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Bradykinin receptor gene variant and human physical performance

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Williams, Alun G., Sukhbir S. Dhamrait, Peter T. E. Wootton, Stephen H. Day, Emma Hawe, John R. Payne, Saul G. Myerson, Michael World, Richard Budgett, Steve E. Humphries, and Hugh E. Montgomery. Bradykinin receptor gene variant and human physical performance. *J Appl Physiol* 96: 938–942, 2004. First published November 7, 2003; 10.1152/japplphysiol.00865.2003.—Accumulating evidence suggests that athletic performance is strongly influenced by genetic variation. One such locus of influence is the gene for angiotensin-I converting enzyme (ACE), which exhibits a common variant [ACE insertion (I)/deletion (D)]. ACE can drive formation of vasoconstrictor ANG II but preferentially degrades vasodilator bradykinin. The ACE I allele is associated with higher kinin activity. A common gene variant in the kinin β_2 receptor (B_2R) exists: the -9 as opposed to $+9$ allele is associated with higher receptor mRNA expression. We tested whether this variant was associated with the efficiency of muscular contraction [delta efficiency (DE)] in 115 healthy men and women, or with running distance among 81 Olympic standard track athletes. We further sought evidence of biological interaction with ACE I/D genotype. DE was highly significantly associated with B_2R genotype (23.84 ± 2.41 vs. 24.25 ± 2.81 vs. 26.05 ± 2.26% for those of $+9/+9$ vs. $+9/-9$ vs. $-9/-9$ genotype; $n = 25$, 61, and 29, respectively; $P = 0.0008$ for ANOVA adjusted for sex). There was evidence for interaction with ACE I/D genotype, with individuals who were ACE II, with B_2R $-9/-9$ having the highest DE at baseline. The ACE I/B_2R -9 "high kinin receptor activity" haplotype was significantly associated with endurance (predominantly aerobic) event among elite athletes ($P = 0.003$). These data suggest that common genetic variation in the B_2R is associated with efficiency of skeletal muscle contraction and with distance event of elite track athletes and that at least part of the associations of ACE and fitness phenotypes is through elevation of kinin activity.

polymorphism; angiotensin-converting enzyme; skeletal muscle; elite athlete

GLOBAL INDEXES OF HUMAN ATHLETIC performance (36), as well as more precise measures of human skeletal muscle function (42), are strongly influenced by genetic as well as environmental factors. To date, few genetic loci of influence have been identified (47). One such is the gene for angiotensin-I converting enzyme (ACE) (15, 28, 30, 44, 45).

Endocrine ACE plays a prominent role in circulatory homeostasis, being responsible for the genesis of the vasoconstrictor angiotensin II (ANG II) and for the degradation of bradykinin, a vasodilator through its action at the bradykinin β_2 receptor (B_2R) BDKRB2. However, the majority of total body ACE is found in tissue compartments (10), where it may serve quite distinct, localized, and varied roles (13). Within such diverse tissues, the presence [insertion (I)], rather than the absence [deletion (D)], of a 287-base pair Alu repeat sequence in the gene for ACE is associated with lower ACE activity (8, 11).

ACE expressed in human skeletal muscle (38) would seem to influence its function: the ACE I allele has been associated with increased training-related gains in fatigue resistance (28) and contractile efficiency (44), and the D allele with improvements in strength (15). Similarly, the I allele is associated with elite endurance performance both at sea level (30) and at altitude (28), and the D allele is associated with performance over shorter distances in runners, rowers, and swimmers (30, 45). However, there is some contradictory evidence regarding the influence of the ACE gene on endurance performance (35, 37, 41).

Such effects may be mediated through alterations in levels of either ANG II or bradykinin. ANG II has recognized effects on metabolism (2) and is a recognized growth factor necessary for the hypertrophy of skeletal muscle in response to mechanical load (19). Levels of bradykinin, meanwhile, are dependent on ACE genotype (29) and may influence skeletal muscle glucose uptake and muscle blood flow (43). Evidence suggests, however, that the allelic association with endurance phenotypes might be partly mediated through alterations in skeletal muscle mechanical and metabolic efficiency (44). Such demonstrable increases in efficiency of exertionally related oxygen utilization (46) might also explain the more powerful association of the I allele with elite mountaineering (where prolonged exercise occurs under hypoxic conditions) than endurance performance at sea level (28, 30).

The absence (-9), rather than the presence ($+9$), of a 9-base pair repeat in exon 1 of the gene encoding the B_2R is associated with higher gene transcriptional activity (1), higher receptor mRNA expression (25), and a reduced cardiac trophic response to exercise training (4). If the effects of ACE on human skeletal muscle function and its role in influencing more global aspects of performance are mediated through bradykinin, then we might anticipate B_2R genotype to be similarly associated with muscle function. We have examined this hypothesis.

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METHODS

Two studies were performed. In each case, there was appropriate army and university ethics committee approval, with written, informed consent obtained from each participant. All subjects and staff were blind to genotype during experimentation and data analysis.

Efficiency of contraction of skeletal muscle. Subjects were drawn from two sources. All were free from significant cardiorespiratory or musculoskeletal disease and were taking no medication. Men ($n = 73$) were drawn from consecutive Caucasian male British army recruits, selected for homozygosity for the ACE I/D variant, and studied at the start of training (44). Female subjects ($n = 42$) were sedentary Caucasian adult volunteers recruited from the staff and students of Staffordshire University, UK. All subjects exercised on an electrically braked cycle ergometer (Lode Rehcor, Lode, Netherlands) at 60 rpm at external power outputs of 40, 60, and 80 W for 3 min per stage. The ergometer was calibrated in accordance with the manufacturer's instructions, and seat and handlebar height and angle were adjusted appropriately for individual subjects. Expired air was analyzed breath by breath (Cardiokinetics measurement cart, Medical Graphics), and heart rate was monitored telemetrically (Polar Electro, Polar, Kempele, Finland). Subjects wore a nose clip and breathed through a low dead space (39 ml) mouthpiece and volume sensor assembly. Gases were drawn continuously from the mouthpiece assembly through a capillary line and analyzed for O_2 and CO_2 concentrations by rapid-response analyzers (O_2: zirconia type; CO_2: infrared absorption). The system underwent satisfactory weekly physiological calibration and was calibrated before each test with gases of known concentration (Medical Graphics). Expiratory volumes were determined by using a bidirectional differential pressure pneumotach (32), which was calibrated before each test with a 3-liter graduated gas syringe (Hans-Rudolph, Kansas City, MO), according to the manufacturer's instructions. The rate of oxygen uptake ($\dot{V}O_2$) and respiratory exchange ratio (RER) data was assessed by two reviewers independently, who confirmed that each subject was in steady state during the third minute of exercise at each stage. The data were then averaged for each 30-s period. For the group as a whole, there were no significant differences between the $\dot{V}O_2$ and RER data for the fifth and sixth 30-s periods at each stage (all $P > 0.05$, paired *t*-tests). Consequently, the mean $\dot{V}O_2$ and RER of the fifth and sixth 30-s periods (i.e., from 2–3 min) at the 40- and 80-W stages for each subject were calculated. A conversion factor dependent on RER was applied to the $\dot{V}O_2$ measured, to give rate of energy expenditure (3). The efficiency of contraction in human skeletal muscle is defined as the energy used per unit power output. A measure of this, delta efficiency (DE), was calculated as the ratio of the change in work performed per minute (i.e., 2,400 J between 40- and 80-W stages) to the change in energy expended per minute, expressed as a percentage (17) and related to B_2R genotype.

Elite athletes. The cohort studied has previously been described in detail (30). In brief, 91 British Olympic-standard runners (48 men, 43 women; 79 Caucasian) were grouped by independent experts according to distance run: ≤200 m (predominantly anaerobic or power), 400–3,000 m (mixed aerobic and anaerobic), and ≥5,000 m (predominantly aerobic or endurance trained) (30).

Genotyping. DNA was isolated from either buccal cells or peripheral blood leukocytes, as previously described (30). B_2R genotype was ascertained with forward 5′-TCTGGCTTCTGGGCTCCGAG-3′ and reverse 5′-AGCGGCATGGGCACTTCAGT-3′ primers and ACE genotype by using a three-primer PCR amplification, with products resolved on a 7.5% polyacrylamide gel by two independent staff blind to all subject data (12). B_2R genotype determination was possible in only 81 of 91 of these runners due to degradation of DNA.

Data analysis. For the whole sample, characteristics were compared between genotype groups and between groups defined by the presence or absence of a specific allele, using one-way ANOVA, two-tailed unpaired *t*-tests, linear trend analysis, and one-way analysis of covariance with gender as covariate. Within each gender, characteristics were compared between genotype groups and between allele groups by using one-way ANOVA, two-tailed unpaired *t*-tests, and linear trend. All data were analyzed by using SPSS version 11 and "intercooled STATA" software version 7.0 (STATA). Frequency of alleles or haplotypes across the competitive distances were compared by χ^2 test for linear trend or by Fisher's exact test, respectively, by using the distance run as the categorical variable. Data are presented as means ± SD. *P* values of <0.05 were considered statistically significant.

RESULTS

Efficiency of contraction of skeletal muscle. B_2R genotype distribution in the group overall ($n = 115$; 29 vs. 61 vs. 25 for −9/−9 vs. −9/+9 vs. +9/+9) was consistent with Hardy-Weinberg equilibrium and similar to that previously reported (1, 4, 25). Characteristics [men: age 19.3 ± 2.5 yr, height 1.78 ± 0.06 m, body mass index (BMI) 22.48 ± 2.29 kg/m^2, women: age 23.2 ± 6.2 yr, height 1.66 ± 0.05 m, BMI 24.16 ± 2.96 kg/m^2] were independent of B_2R genotype. Male characteristics were similar to those of the consecutive cohort from whom they were drawn.

Mean cohort DE was 24.98 ± 2.77%, but tended to be lower in women than in men (23.98 ± 2.52 and 24.98 ± 2.77%, respectively, $P = 0.058$). There was no association of age, height, mass, or BMI with DE. DE was highly significantly associated with B_2R genotype (23.84 ± 2.41 vs. 24.25 ± 2.81 vs. 26.05 ± 2.26% for those of +9/+9 vs. +9/−9 vs. −9/−9 genotype, $P = 0.003$ by ANOVA, $P = 0.002$ for linear trend; $P = 0.001$ for +9 allele vs. −9/−9 carriers; Fig. 1). This significance increased after adjustment for gender ($P = 0.0008$ for ANOVA, $P = 0.0011$ linear trend), and the data remained significant after adjustment for all demographic data ($P = 0.003$ for ANOVA). Multivariate analysis, including gender as a covariate, suggested that B_2R genotype accounted for 11.2% of the interindividual variability in DE.

As previously reported, there was no association between ACE genotype and DE (44). We sought to examine whether there was any biological interaction between ACE and B_2R genotypes in influencing DE (Fig. 2). Among the 45 subjects of ACE DD genotype, DE tended to be highest among B_2R −9/−9 homozygotes (23.45 ± 2.81 vs. 24.06 ± 3.15 vs. 25.30 ± 1.65% for +9/+9 vs. +9/−9 vs. −9/−9), although, due to the substantial reduction in sample size, this difference was not statistically significant ($P = 0.233$ for ANOVA , $P = 0.097$ for linear trend, $P = 0.104$ for +9 allele vs. −9/−9 carriers). However, B_2R genotype significantly influenced DE

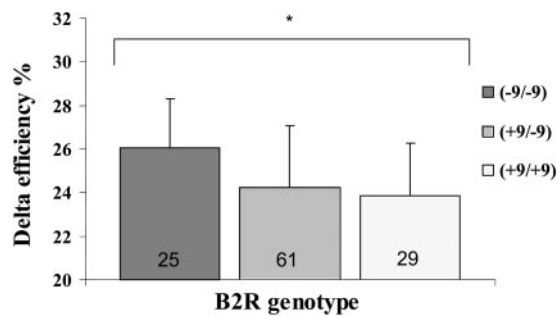

Fig. 1. Delta efficiency according to bradykinin β_2 receptor (B_2R) (+9/−9) genotype in 115 sedentary men and women. Values are means ± SD. *$P = 0.0008$ for ANOVA, $P = 0.0011$ linear trend adjusted for gender. No. of subjects in each group is displayed at the base of each bar.

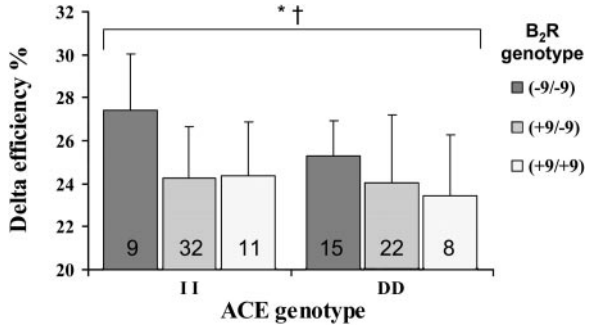

Fig. 2. Delta efficiency in 115 men and women differs significantly across angiotensin-I converting enzyme (ACE) and BDKRB2 haplotypes. Values are means ± SD. *$P = 0.0045$ for haplotypes ranked above, adjusted for gender. †$P = 0.0007$ for comparison of II (−9/−9) vs. DD (+9/+9) adjusted for gender. I, insertion; D, deletion. No. of subjects in each group is displayed at the base of each bar.

for those individuals who were of ACE II genotype (24.34 ± 2.51 vs. 24.26 ± 2.41 vs. 27.41 ± 2.61% for +9/+9 vs. +9/−9 vs. −9/−9, $P = 0.005$ by ANOVA, $P = 0.013$ for linear trend; $P = 0.0008$ for +9 allele vs. −9/−9 carriers). DE was associated with ACE/B_2R ranked haplotypes ($P = 0.0045$ for linear trend adjusted for gender, haplotypes ranked according to Fig. 2). DE was significantly higher in individuals with the highest predicted kinin receptor activity (ACE II, B_2R −9/−9) compared with lowest kinin receptor activity (ACE DD, B_2R +9/+9; $P = 0.0007$ ANOVA adjusted for gender).

B_2R genotype in elite athletes. The B_2R genotype frequency was in Hardy-Weinberg equilibrium, and rare (−9) allele frequency [0.46 (range 0.39–0.54)] was similar to previous reports (1, 4, 25). Among the 81 runners, analysis revealed a linear trend of increasing −9 allele frequency with distance run. The proportion of −9 alleles increased from 0.382 to 0.412 to 0.569 for those athletes running ≤200 m ($n = 17$), 400–3,000 m ($n = 35$), and ≥5,000 m ($n = 29$), respectively ($P = 0.06$ for linear trend; $P = 0.04$ for comparison of ≤5,000 vs. ≥5,000 m). ACE and B_2R haplotypic analysis demonstrated a significant relationship with distance run (≤5,000 vs. ≥5,000 m), both overall ($P = 0.001$ Fisher's exact test) and for Caucasians only ($P = 0.003$), with a greater proportion of "low kinin receptor activity" (ACE D allele, B_2R +9 allele) in events <5,000 m and, conversely, a greater proportion of "high kinin receptor activity" haplotypes (ACE I allele, B_2R −9 allele) competing in events >5,000 m (Fig. 3).

DISCUSSION

Our data suggest that the B_2R −9 (rather than +9) allele is associated with higher skeletal muscle metabolic efficiency and also with endurance athletic performance. Moreover, these associations were greatest among individuals with highest kinin receptor activity as marked by the ACE I (high kinin ligand generation) allele (29) and B_2R −9 (high receptor expression) allele (25). Such data support recent linkage analyses, which suggest an effect of a locus near to the B_2R gene on performance-related phenotypes, such as cardiac output and stroke volume (34).

Such data are important for two reasons. First, it has been suggested that the ACE I/D polymorphism is in strong allelic association with functional variants in adjacent genes (such as that for growth hormone), and that these (and not ACE phenotype) are responsible for the observed associations with ACE genotype (35). However, our data suggest that this is not the case, given the demonstration of a similar (and biologically plausible) effect of a downstream receptor. In this regard, our data support past studies suggesting such linkage disequilibrium to be unlikely (28, 44). Second, the ACE I allele has been associated with increased metabolic efficiency (44) and with endurance performance (18, 28, 30), and these are the first data to implicate a specific underlying mechanism. At least some of these associations between ACE genotype and performance seem to be mediated through alterations in kinin activity at the B_2R, given that the ACE I allele is associated with increased kinin activity (29) and that a genetic marker of higher kinin receptor expression (1, 25) is now associated with the same performance phenotypes. Association with other genetic variants (such as the −58CT promoter variant) or haplotypes in the B_2R gene should be sought as confirmation of these data. Furthermore, in vivo work is also required to relate the ACE/B_2R haplotypes to kinin metabolism and responses. However, these haplotypic data do support our previous observation relating these ACE/B_2R haplotypes to prospective exercise-induced left ventricular growth (4).

Skeletal muscle contains a complete kallikrein-kinin system (26), can liberate kinins locally (23), and expresses functional B_2 receptors (14, 33). However, it is not yet clear precisely how kinin activity affects the endurance performance phenotypes studied here. Bradykinin generated within exercising skeletal muscle (23) may influence muscle blood flow and skeletal muscle glucose uptake (43). In fact, through the B_2R (40), bradykinin enhances insulin-stimulated tyrosine kinase activity of the insulin receptor, with subsequent GLUT-4 translocation in skeletal muscle tissue during exercise (40). B_2R activation can lead to transient rises in inositol 1,4,5-trisphosphate (33), which is involved in excitation coupling of skeletal muscle (16, 20) via increases in cytoplasmic calcium (24). This process is enhanced both by insulin (21) and by inhibition of ACE (22). Bradykinin-induced nitric oxide (NO) generation may also modulate mitochondrial respiratory control (27). NO is a vasodilator that, at physiological concentrations, reversibly inhibits cytochrome-*c* oxidase (mitochondrial complex IV) in

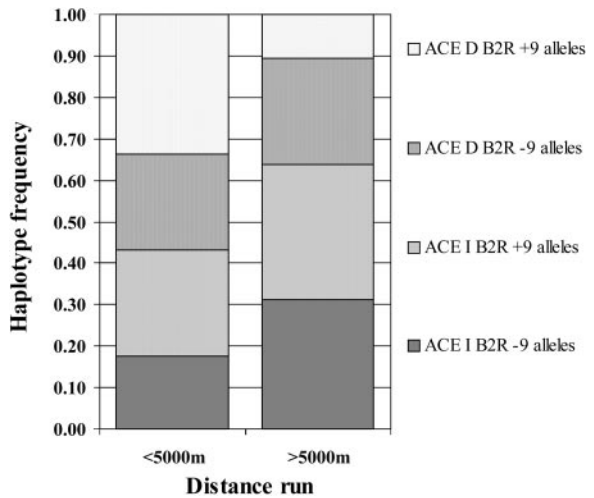

Fig. 3. ACE/BDKRB2 haplotypes differ significantly in 81 Olympic standard runners stratified by distance run ($P = 0.001$).

competition with oxygen (6) and thus reduces $\dot{V}_{O_2}$ in skeletal muscle and heart mitochondria (6, 31). It has been suggested that the interplay between NO and oxygen allows cytochrome-*c* oxidase to act as an oxygen sensor within cells (7). NO donors have also been shown to reversibly inhibit oxygen utilization in rat skeletal muscle mitochondria (6). Tissue and whole animal studies have shown that kinins can suppress oxygen consumption via endogenous NO production in skeletal (39) and cardiac muscle (49), an effect mimicked by ACE inhibition and prevented by blockade of B_2R (49). It may also be that B_2R genotype influences skeletal muscle fiber type. The relative proportion of type I (slow twitch, oxidative) to type IIA (fast oxidative) and type IIB (fast glycolytic) skeletal muscle fibers has a strong influence on propensity to endurance or sprint performance (9) and also influences DE (9), whereas ACE I/D genotype has recently been associated with fiber-type distribution (48).

Conversely, such a role for bradykinin does not exclude a contribution for ANG II in mediating the effects of ACE. Chronic ANG II infusion results in profound metabolic cachexia in rodents (2), with muscle catabolism and increased energy expenditure allied with changes in oxygen consumption (5). As a powerful growth factor, it is also necessary for the hypertrophy of skeletal muscle in response to mechanical load (19).

Evidently, further studies are required to confirm these observations among other comparable groups of athletes. The association of genotype with relative ranked performance among endurance athletes should also be sought. Such studies should also include those of other ages and race. The small number of ACE ID heterozygotes ($n = 18$) restricted the ability to assess the ACE/B_2R haplotypic association with DE within this group. This inability in no way weakens our observations, but further studies should be performed if allele codominant influences on haplotype response are to be sought. In addition, no single gene will determine (exclusively) propensity to a given sporting discipline, and any association does not demonstrate the underlying mechanism of causation. By combining association study of phenotype class with a mechanistic study, we have attempted to overcome such problems. However, these data do suggest that bradykinin, acting via the B_2R, has a role in regulating skeletal muscle performance. The implications of such findings go beyond sports alone and may extend to the management of patients with cardiovascular, respiratory, and metabolic diseases, in which muscle function is adversely affected.

GRANTS

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