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Abstract
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Introduction: Susceptibility to decompression sickness (DCS) is characterised by a wide inter-individual variability whose origins are still poorly understood. This hampers reliable prediction of DCS by decompression algorithms. We previously selectively bred rats with a 3-fold greater resistance to DCS than standard rats. Based on its previously reported relation with decompression outcomes, we assessed whether modification in vascular function is associated with resistance to DCS.
Methods: The arterial pressure response to intravenous administration of acetylcholine (ACh, 5 $\mu g.kg^{-1}$) and adrenaline (5 and 10 $\mu g.kg^{-1}$) was compared in anaesthetised DCS-resistant rats (seven females, seven males) and standard Wistar rats (seven females, 10 males) aged 14–15 weeks. None of these rats had previously undergone hyperbaric exposure.
Results: There was a non-significant tendency for a lower diastolic (DBP) and mean blood pressure (MBP) in DCS-resistant rats. After ACh administration, MBP was significantly lower in resistant rats, for both males ($P = 0.007$) and females ($P = 0.034$). After administration of adrenaline 10 $\mu g.kg^{-1}$, DCS-resistant rats exhibited lower maximal DBP ($P = 0.016$) and MBP ($P = 0.038$). Systolic and pulse blood pressure changes did not differ between groups in any of the experiments.
Conclusions: Resistance to DCS in rats is associated to a trend towards a lower vascular tone but not blood pressure reactivity. Whether these differences are a component of the susceptibility to DCS remains to be confirmed.

Introduction

Susceptibility to decompression sickness (DCS) is characterised by a wide interindividual variability in humans. This is documented both by empirical data which have shown that multiple divers can execute the exact same dive profile but not all of them will experience symptoms[1] and by experiments employing animal models of DCS which provide many examples of this huge inter-individual variability for the occurrence of DCS.[2,3]

This could be partly explained by interindividual variability in post-dive venous gas emboli (VGE) formation.[4,5] However, although the occurrence of DCS correlates with VGE detected post-dive,[1] this correlation is weak. These observations clearly show that for the same hyperbaric exposition, the probability of DCS depends on many factors which drive the formation of VGE and/or modulate their power to trigger DCS. Indeed, for a given dive profile the risk of DCS is influenced by many individual factors including body composition,[6] the presence of right-to-left shunts (such as patent foramen ovale)[7] or, in animal models of DCS, hydration.[8] A more complete overview of DCS risk factors is provided elsewhere.[9] Physiological variables including inflammation,[10,11] coagulation,[12,13] oxidative stress,[14] and vascular dysfunction,[14,15] have also been claimed to modulate susceptibility for DCS. However, little consensus has been reached and the primary physiological variables that drive resistance to DCS remain to be specified. A consequence is that not all DCS can be predicted by decompression algorithms based on theoretical models of saturation and bubble formation in divers.[6]

There is a body of data which suggest that the vascular system also might influence both the amount of VGE formed after a dive and the probability of DCS. Indeed, one *in vitro* study showed that bubbles can form at active hydrophobic spots located at the surface of the endothelium.[16] Administration of nitric oxide (NO) donors decreases both the number of VGE detected after a dive[17] and the risk of DCS in animal models,[18,19] whereas inhibition of NO synthase increases it.[20,21] Chronic administration of angiotensin converting enzyme inhibitor before the dive reduces the occurrence of DCS in rats,[22] consistent with a post-dive decrease of angiotensin II in animals with no symptoms of DCS but not those with DCS.[14] Lastly, one study reported significant differences in basal total arterial compliance and stable metabolites of NO in the plasma between divers with low and high bubble grades.[23] Taken together, these data suggest that the viscoelastic properties of the vascular system might influence the susceptibility to DCS.

Our group initiated a large-scale artificial selection program with Wistar rats based on their resistance to DCS, and reported a threefold decrease in DCS occurrence.[24] This selection program now provides a population with significantly increased spontaneous resistance to DCS. First investigations showed that, when compared to standard Wistar rats, these animals exhibit increased leukocyte counts, lower coagulability and lower mitochondrial basal oxygen consumption,[25] as well as modifications of the gut microbiome.[26] At the vascular level, we observed decreased *in vitro* vasorelaxation of the aorta in response to NO donor administration, and no differences in vasoconstriction elicited by phenylephrine or KCl.[25]

Based on the previously reported association of vascular function with decompression outcomes and the apparent contradiction with the lower vasorelaxation capacity observed in our selected animals, we assessed whether increased resistance to DCS is associated with *in vivo* modification in vascular function. To this end we compared arterial pressure response to acetylcholine (ACh) and adrenaline administration in DCS-resistant and standard Wistar rats.

Methods

ETHICAL APPROVAL

The protocol described in this study was conducted in accordance with the Directive 2010/63/EU of the European Parliament and of the council on the Protection of animals used for scientific purposes, and with the French national laws R214-87 to R214-137 of the Rural Code and subsequent modifications. It followed the 3Rs and was approved by the Ethics Committee of the Université de Bretagne Occidentale for Animal Experimentation (approval no. APAFIS#10838-2017072817299340v1 and APAFIS#15628-2018061516233394v3).

ANIMALS

Fourteen DCS-resistant animals (seven females and seven males), aged 14–15 weeks old, bred at the university animal house, were used in this study. They were compared to 17 age-matched standard Wistar rats (seven females and 10 males), i.e., the same as those we used for the founding stock, obtained from the same breeder (Janvier Labs, St Genêts, France). Because the aim was to assess any difference in cardiovascular function associated with resistance to DCS independently of persistent physiological modifications induced by diving itself,[27,28] none of these rats were previously exposed to hyperbaric conditions. The standard rats were acclimated with the facility for at least two weeks. All animals were housed three per cage under controlled temperature (21 ± 1°C) and lighting (12 h of light per day, 0600–1800) at the university animal housing facility until the day of the experiment. They were fed standard rat chow and water *ad libitum*.

ARTERIAL PRESSURE

Following anaesthesia, a temperature probe was inserted rectally and animals were placed in supine position on a warming pad (Z31SY, Ascon tecnologic, Italy) to maintain central body temperature in a normal range (37.5 ± 0.5°C). A 2 cm cervical incision was performed, followed by a tracheostomy (2 mm diameter polyethylene tube). An arterial catheter (Leader Flex 22 G, 0.7 × 40 mm, Vygon, France) was inserted in the right carotid allowing continuous intra-arterial pressure monitoring. A venous catheter was inserted in the left jugular vein (Leader Flex 22 G, 0.7 × 40 mm, Vygon, France) for infusion of drugs. Vital signs (heart rate, invasive arterial pressure and body temperature) were continuously recorded during the procedure (MP35, BIOPAC Systems, Inc. Varna, Bulgaria).

Acetylcholine (5 $\mu g \cdot kg^{-1}$, Sigma, A6625-25G) was first administered. After blood pressure returned to basal values, adrenaline (5 $\mu g \cdot kg^{-1}$, Sigma, E4250-1G) was administered, followed by a 10 $\mu g \cdot kg^{-1}$ dose when a stable blood pressure was reached again. All traces were displayed on a personal computer using Biopac Student Lab Pro 3.7.1 (BIOPAC Systems, Inc. Goleta CA, USA) and stored for later analysis. Diastolic (DBP) and systolic (SBP) blood pressure were measured before administration of each drug and after injection. For each point, mean blood pressure (MBP) was calculated according to the formula MBP = DBP + 1/3 (SBP – DBP). Maximal changes in systolic, diastolic, pulse and mean pressure were determined for each drug and dose.

STATISTICAL ANALYSIS

All data were analysed using Statistica™ software (v. 13, StatSoft France, 2017). Because results were not all parametrically distributed, as assessed with a Shapiro-Wilk test, we used a Kruskal-Wallis ANOVA by ranks test on four independent groups: standard females (StF), standard males (StM), resistant females (ReF) and resistant males (ReM). When a significant difference was detected between groups a Mann-Whitney U post-hoc analysis was run. Differences were considered significant at $P < 0.05$. Data were reported as median (interquartile range [IQR]).

Results

ACETYLCHOLINE

Blood pressure values before and after administration of ACh are presented in Table 1.

Before administration of ACh, no statistically significant difference between groups was detected for SBP and pulse pressure (PP). There was a tendency for a lower DBP and MBP in DCS-resistant than in standard rats, although the differences between groups did not reach statistically significant threshold either.

Table 1

Changes in median (IQR) blood pressure (mmHg) elicited by acetylcholine; DBP – diastolic blood pressure; Max – maximum; MBP – mean blood pressure; PP – pulse pressure; ReF – female DCS-resistant Wistar rats; ReM – male DCS-resistant Wistar rats; SBP – systolic blood pressure; StF – female standard Wistar rats; StM – male standard Wistar rats

Pressure measure	StF		StM		ReF		ReM		*P*-value	
	Basal	ACh	Basal	ACh	Basal	ACh	Basal	ACh	Basal	ACh
DBP	83.7 (65.1–91.6)	38.7 (37.1–41.1)	83.2 (71.9–104.9)	39.2 (37.0–40.5)	59.9 (59.3–90.4)	31.8 (28.3–37.0)	69.5 (63.2–78.1)	34.8 (31.1–41.5)	0.055	0.050
SBP	108.7 (93.4–114.9)	71.1 (70.3–71.5)	108.7 (97.1–133.6)	72.2 (66.5–80.0)	88.6 (85.9–116.6)	65.1 (59.5–70.4)	99.9 (88.2–107.9)	69.5 (57.4–73.9)	0.186	0.128
MBP	92.0 (74.5–99.3)	49.2 (48.3–49.5)	91.7 (80.3–118.6)	48.5 (47.5–53.9)	69.5 (65.0–99.2)	42.8 (39.5–48.1)	77.5 (74.3–88.0)	45.4 (43.4–46.4)	0.066	0.006
PP	25.0 (23.3–28.3)	33.5 (32.4–34.5)	25.2 (24.0–30.5)	30.0 (27.8–38.1)	26.6 (24.6–28.7)	33.1 (32.5–33.5)	30.0 (14.9–33.3)	34.7 (19.9–42.8)	0.875	0.877

Intravenous administration of ACh 5μg.kg^{-1} elicited hypotension in all groups. The Kruskal-Wallis analysis indicated that minimal MBP values after the administration of ACh were significantly different between groups. Post-hoc comparisons indicated that the post-ACh MBP values were significantly lower in resistant than standard animals, for both males (P = 0.007) and females (P = 0.034). The other blood pressure parameters after administration of ACh were not different between groups, although there was a non-statistically significant trend for lower DPB in DCS-resistant individuals than in standard rats. Nevertheless, for all blood pressures, the differences between the basal values and those measured after ACh administration were not different.

ADRENALINE

Kruskal-Wallis analysis indicated significant differences between groups for values of DBP, SBP and MBP obtained both before and after administration of adrenaline 5 μg.kg^{-1}, but not for PP (Table 2).

No differences were detected between groups for arterial pressures before administration of adrenaline 10 μg.kg^{-1}, whereas there were statistically significant differences between groups after injection of the drug for DBP and MBP but not SBP and PP (Table 3). Post-hoc testing indicated that maximum DBP was significantly lower in females rats resistant to DCS than in standard rats (P = 0.030). However, as was the case for ACh administration, the differences in blood pressures between the basal values and those measured after adrenaline administrations were not different between groups.

Discussion

We found lower MBP values after administration of ACh in DCS-resistant than standard rats of both sexes. In contrast, after administration of 10 μg.kg^{-1} adrenaline the hypertensive response was weaker in DCS-resistant than standard rats, as indicated by lower maximum values of DBP and MBP, which was more evident in females. However, the amplitude of the responses to both ACh and adrenaline were not different between resistant and standard animals. This was probably because of a trend (although non-significant) to lower basal pressures in resistant animals.

Susceptibility to DCS is characterised by substantial interindividual variability, which is particularly well documented in animal models.[2,3] Such variability also exists in divers[29] and is one of the causes of so-called 'undeserved' DCS since current decompression algorithms cannot take it into account. Indeed, one study reported that 97.5% of the DCS cases recorded in the DAN DSL database occurred without violation of the algorithm recommendations.[6] This 'probabilistic' character of the susceptibility to DCS also hampers studies of its determinants. To overcome this limitation we selectively bred Wistar rats based on their resistance to DCS. Indeed, the ratio of asymptomatic animals

Table 2

Changes in median (IQR) blood pressure (mmHg) elicited by adrenaline 5 μg.kg^{-1}; DBP – diastolic blood pressure; Max – maximum; MBP – mean blood pressure; PP – pulse pressure; ReF – female DCS-resistant Wistar rats; ReM – male DCS-resistant Wistar rats; SBP – systolic blood pressure; StF – female standard Wistar rats; StM – male standard Wistar rats

Pressure measure	StF		StM		ReF		ReM		*P*-value	
	Basal	Max	Basal	Max	Basal	Max	Basal	Max	Basal	Max
DBP	79.4 (70.7–87.5)	136.7 (135.0–165.0)	89.9 (77.9–95.7)	148.9 (144.7–152.7)	65.3 (48.6–70.1)	121.2 (98.0–123.2)	80.3 (58.8–104)	129.9 (117.6–142.6)	0.026	0.010
SBP	101.3 (94.7–111.1)	199.4 (197.4–201.6)	119.2 (111.5–131.2)	196.1 (185.8–208.8)	91.1 (79.0–94.7)	168.0 (130.7–170.6)	102.1 (96.1–108.4)	171.3 (162.8–185.8)	0.088	0.002
MBP	86.0 (80.0–95.4)	159.1 (156.2–177.2)	99.7 (88.1–107.6)	167.1 (161.9–171.0)	74.6 (58.8–77.0)	137.6 (108.9–143.1)	85.6 (71.3–94.8)	145.6 (135.6–155.3)	0.018	0.001
PP	23.5 (20.5–27.8)	57.5 (36.6–61.7)	29.3 (24.3–34.3)	51.4 (34.2–64.4)	26.7 (21.7–28.0)	33.0 (29.6–49.4)	25.8 (15.8–31.3)	38.6 (29.0–54.2)	0.651	0.516

Table 3

Changes in median (IQR) blood pressure (mmHg) elicited by adrenalin 10 μg.kg^{-1}; DBP – diastolic blood pressure; Max – maximum; MBP – mean blood pressure; PP – pulse pressure; ReF – female DCS-resistant Wistar rats; ReM – male DCS-resistant Wistar rats; SBP – systolic blood pressure; StF – female standard Wistar rats; StM – male standard Wistar rats

Pressure measure	StF		StM		ReF		ReM		*P*-value	
	Basal	Max	Basal	Max	Basal	Max	Basal	Max	Basal	Max
DBP	108.7 (83.7–111.2)	151.4 (145.5–159.5)	75.4 (73.9–98.7)	162.1 (159.0–166.0)	96.3 (63.7–114.7)	136.9 (129.4–141.8)	85.8 (57.7–107.9)	148.7 (133.0–166.4)	0.242	0.016
SBP	137.6 (109.4–150.9)	210.4 (204.6–223.7)	103.5 (99.0–129.7)	211.7 (193.4–222.3)	128.5 (107.5–148.4)	205.2 (200.8–215.3)	110.4 (96.4–126.3)	192.2 (177.8–204.4)	0.243	0.306
MBP	118.3 (88.3–124.4)	173.8 (169.1–178.2)	83.4 (82.4–109.1)	176.0 (175.1–185.8)	107.1 (78.3–128.9)	160.7 (157.5–161.5)	94.0 (70.6–114.0)	166.2 (157.0–175.0)	0.253	0.038
PP	33.7 (25.7–35.3)	57.4 (27.6–70.7)	28.6 (24.6–31.4)	41.7 (36.4–62.7)	32.9 (29.9–42.5)	59.9 (59.0–76.2)	29.9 (18.4–38.7)	37.0 (25.8–57.1)	0.545	0.509

rose from 35% in the non-selected Wistar rats to 80% and 72% in selected females and males, respectively.[25] Now that we have a population that is significantly different from normal in its resistance to DCS, our objective is to investigate the physiological characteristics of these individuals that may drive this resistance.

It is now well accepted that the risk of DCS depends not only on the amount of VGE formed during and after decompression but also the ability to cope with them, both being influenced by individual factors. Vascular function is one of a number of physiological risk factors proposed.[10–14] For instance, one study found that divers with lower bubble grades after a dive also had lower SBP and PP before the dive.[23] In keeping with these previous data, although the difference between groups did not reach statistical significance in the present experiment, we also observed that before any intervention (i.e., before administration of ACh) the DCS-resistant rats tended to have lower diastolic and mean blood pressure than the standard rats.

We found both greater hypotension in response to ACh and weaker adrenaline-induced hypertension in the rats resistant to DCS. Moreover, we observed these differences for DBP and MBP only, and not for SBP or PP. Since the changes between basal and post-infusion blood pressures were not different, it seems plausible that resistance to DCS could be associated with a general trend towards lower total peripheral vascular resistance but not vascular reactivity. One study reported that mean arterial blood pressure was increased in anaesthetised rats during a simulated air dive at 600 kPa, which was due to an increase in total peripheral vascular resistance which developed within five minutes.[30] This hypertensive response to hyperbaric exposure is confluent with an earlier study which reported decreased blood flow in skeletal muscles of Wistar rats exposed to 500 kPa He-N_2-O_2.[31] It is therefore plausible that the shift in the blood pressure observed in our DCS-resistant animals would at least partially counteract the hypertensive effect of diving by limiting the maximal total peripheral vascular resistance at depth. This is still to be confirmed and, even if so, whether this represents an advantage for the resistance to DCS remain to be determined. However, we showed previously that chronic treatment with nifedipine, which lowers arterial pressure, before the dive did not influence the risk of DCS in rats.[22] This suggests that factors that affect blood pressure, rather than the blood pressure itself, may influence resistance to DCS.

This hypothesis agrees with the pre-dive higher plasma concentration of NO metabolites previously reported in divers who produce lower grade bubbles after the dive.[23] It is also confluent with previous studies showing that the administration of NO donors decreases both the amount of VGE detected in humans after a dive[17] and the risk of DCS in animal models,[18,19] whereas inhibition of the NO synthase increases it.[20,21] Similarly, chronic administration of angiotensin converting enzyme inhibitor, but not angiotensin receptor antagonists, before the dive reduces occurrence of DCS in rats.[22] This result is confluent with the post-dive decrease of angiotensin II in animals with no symptoms of DCS but not those with DCS[14] and with the decreased plasma concentrations of adrenaline and noradrenaline in humans after a dive.[32] Unfortunately, we did not measure circulating concentrations of NO, angiotensin II or adrenaline in this study. However, we previously reported decreased coagulation tendency, a function influenced by both NO and angiotensin II, in male rats selected for their resistance to DCS.[25] This remains to be confirmed.

LIMITATIONS

In this study, we used standard Wistar rats obtained from an approved provider as control rats. Even if the DCS-resistant animals were derived from animals of the same Wistar strain obtained from the same provider, the standard and resistant animals used for this study were not bred in the same conditions since their birth. This might have influenced physiological parameters independently from the resistance to DCS. However, standard rats were kept for two weeks before the experiments which probably limited this potential bias. Additionally, our previous experiments showed that it is unlikely that our breeding conditions alone induced such a resistance.[25] Another limitation arises from our approach which compared animals of differing resistance to DCS but which were not exposed to a simulated dive. It is therefore possible that the differences we found between these groups may represent collateral modifications only. To experimentally question the relationship between these alterations of the vascular function and resistance to DCS is the subject of continued investigation by our research group and others.

Conclusion

This study revealed a possible shift towards lower basal blood pressure in rats animals bred to be resistant to DCS with no difference in responses to hypo- and hypertensive drugs when compared to standard rats. These differences are compatible with differences in vasoactive circulating factors and might represent a possible mechanism of DCS-resistance.

Currently-used decompression procedures based on calculated algorithms are presently considered to be relatively safe. Nevertheless, the fact that DCS still occurs even without violation of the algorithm recommendations[6] indicates that, for at least a proportion of the diver population, current algorithms are not conservative enough. It is now well recognised that improvements in decompression algorithms based primarily on biophysical models, may be possible by identifying and modifying a diver's individual risk factors.

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