR2* and *BDKRB2* genes

<table>
<thead>
<tr>
<th>Gene</th>
<th><strong>Control level</strong></th>
<th><strong>Face Immersion 2</strong></th>
<th><strong>Recovery 2</strong></th>
<th><strong>Face Immersion 3</strong></th>
<th><strong>Recovery 3</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase</strong></td>
<td><strong>Gene</strong></td>
<td><strong>G/A (n=23)</strong></td>
<td><strong>G/G (n=43)</strong></td>
<td><strong>A/A (n=3)</strong></td>
<td><strong>G/A (n=13)</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.20±1.62 (±1.38**</td>
<td>1.27±0.52</td>
<td>2.44±1.04</td>
<td>1.49±1.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.38±0.33</td>
<td>0.35±0.16</td>
<td>0.51±0.28</td>
<td>0.34±0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.15±1.05</td>
<td>0.86±0.98</td>
<td>1.22±1.38</td>
<td>1.09±1.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.37±0.38</td>
<td>0.35±0.08</td>
<td>0.50±0.3</td>
<td>0.29±0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.20±0.19</td>
<td>0.86±0.08</td>
<td>1.57±0.83</td>
<td>0.93±0.82</td>
</tr>
</tbody>
</table>

All of the data are the group means ± SD. *p<0.05, **p<0.01 (Mann-Whitney test for paired depended samples) when compared to the combination between the different alleles of the same gene in the same state (control, face immersion, recovery) except for the REN A/A group.
Table 5. Pulse transit time characteristics in the subjects with a polymorphism in the AGTR1, BDKRB2 and ACE genes

<table>
<thead>
<tr>
<th>Phase</th>
<th>Gene</th>
<th>Genotype</th>
<th>A/C (n=27)</th>
<th>A/A (n=35)</th>
<th>C/C (n=4)</th>
<th>T/C (n=13)</th>
<th>C/C (n=22)</th>
<th>T/T (n=36)</th>
<th>I/I (n=28)</th>
<th>I/D (n=23)</th>
<th>D/D (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control level</td>
<td>AGTR1</td>
<td></td>
<td>209.5±20.6</td>
<td>220.9±20.5</td>
<td>206.5±20.5</td>
<td>215.4±17.3</td>
<td>214.1±17.9</td>
<td>217.6±27.1</td>
<td>217.6±22.8</td>
<td>214.5±20.6</td>
<td>213.2±18.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BDKRB2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ACE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Face Immersion 2</td>
<td></td>
<td></td>
<td>185.0±24.0</td>
<td>197.8±22.7</td>
<td>191.0±7.3</td>
<td>196.5±15.9</td>
<td>190.8±16.7</td>
<td>181.7±18.3</td>
<td>201.9±24.3</td>
<td>184.6±18.2</td>
<td>188.9±17.9</td>
</tr>
<tr>
<td>Recovery 2</td>
<td></td>
<td></td>
<td>191.4±31.1</td>
<td>197.4±26.2</td>
<td>192.3±6.7</td>
<td>195.1±19.7</td>
<td>193.7±22.0</td>
<td>197.5±18.9</td>
<td>195.6±22.7</td>
<td>198.8±15.1</td>
<td>189.6±13.5</td>
</tr>
<tr>
<td>Face Immersion 3</td>
<td></td>
<td></td>
<td>192.1±25.5</td>
<td>199.8±22.7</td>
<td>213.7±30.6</td>
<td>202.3±3.5</td>
<td>188.8±5.6</td>
<td>196.8±3.9</td>
<td>208.3±25.4</td>
<td>194.1±19.7</td>
<td>185.9±16.1</td>
</tr>
<tr>
<td>Recovery 3</td>
<td></td>
<td></td>
<td>184.1±36.7</td>
<td>197.0±18.7</td>
<td>192.3±9.2</td>
<td>194.7±14.2</td>
<td>193.4±22.0</td>
<td>180.6±48.9</td>
<td>196.6±20.1</td>
<td>191.3±22.5</td>
<td>184.0±11.3</td>
</tr>
</tbody>
</table>

All of the data are the group means ± SD. *p<0.05, **p<0.01 (Mann-Whitney test for paired depended samples) when compared to the combination between the different alleles of the same gene in the same state (control, face immersion, recovery) except for the AGTR1 C/C group.
Table 6. Changes in the systolic blood pressure (mm Hg. V) during face immersion in the water and during recovery

<table>
<thead>
<tr>
<th></th>
<th>Control level</th>
<th>Face Immersion 2</th>
<th>Recovery 2</th>
<th>Face Immersion 3</th>
<th>Recovery 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>114,3</td>
<td>131,8</td>
<td>117,9</td>
<td>132,8</td>
<td>115,4</td>
</tr>
<tr>
<td>SD</td>
<td>10,1</td>
<td>18,7</td>
<td>11,9</td>
<td>19,3</td>
<td>10,3</td>
</tr>
<tr>
<td>p-value, Control level - Face Immersion 2</td>
<td>0,001</td>
<td></td>
<td></td>
<td></td>
<td>0,001</td>
</tr>
<tr>
<td>p-value, Face Immersion 2 - Recovery 2</td>
<td></td>
<td>0,001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-value, Recovery 2 - Face Immersion 3</td>
<td></td>
<td></td>
<td></td>
<td>0,001</td>
<td></td>
</tr>
</tbody>
</table>
Table 7. Changes in the diastolic blood pressure (mm Hg. V) during face immersion in the water and during recovery

<table>
<thead>
<tr>
<th></th>
<th>Control level</th>
<th>Face Immersion 2</th>
<th>Recovery 2</th>
<th>Face Immersion 3</th>
<th>Recovery 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>71.3</td>
<td>89.8</td>
<td>70.9</td>
<td>86.8</td>
<td>70.4</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>10.1</td>
<td>18.7</td>
<td>11.9</td>
<td>19.3</td>
<td>10.3</td>
</tr>
<tr>
<td><strong>p-value,</strong></td>
<td></td>
<td></td>
<td><strong>p-value,</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Control level</strong></td>
<td></td>
<td></td>
<td><strong>Face</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Immersion 2</strong></td>
<td></td>
<td></td>
<td><strong>Immersion 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Recovery 2</strong></td>
<td>0.001</td>
<td></td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Face</strong></td>
<td></td>
<td></td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Immersion 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Recovery 3</strong></td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
I-allele: 497bp

D-allele: 210bp