Unchanged [3H]ouabain binding site content but reduced Na+-K+ pump α2-protein abundance in skeletal muscle in older adults

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McKenna MJ, Perry BD, Serpiello FR, Caldow MK, Levinger P, Cameron-Smith D, Levinger I. Unchanged [3H]ouabain binding site content but reduced Na+-K+ pump α2-protein abundance in skeletal muscle in older adults. J Appl Physiol 113: 1505–1511, 2012. First published August 30, 2012; doi:10.1152/japplphysiol.01032.2011.—Aging is associated with reduced muscle mass, weakness, and increased fatigability. In elderly can lead to increased risk of falls, reduced mobility, poor health, and increased susceptibility for developing metabolic diseases, such as obesity and type 2 diabetes.

The Na+-K+ pump (NKA) is a ubiquitously expressed protein in all mammalian cells (6). In skeletal muscle, the NKA regulates Na+-K+ concentration gradients across the sarcolemmal and transverse tubular membranes, thereby modulating membrane potential, excitability, as well as force (9, 55). The NKA in skeletal muscle is highly regulated at rest and during exercise by a variety of hormonal, ionic, redox, acid-base, and contractile factors (9, 38, 42, 56). Although the NKA plays a vital role in regulating muscle Na+-K+ homeostasis, the control of membrane excitability is complex, particularly during muscle contraction, where changes in Cl− conductance and acidosis play additional modulatory roles (37, 48, 49).

The NKA family comprises four α- (α1-4) and three β- (β1-3) NKA isoforms, with both α- and β-subunits required for NKA activity (6). A further regulatory protein, phospholemman, is also associated with and modulates NKA activity in skeletal muscle (53). In rat muscles, fiber-type specificity of NKA α- and β-isofrom expression has been observed (24), and the α2-isoform accounts for up to 85% of the total α-subunits expressed (19). Both α1- and α2-isoforms were found to affect muscle force in α1- and (perinatal) α2 knockout mice, with stronger function for α1 in the maintenance of membrane excitability and for the more abundant α2-isoforms in contractility (22, 52). Thus any decline in NKA α1- and/or α2-isoform expression may contribute to impaired muscle function, via a decline in muscle excitability and contractility. Human skeletal muscle expresses three NKA α- (α1-3) and three β- (β1-3) isoforms at mRNA and protein levels (42, 45, 50), with α2-mRNA also detected at very low levels (45). The exact roles of these NKA isoforms remains unclear in human skeletal muscle function.

The effects of aging on skeletal muscle NKA are also unclear. Muscle-specific changes in NKA isoform abundance and in ouabain binding have been reported with aging in rat muscles, but the findings between studies are not always consistent (30, 43, 57, 61). The [3H]ouabain binding site content reflects binding to, and thus full quantification of, the functional NKA capacity (12). In rodent muscle, the [3H]ouabain binding detects the predominant NKA α2-isoform and thus is insensitive to the α1-isoform (9). In rat soleus muscle, the [3H]ouabain binding site content measured in intact or cut portions of muscles fell by ~35% between 12 and 86 wk of age (30). In contrast, [3H]ouabain binding measured in rat muscle homogenates was increased in red gastrocnemius muscle, by 118%...
between 6 and 30 mo, while a 30% decline was found in white gastrocnemius muscle (57). In human muscle, ouabain binds to all α-isoforms with high affinity, and thus the \[^{[3]H}\]ouabain binding site content is a measure of total NKA content in muscle. Few studies have investigated the effects of aging on NKA content in skeletal muscle in humans. No significant differences were reported between elderly and young adults (31, 46) or between two separate studies in the same laboratory (9, 14). Although the muscle \[^{[3]H}\]ouabain binding did not differ significantly between six young (mean age 28 yr) and six older controls (mean age 68 yr), a trend for a 14% lower NKA content was reported in the elderly (31). No age effect was seen in \[^{[3]H}\]ouabain binding measured in 9 healthy individuals ranging from 25 to 53 yr and 11 healthy individuals from 53 to 80 yr (46). Furthermore, no evidence of an aging effect was found with similar muscle \[^{[3]H}\]ouabain binding values in children (0–8 yr) to previously determined values for adults (29). Nonetheless, a possible aging-related decline in NKA content or function in human muscle is evidenced by impaired K\(^+\) regulation, where elderly individuals exhibited a greater rate of rise in venous plasma K\(^+\) concentration during exercise (15) and a reduced terbutaline-stimulated K\(^+\) uptake into forearm muscles (16). A greater rise in muscle interstitial K\(^+\) concentration in stimulated muscles was also found in old than in young rats (36).

Aging induces changes in NKA isoform expression in rat muscles. Similar directional changes were observed in three studies that compared rats aged between 6 mo (57, 61) or 16 mo (43) vs. 30 mo, comprising increased α₁, α₂, and β₁, and decreased α₂ and β₂-isoform abundance (43, 57, 61). However, these changes were qualitatively highly inconsistent for some isoforms. Importantly, nothing is known about the impacts of aging on NKA isoform gene expression or protein abundance in human muscle. Such information may be important in understanding \[^{[3]H}\]ouabain binding in human muscle, since, in rats, aging was associated with reduced α₂, but with large increases in α₁, abundance. However, changes in \[^{[3]H}\]ouabain binding with aging in rat muscles surprisingly did not correspond to the decline in α₂-isoform abundance (57).

Therefore, this study investigated the effects of aging on skeletal muscle NKA content and on NKA isoform gene expression and protein abundance in humans. We hypothesized that both the \[^{[3]H}\]ouabain binding site content and the α₂-isoform abundance would be reduced in skeletal muscle in the elderly, compared with young individuals.

**METHODS**

**Participants**

Seventeen older adults (Elderly; 9 men and 8 women; age 66.8 ± 6.4 yr, mean ± SD) and 16 young individuals (Young; 8 men and 8 women; 23.9 ± 2.2 yr, P < 0.001 between groups) participated in the study. Height was similar between groups (Elderly, 168.6 ± 7.6 cm vs. Young, 171.6 ± 9.1 cm, P = 0.30), whereas the Elderly had higher body mass (82.7 ± 16.9 kg vs. 71.2 ± 15.0 kg, P = 0.046) and body mass index (29.0 ± 4.7 vs. 23.9 ± 3.2 kg/m², P < 0.001), respectively. Some people from both groups were recreationally active. The two groups reported a similar weekly duration of physical activity (Elderly, 11.6 ± 10.2 vs. Young, 7.4 ± 8.2 h/wk, P = 0.33), although the Young were typically engaged in a higher intensity of exercise (e.g., running rather than walking). Elderly participants receiving medications, included anti-diabetic (n = 2), anti-cholesterol (n = 2), β-blockers (n = 3), angiotensin II receptor antagonist (n = 2), platelet inhibitor (n = 1), angiotensin I-converting enzyme inhibitor (n = 3), and aspirin (n = 2). No Young participants received medication. Each participant received written and verbal explanations about the nature of the study before signing informed consent. The study protocol was approved by the Victoria University Human Research Ethics Committee.

**Test Procedures**

Participants underwent anthropometric (height, mass) and strength measurements, performed an incremental exercise test to determine peak oxygen uptake (VO₂peak), and underwent a vastus lateralis muscle biopsy procedure.

**Muscle strength.** Isometric muscle strength was measured in a smaller cohort (Elderly, n = 7; Young, n = 14) in the leg from which the muscle biopsy was taken (usually nondominant). Muscle force was measured using a nonextendable strain gauge, attached to the participant’s leg using a webbing strap with a Velcro fastener. The participant sat in a tall chair with a strap around the lower leg and with the hip and knee joint angles at a 90° angle. The distance from the knee joint to the strap around the ankle was measured with a tape measure and used for the calculation of torque (Nm). The participant exerted maximal force against the strap assembly for 3 s during each of three trials, with the highest torque used for analyses and torque expressed normalized for body mass.

**Rating of perceived exertion-limited VO₂peak test.** The VO₂peak was assessed during a rating of perceived exertion (RPE)-limited graded exercise test on a cycle ergometer (Cybex MET 100, Cybex Metabolic Systems, Ronkonkoma, NY), as described previously (34). Participants commenced cycling at 25 W, then at 40 W in the 2nd min, and followed thereafter increments of 20 W each minute. The tests were terminated when participants’ RPE reached “very hard”, as indicated on a Borg scale = 17 (7). The O₂ uptake for each 15-s interval was measured by an automated gas analysis system (Medgraphics, Cardio2 and CPX/D System with Breezeex Software, 142090–001, Revia, MN). The system was calibrated before each test, with the gas analyzers adjusted for precision gases and the turbine flow measures by a standard 3-liter syringe.

**Muscle biopsy.** A resting muscle sample was taken from the vastus lateralis under local anesthesia, utilizing the needle biopsy technique (39). Samples were immediately frozen in liquid nitrogen and then stored in −80°C until analyses.

**Muscle Analyses**

**Muscle \[^{[3]H}\]ouabain binding site content.** Twenty milligrams of muscle sample were used in the \[^{[3]H}\]ouabain binding content analysis, as was previously described (46, 50). In brief, each sample was washed for 2 × 10 min at 37°C in vanadate buffer (250 mM sucrose, 10 mM Tris-HCl, 3 mM MgSO4, 1 mM NaVO4; pH 7.3). Following the washout period, muscle samples were incubated for 2 h at 37°C in vanadate buffer with the addition of \[^{[3]H}\]ouabain (2.0 μCi/ml and 10–6 M, PerkinElmer, Boston, MA) were added before liquid scintillation counting of \[^{[3]H}\]ouabain. The content of \[^{[3]H}\]ouabain binding site content was calculated on the basis of the sample wet weight and specific activity of the incubation buffer and samples and expressed as picomoles per gram wet weight. The final \[^{[3]H}\]ouabain content was then calculated, accounting for unspecific binding, correction factor for im猝ority of \[^{[3]H}\]ouabain, loss \[^{3}H\]-bound ouabain during washout, and incomplete saturation, as previously described (46, 50). Due to small muscle biopsy samples, \[^{[3]H}\]ouabain...
binding analyses were measured in 14 Young and 8 Elderly individuals.

RNA extraction and RT-PCR. Total cellular RNA was extracted using a modification of the phenol-chloroform extraction and isopropanol precipitation protocol, using the TOTALLY RNA Kit (Applied Biosystems, Foster City, CA), as described previously (58). RNA quality and concentration were determined using the NanoDrop 1000 Spectrophotometer (Thermo Scientific). First-strand cDNA was generated from 1.0 μg total RNA using high-capacity RNA-to-cDNA kit (Applied Biosystems). RT-PCR was performed using the Applied Biosystems 7500 Real Time PCR machine (Applied Biosystems), and PCR was performed in duplicate with reaction volumes of 14 μl, containing Power SYBR Green (Applied Biosystems), forward and reverse primers, and cDNA template (1.25 ng/μl). Data were analyzed using a comparative critical threshold (Ct) method, where the amount of target normalized to the amount of endogenous control relative to control value is given by 2^-ΔΔCt (Applied Biosystems). The efficacy of TBP (NM_003194) as an endogenous control was examined using the equation 2^-ΔΔCt. No changes in the expression of this gene were observed (data not shown), so it was considered an appropriate endogenous control for this study. NKA primers were used as previously described (42). Muscle NKA gene expression analyses were performed on samples from 17 Elderly (except β3, where n = 16) and 12 Young individuals.

Western blotting. Forty milligrams of frozen muscle samples were used for NKA immunoblot analyses (42). Skeletal muscle proteins were extracted in ice-cold buffer containing 20 mM Tris pH 7.8 (Bio-Rad Laboratories, Hercules, CA), 137 mM NaCl, 2.7 mM KCl (Merck, Kilsyth, Australia), 1 mM MgCl2, 5 mM Na2HPO4, 10 mM NaF, 1% Triton X-100, 10% glycerol (Ajax Finechem), 0.5 mM Na3VO4, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 200 mM PMSF, 1 mM DTT, 1 mM benzamidine. All reagents were analytic grade (Sigma-Aldrich, St. Louis, MO), unless otherwise specified. Samples were homogenized (1:37.5 dilution) for 20 s, using a tissue homogenizer (TH220, Omni International, Kennesaw, GA). Homogenates were rotated for 60 min at 4°C, and protein concentration of the homogenates was determined using a commercially available kit (DC Protein Assay, Bio-Rad Laboratories, Hercules, CA).

Studies utilizing repeated centrifugation of muscle and membrane separation control in very low recovery of Na⁺-K⁺-ATPase enzymes, thereby yielding a final sample that may be unrepresentative of the whole muscle Na⁺-K⁺-ATPase population (20). Hence, muscle sample analyses did not include any membrane isolation steps, to maximize recovery of Na⁺-K⁺-ATPase enzymes (42). Aliquots of the muscle lysate (homogenate) were mixed with Laemmli sample buffer, and proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (6–20% gradient gels), for 80 mA and overnight at 16 mA, in a standard vertical electrophoresis unit (SE 600 Chroma, Hoefer, San Francisco, CA). For the analysis of protein abundance of α1, α2, β1, and β3 Na⁺-K⁺-ATPase isoforms, 60 μg of total protein per sample were loaded in each gel, while 20 μg were loaded for α2 and β1. Following electrophoresis, proteins were transferred to polyvinylidene fluoride membranes (Bio-Rad) for 3 h at 320 mA using a semidry blotter (TE70X, Hoefer). Membranes were blocked in TBST buffer (10 mM Tris, 100 mM NaCl, 0.02% Tween-20) containing 7.5% nonfat milk, for 1 h at room temperature. After being washed (3 × 10 min in TBST), membranes were incubated with the appropriate primary antibody overnight at 4°C. Primary antibodies were incubated in Tris buffered saline buffer containing 0.1% NaN3 and 0.1% albumin bovine serum. All membranes were incubated with the same amount of dilution buffer.

The protein abundance was determined semiquantitatively, assessed as the chemiluminescence intensity of the specific protein of interest, against a normalized (constitutively expressed protein) band intensity (determined in pixelation intensity). This value is then expressed as arbitrary unit (as a fold change relative to the constitutively expressed protein). It is not possible to establish molarity as pure protein standards for the Na-K-ATPase are not available for human proteins. Typical of this Western blotting protocol, stripped blots are Ponceau stained to confirm complete transfer.

To determine NKA protein abundance, membranes were incubated with antibodies for NKA α1 (monoclonal α6F, developed by D. Fambrough, obtained from the Developmental Studies Hybridoma Bank, and maintained by the University of Iowa), NKA α2 (polyclonal anti-HERED, kindly donated by T. Pressley, Texas Tech University), NKA α3 (Thermo Scientific, Rockford, IL, no. MA3–915), NKA β1 (Thermo Scientific, no. MA3–930), NKA β2 (Becton Dickinson Bioscience, San Jose, CA, no. 610915), and NKA β3 (Becton Dickinson, no. 610993), as earlier described (42). The abundance of all proteins was normalized for loading control with GAPDH (Santa Cruz Biotechnology, no. FL-335). Following incubation with the primary antibodies, membranes were washed in TBST buffer (3 × 10 min) and incubated with the appropriate anti-rabbit (PerkinElmer, no. NEF812001EA) or anti-mouse (PerkinElmer, no. NEF822001EA) horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Secondary antibodies were diluted 1:5,000 in TBST buffer containing 5% nonfat milk. After the membranes were washed in TBST, immunoreactive proteins were detected using chemiluminescence reagents (Immobilon HRP Substrate, Millipore, Billerica, MA) and quantified by densitometric scanning (VersaDoc Imaging System, BioRad). NKA protein abundance analyses were performed in 13 Elderly and 15 Young individuals.

Statistical Analyses

All data are reported as means ± SD. Independent t-tests and effect size (ES) with 90% confidence intervals were used to calculate differences between groups using a custom spreadsheet (23). Statistical significance was accepted at P < 0.05. Log-transformation was performed on variables that were not normally distributed to reduce bias. Magnitudes of change was classified as small, 0.2–0.6; moderate, 0.6–1.2; large, 1.2–2.0; and very large, 2.0–4.0 (4, 23). Effects with less certainty (magnitude of <75%) were classified as no meaningful difference (4, 23). Spearman correlation coefficients were used to examine correlations between NKA isoforms, [3H]ouabain binding concentration, and V0peak.

RESULTS

Functional Tests: RPE-Limited V0peak and Muscle Strength

The Elderly had a 36.6% lower RPE-limited V0peak than the Young (24.9 ± 4.9 vs. 39.3 ± 7.6 ml·kg⁻¹·min⁻¹, P = 0.000). Muscle strength was 36.3% lower in Elderly than Young (1.55 ± 0.44 vs. 2.44 ± 0.46 Nm/kg, P = 0.000).

Muscle [3H]Ouabain Binding Site Content

The muscle NKA content did not differ between Elderly and Young (352.5 ± 76.2 vs. 350.0 ± 108.0 pmol/g wet weight, P = 0.96).

Muscle NKA Isoform Gene Expression

No significant differences were observed between groups for α1, α2, α3, β1, or β2-mRNA expression (P > 0.18, small ESs and magnitudes of difference <75%, Table 1). However, the β3-mRNA was higher in Elderly compared with Young (P = 0.011, ES 0.75 ± 0.58 arbitrary units, Table 1).

Muscle NKA Isoform Protein Abundance

No differences were observed between groups for NKA α1- or α3-protein abundance (P > 0.76, small ES and magnitude of difference <75%, Fig. 1). The NKA α2-protein abundance was...
24.4% less in Elderly compared with Young (P = 0.049, ES −0.74 ± 0.63 arbitrary units, Fig. 1). No differences were found between groups for β1- or β2-protein abundance (P > 0.20, small ES and magnitude of difference <75%, Fig. 2). The NKA β1-protein abundance was 23.0% lower in Elderly compared with Young (P = 0.041, ES −0.74 ± 0.63 arbitrary units, Fig. 2).

Correlations

Age was correlated with β3-mRNA (r = 0.47, P = 0.01) and negatively correlated with VO2peak (r = −0.73, P < 0.001) and strength (r = −0.69, P = 0.001) and tended to be negatively correlated with α2-protein abundance (r = −0.33, P = 0.089). The VO2peak tended to be correlated with α2-protein (r = 0.39, P = 0.069) and was negatively correlated with α1-protein (r = −0.41, P = 0.055). The [3H]ouabain binding tended to be correlated to α1-protein (r = 0.43, P = 0.06), but not with α2-protein.

DISCUSSION

This is the first study to investigate the impact of aging on muscle NKA isoform expression in human skeletal muscle and, when combined with measures of [3H]ouabain binding, yields two main novel findings. First was the apparently paradoxical finding that the Elderly exhibited an unchanged [3H]ouabain binding site content, but that this was accompanied by a 24% reduction in the muscle NKA α2-protein abundance in muscle relative to the Young participants. Second, the muscle NKA β3-protein abundance was also reduced in the Elderly, compared with Young. The broader context for understanding these changes and their implications is the marked decline in musculature, strength, and exercise performance evident in the Elderly.

It is well established that the aging process is accompanied by a steady decline in muscle mass and strength, which becomes increasingly apparent after about 50 yr of age (32, 54, 60) and with an 8–10% reduction in maximum oxygen consumption each decade after around 30 yr (47). The 36–37% lower VO2peak and strength in the Elderly is consistent with the mean age difference of around 4 decades and clearly indicates a pronounced impairment of muscular function in these individuals. An inability to sustain muscular and aerobic power may also cause posture and mobility problems and is associated with a reduced quality of life and activities of daily living in the elderly (59). Thus factors that may adversely influence the capacity to perform repeated muscular contractions are of greater importance in the elderly than in the young. Numerous studies in isolated rat muscles have established that the NKA plays a vital role in regulating muscle Na⁺/K⁺ exchange and membrane potential during repeated contractions, as well as under fatiguing conditions, and thereby NKA facilitates preservation of muscle excitability and contractility (8, 9, 11). Studies in humans have also confirmed the important role of muscle NKA in regulating muscle and systemic Na⁺/K⁺ homeostasis and in attenuating muscular fatigability during exercise (26, 27, 37, 44, 55). Thus any adverse impacts of aging on muscle NKA could potentially have important adverse implications for muscle function in the aged.

Based on findings in animal studies (30), as well as a tendency in one human study (31), we hypothesized that the skeletal muscle [3H]ouabain binding site content would be reduced in the Elderly compared with the Young, but no such difference was found. We included [3H]ouabain binding as the expected critical measurement, and, therefore, our finding of no decrease was surprising. This finding is, however, consis-
tent with two previous reports (31, 46), which examined six to nine older individuals and/or involved measurements in a clinical elderly population. Due to limited muscle sample availability, we measured [3H]ouabain binding site content in only eight Elderly participants; thus our study could also be criticized for potentially being underpowered. However, our values for Young adults (~350 pmol/g wet wt) were comparable to other muscle samples collected in our laboratory and analyzed either in our laboratory or in that of colleagues (means ~330–350 pmol/g wet wt) (17, 33, 50); although the SD for NKA content was somewhat higher in this study, there was no tendency for a decline evident in the aged population. Thus, taken together, this and the two published studies comparing a young with an elderly cohort all showed no difference in [3H]ouabain binding site content, and hence this was not reduced with aging. Nonetheless, further studies with a larger sample size are needed to conclude this.

Consistent with our hypothesis, we show for the first time that the NKA α2-protein abundance was reduced by 24% with aging in human vastus lateralis muscle. This is consistent with the 12–50% decline in NKA α2 with aging in red and white gastrocnemii and in soleus muscles in rats (43, 57, 61). Given that the α2 isoform is by far the most abundant (up to 85%) of the α-NKA isoforms in rat muscles (19) and is involved in the maintenance of muscle force and membrane excitability (10, 11, 22, 52), this finding has important implications for understanding the decline in muscle function in older humans. A 24% reduction in NKA α2-protein abundance with aging could suggest a reduced muscular fatigue resistance. There is evidence with aging for both an increased muscle fatigability (especially in high-intensity or high-velocity dynamic contractions) and a fatigue resistance (especially during slowed voluntary contractions) (1, 28). One limitation of our study is that no measurements of fatigability per se were undertaken. Thus we cannot conclude that the reduced NKA α2 with aging is linked with greater fatigability. Furthermore, in human muscle, the relative abundance of α2 compared with the other α isoforms, as well as the exact role(s) of α2, are not yet known. Furthermore, although the reduction in NKA α2-protein abundance with aging had a moderate ES, the percent decline was relatively small, and other aging studies have reported variable relative changes in NKA isoform proteins with aging. To maximize the recovery of NKA, we utilized a complete muscle homogenate for these measures to avoid the well-known loss of protein during centrifugation and separation steps (20). However, we do not know the exact amount of protein that is specifically NKA that is injected into each well during Western blot measures. In addition, these immunoblots detect differences in the relative abundance of NKA α2-protein in Elderly vs. Young muscle and thus do not quantify molar differences. Further studies utilizing quantitative techniques are required to verify these findings.

Since α2 is the dominant α isoform in skeletal muscle, proportional declines in NKA α2-protein abundance and [3H]ouabain binding were anticipated with aging, but results were divergent, with reduced α2-protein abundance but unaltered [3H]ouabain binding site content. Several factors might be considered to explain this, including increased relative membrane density of the fiber, compensatory upregulation of other α isoforms, selective type II fiber loss affecting α2 abundance, different fiber and isoform affinity for ouabain, and differences in muscle water content and variability using the Western blot technique. The most likely possibility is that, due to the smaller fiber size in aged muscle, the relative membrane density of the fiber is actually increased. In porcine muscle, [3H]ouabain binding site content was related to muscle fiber plasma membrane area, being higher in smaller muscle fibers (21). In aging rats, the muscle [3H]ouabain binding site content varied in accordance with both muscle size as well as the number of pump receptors (30). Fiber size was not ascertained in this study, but a reduced fiber cross-sectional area is expected in aged muscle (54). With aging over 4 decades as in this study, a decline in muscle fiber cross-sectional area of around 10–30% could be anticipated for type II fibers (13, 25). Thus, with an expected decline in muscle fiber size with aging, and in the absence of any other change, one might expect to see a small increase in [3H]ouabain binding. This was not seen, and indeed no change in [3H]ouabain binding site content was detected. Thus the absence of a higher (or tendency to a higher) [3H]ouabain binding site content in aged muscle is, in fact, consistent with a possible decline in NKA content with aging. This means that the [3H]ouabain binding site content findings are then consistent with the 24% decline of NKA α2 detected in the aged muscle. In human muscles, the [3H]ouabain binding capacity measures total NKA content, since ouabain binds to all α isoforms with high affinity. Hence another possibility is that α1 and/or α3 were upregulated with aging, perhaps as a compensatory response to reduced NKA α2. However, this was not the case, since neither α1 nor α3 differed between Young and the Elderly. This is clearly different to the rat where the NKA α1-protein abundance was increased with age in red (by 25% to as much as sevenfold) and white (by 77% to twofold) gastrocnemii muscles and also in soleus and extensor digitorum longus muscles (43, 57, 61). As aging results in a predominant loss of type II fibers (54), this also raises the possibility of a fiber-selective loss of NKA α2 isoform; however, this is unlikely, given the similar NKA α2 in red and white muscle in the rat muscles (24) and the lack of a relationship between NKA content and fiber type in human muscle (17). A further possibility is that the discrepancy between decreased α2 abundance and unaltered [3H]ouabain binding site content with aging reflects the different reference base used for each measurement, being per microgram protein and per gram wet muscle, respectively. A difference in muscle water content seems unlikely in explaining this, since this did not differ in muscle obtained from young (5) vs. older individuals (3) of similar age range. The immunoblotting measures are less sensitive to differences in fiber size, since the amount of protein added is proportional to the muscle protein concentration. Therefore, a decline in relative abundance of NKA α2, as detected here, clearly points to and is consistent with a lesser α2 with aging. A further possible explanation is that any α2 not embedded in plasma or t-tubular membranes may also decline with age. The exact reasons for these findings cannot be solved without further detailed investigation of fiber cross-sectional area and NKA α2 localization. In summary, an expected decrease in fiber size and increased membrane-to-fiber area ratio with aging would typically increase the [3H]ouabain binding content, but this increase did not occur due to an underlying reduction in muscle NKA α2-protein abundance with aging. However, we cannot exclude the possibility that our findings of reduced NKA α2-protein abundance in aged muscle were
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expression and protein abundance are novel. An interesting observa-
question to resolve.

Our findings of aging effects on NKA isoform gene expres-
sion and protein abundance are novel. An interesting observa-
tion was that the β3-mRNA was greater, whereas the β3-
protein abundance was 23% less in the Elderly than in the Young. Little is known about the function of β3 in human muscle. However, β3 adaptability has been demonstrated previously at the mRNA, but not protein, level. While muscle β3-mRNA was increased after intense exercise (42) and in resting muscle after training (2), β3-protein abundance was not changed. The muscle β3-mRNA was higher in recreationally active young men than women (41). Since β3 is anticipated to be expressed only at very low levels in muscle, the significance of this finding is not clear. No difference in the mRNA of other NKA isoforms was found with aging. We found no effect of aging on NKA α3-protein abundance, and this does not appear to have been studied in other species. Our findings of a lack of difference with aging in the NKA β1, β2-, or β3-isoform protein abundance clearly differs from previous findings in rat muscle. In rats, NKA β1 was increased with aging in red (by 1.4- and 3-fold) and white (by 2.4- and 3.5-fold) gastrocnemii muscles in two (57, 61) of three studies (43, 57, 61); β2 was more consistently decreased in red (by 57–77%) and white (by 36–80%) gastrocnemii muscles (43, 57, 61), while β3 was increased in both red (by 1.1- and 2.2-fold) and white (by 2.3- and 7.9-fold) gastrocnemii muscles (43, 61). The reasons for this apparent species difference are not clear, but we note considerable inconsistencies in the relative changes and across muscle types for some isoforms with aging in rat muscle.

In conclusion, elderly individuals are characterized by re-
ductions in muscle NKA α2- and NKA β2-protein abundance
compared with young individuals, whereas aging was without
effect on [3H]ouabain binding content. A greater β3-mRNA
was found in elderly muscle, but aging did not modify the gene
or protein expression of other NKA isoforms in muscle. Future
studies are required to confirm this lower NKA α2 with aging
and any possible relationship with muscle fatigability in aging
individuals. This problem is important since fatigue is an
important contributor to the reduction in capacity to perform
activities of daily living with aging.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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