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Examining the effects of age, sex, and body mass index on normative median motor nerve excitability measurements

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HIGHLIGHTS

- Evidence for biophysical differences between male and female motor axons.
- Properties mediated by the nodal KCNQ channel are enhanced in females.
- Age has a small effect on motor axonal excitability in adults.

ABSTRACT

Objectives: The purpose of this study was to build a large reference database of excitability measures in normal subjects and to examine the effects of age, sex, and BMI.
Methods: One hundred and five healthy subjects had median motor nerve excitability testing performed at the wrist using the automated threshold-tracking program, QTRAC. Statistical linear regression was used to explore relationships between nerve excitability and the independent variables.
Results: The main effect of age is a reduced superexcitability. Lesser effects are flattening of the normalized stimulus response curve and reduction in threshold change following strong hyperpolarizing currents. Females have lower thresholds than males and small but significant differences in voltage-gated potassium channel (KCNQ) mediated properties (late subexcitability, accommodation half time, and threshold undershoot following depolarizing electrotonus), as well as a small increase in superexcitability. BMI has no influence on nerve excitability data and does not explain sex-related differences in threshold.
Conclusions: Age and sex have few and small effects on excitability parameters.
Significance: The expression of nodal KCNQ channels appears to be greater in females. Age-related increases in subexcitability may be attributable to changes in the muscle fibre and not the nerve.

1. Introduction

Nerve conduction studies (NCS) are influenced by age; in adulthood this is largely determined by reductions in the number of motor and sensory axons, changes in myelination, and reconfiguration of the motor unit (Swallow, 1966; Kawamura et al., 1977; Roos et al., 1997). Sex-related differences in NCS are not as pronounced, and although digital sensory responses are often highest in young females, and greater stimulus intensities may be required to achieve maximal responses in men (Kiernan et al., 2001a), peripheral axons are not known to differ biophysically between the sexes. It has been suggested that soft tissue characteristics between men and women explain differences in threshold to stimulation (Kiernan et al., 2001a,b) although no group has formally tested the hypothesis by measuring soft tissue markers such as body mass index (BMI).

Nerve excitability testing is a novel research and investigational tool, which is complimentary to conventional NCS, and which contributes insight into the pathophysiology of peripheral nerve disorders (Bostock et al., 1998; Nodera and Kaji, 2006). A few studies have examined the effects of age and sex on axonal membranes using nerve excitability techniques (Kiernan et al., 2001a; Jankelowitz et al., 2007; Bae et al., 2008). Although there is agreement that age exerts some influence on excitability, specific findings from these studies have been inconsistent.

We measured multiple excitability data as well as baseline BMI in 105 normal subjects to better define the effects of age, sex, and soft tissue characteristics on nerve excitability and to contribute a large normative dataset that can be used by other groups.

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2. Methods

2.1. Subjects

Nerve excitability studies were carried out in 105 healthy volunteers without a history of peripheral neuropathy or known risk factors for neuropathy. Subjects were recruited from community groups, and from hospital contacts (medical students, hospital staff, patients' relatives) and were aged from 19 to 82 years (51 female, 54 male). All participants provided informed written consent, and the study was approved by the Medical Ethics Committee of St. Vincent's University Hospital.

2.2. Neuropathy screening

A detailed clinical history, focused neurological examination and NCS were used to screen for and exclude carpal tunnel syndrome, peripheral neuropathy, and any predisposing conditions such as alcohol excess, neurotoxic medication or diabetes. NCS comprised unilateral conduction studies of superficial radial, sural, median and posterior tibial nerves; these were performed by a single operator (JMcH) using a Keypoint® Electromyograph (Model 9031A006 Alpine Biomed). In addition, dedicated ulnar to median sensory comparisons were performed in any subjects with symptoms suggestive of CTS, and as a matter of routine in all subjects over the age of 60 years. Height and weight were recorded and BMI calculated in all cases according to the formula BMI = mass (kg)/[height(m)]^2.

2.3. Excitability studies

Nerve excitability studies were performed on the right median motor nerve at the wrist using non-polarisable Ag–AgCl electrodes (Skintact, Innsbruck, Austria). Compound muscle action potentials (CMAPs) were recorded from abductor pollicis brevis (APB) muscle using 9 × 6 mm pre-gelled Ag–AgCl disposable surface electrodes (Alpine Biomed, Fountain Valley, California). Subjects were tested in a relaxed seated position with the hand resting on a pillow. Skin temperature was monitored with a thermistor at the beginning and end of testing and the hand was covered by a blanket or sheet in an effort to achieve a temperature of at least 32 °C.

Excitability measurements were performed using the TRONDE protocol of the QTRAC program (©Prof Hugh Bostock, Institute of Neurology, London), which compares threshold, defined as the stimulus current required to achieve a target response (40% of maximum CMAP), under a variety of conditions (Bostock et al., 1998; Kiernan et al., 2000). The following excitability indices were measured in order: stimulus response curve (SRC), strength duration time constant (SDTC), depolarizing and hyperpolarizing threshold electrotonus, recovery cycle, and the current threshold (*IV*) relationship. The EMG signal was sampled at 10 kHz, and filtered between 2 Hz and 10 kHz using a Keypoint® electromyograph (model 9031A006, Alpine Biomed, Skovlunde, Denmark), connected to a BNC-2120 data acquisition board (National Instruments) and a personal computer running QTRAC. Stimulus waveforms were generated by the test computer and converted to current by a DS-5 isolated linear bipolar constant-current source (Digitimer, Welwyn Garden City, UK) with a maximal output ±50 mA.

Under control of the TRONDE protocol, stimuli were delivered every 0.8 s during the recording. Stimulus duration was varied step-wise from 0.2 to 1 ms for measurement of SDTC and was fixed at 1 ms for all other test and supramaximal conditioning stimuli. The stimulus response curve (SRC) was first plotted in order to set the target for tracking and to define the tracking step, which is calculated from the slope of the curve.

SDTC describes the relationship between stimulus duration and stimulus charge and is dependent both on passive membrane properties and the behaviour of inward persistent sodium channels (Bostock and Rothwell, 1997). Rheobase is the estimated threshold for a current of infinitely long duration and is related to SDTC by Weiss's law (Bostock et al., 1998). SDTC and rheobase are calculated by measuring threshold for stimuli from 0.2 to 1 ms and plotting stimulus charge versus duration.

Threshold electrotonus (TE) and the *IV* relationship examine threshold in response to prolonged hyperpolarizing and depolarizing currents, which indirectly interrogate membrane potential and the functions of voltage dependent ion channels. In TE, 100 ms conditioning currents are applied across the stimulating electrodes (set at ±20, and 40% of threshold current), and changes in threshold are serially measured by applying different conditioning-test stimulus intervals (0–200 ms). Depolarizing TE (TE_D) provides information about the inward rectifying slow voltage-gated potassium conductance (gK_S), and hyperpolarizing TE (TE_H) reflects the outward rectifying hyperpolarization activated cation current (I_H). The *IV* relationship also describes rectifying channel function in response to prolonged 200 ms currents, which range from +50% to −100% of threshold in 10% steps. Unlike TE, however, the *IV* relationship describes the maximal extent of threshold changes rather than the time course of those changes.

The period of threshold changes following a supramaximal-conditioning stimulus is known as the recovery cycle (RC) and it is dependent on a number of nodal and internodal properties. The RC can be plotted by measuring threshold at intervals between 2 and 200 ms following a conditioning stimulus giving rise to a characteristic curve. After a brief period of absolute refractoriness, the axon enters a relatively refractory period (RRP), followed by a superexcitable, then subexcitable phase before finally returning to normal. QTRAC uses on-line subtraction of the conditioning stimulus response from the conditioning stimulus plus test response to minimize artifact and provide a reliable measure of threshold.

2.4. Statistical methods

Data were presented as means ± standard error of the mean (SEM). Statistical analysis of the data was performed using QTRAC-P (©Prof Hugh Bostock, Institute of Neurology, London) with some additional analysis using SPSS version 12 (SPSS, Chicago, Illinois).

Student's *t*-tests (or Mann–Whitney *U* tests for non-parametric data) were applied to examine mean differences between the male and female groups, between subjects <40 and >60 years, and between high and low-BMI groups using QTRAC-P. In order to appreciate the effects of automatic data correction on group differences, *t*-tests and *U*-tests were applied both before and after automatic adjustment of the excitability data for differences in age, BMI, sex, and temperature using QTRAC-P. For example sex differences were examined without correction and after correction for the other three variables: age, BMI, and temperature. A similar approach was used for age and BMI comparisons (Table 1). The interpretation of significant group differences was guided by the following set of principles. Firstly, real differences should be apparent in comparison of the raw data without correction and should not disappear after correction. Secondly, the level of significance should ideally be less than 0.01 for raw comparisons to allow for the effects of multiple comparison, with any sustained differences at a significance of $p < 0.05$ being regarded as trends.

In a complimentary approach, univariate linear regression, and stepwise statistical multiple regression analyses were applied to