

DETERMINATION OF THE CHRONAXIE AND RHEOBASE OF DENERVATED LIMB MUSCLES IN CONSCIOUS RABBITS

Z. Ashley*, H. Sutherland*, H. Lanmuller**, E. Unger**, F. Li*, W. Mayr**, H. Kern***, JC. Jarvis*, S. Salmons*

* Muscle Research Group, Department of Human Anatomy & Cell Biology, The Sherrington Building, University of Liverpool, Liverpool, L69 3GE, UK

** Department of Biomedical Engineering & Physics, Medical University, AKH, A-1090, Vienna, Austria

*** Ludwig Boltzmann Institute of Electrostimulation and Physical Rehabilitation, Department of Physical Medicine, Wilhelminenspital, Vienna, Austria

Abstract

Measurements of the rheobase and chronaxie can be used to define the excitability of nerves and muscles. The aim of this study was to obtain a record over many weeks of changes in the rheobase and chronaxie of denervated rabbit tibialis anterior muscle (TA).

A custom-built electronic stimulator was implanted into the peritoneal cavity of New Zealand White rabbits. Large stainless steel electrodes were placed on the denervated TA muscle. Rheobase and chronaxie were measured non-invasively at weekly intervals by means of a laptop PC, which communicated with the stimulator via a radio-frequency link. At each setting the denervated TA was palpated manually to detect the response of the muscle.

During the first few days after denervation the rheobase increased transiently to 0.8 ± 0.13 mA, approximately twice the value for normal innervated muscle, then decreased to normal for the remainder of the experimental period. Chronaxie underwent a significant 3-fold increase from 4.5 ± 1.1 ms to 14.1 ± 1.1 ms during the first two weeks of denervation and remained elevated throughout.

The custom-built implantable electronic stimulator allowed changes in muscle excitability to be studied over a long period of denervation within individual animals, providing an accurate assessment of the time course of denervation-induced changes in muscle excitability.

Introduction

The excitability of a tissue can be defined by the relationship between stimulation amplitude and stimulation duration, otherwise known as the strength-duration curve. There are two points on this curve that can be used to define the excitability of the tissue, the rheobase and the chronaxie.

The rheobase of an excitable tissue is the minimum stimulus amplitude needed to elicit a response at infinitely long pulse durations; the chronaxie is the minimum pulse duration for a response when the stimulus amplitude is twice the rheobase (Fig. 1).

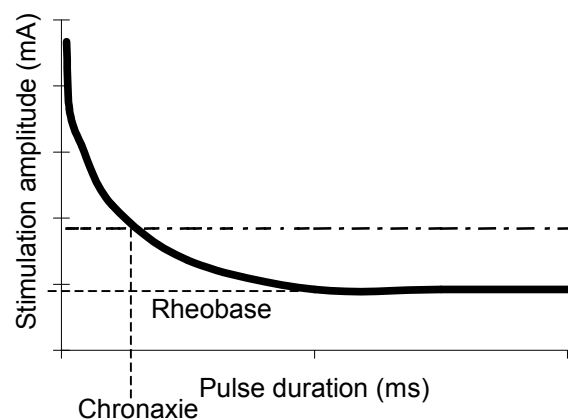


Figure 1: Typical strength-duration curve obtained from skeletal muscle. The rheobase is the asymptote to the lower portion of the curve. The chronaxie is the pulse width required to produce a response at twice the rheobase.

Denervation of skeletal muscles has been shown to result in many changes in structure and function. One of these changes is the decrease in the excitability of the muscles, associated with a large increase in the chronaxie [1]. Denervated muscles do not respond at all to short stimulating impulses; they require pulses of longer duration than those that are adequate for innervated muscles.

The use of electrical stimulation to alleviate the severe atrophy arising from denervation must take account of changes in muscle excitability. This study was designed to investigate the long-term changes in excitability of denervated muscle in order to elucidate the stimulation pattern that is necessary for the restoration of denervated muscle by electrical stimulation.

Material and Methods

Surgical procedures

All procedures were carried out in accordance with the Animals (Scientific Procedures) Act 1986, which governs the use of experimental animals in the UK.

Six New Zealand White rabbits (2.5 – 3 kg) were operated under inhalational anaesthesia (2% Isoflurane). All surgical procedures were carried out with full aseptic precautions.

The ankle dorsiflexor muscles of the left hind limb were denervated by avulsion and ligation of the motor portion of the common peroneal nerve. The proximal stumps were placed into a silicone rubber tube, with two ligatures placed tightly around the nerve. The tube was then reflected 90° from its original position and secured to underlying fascia. A custom-built electronic stimulator was placed into the peritoneal cavity and secured to the abdominal wall. Leads were routed subcutaneously to large stainless steel foil electrodes on the proximal superficial and deep distal surfaces of the TA muscle. The animals were allowed to recover from anaesthesia and inspected daily.

Measurements

The rheobase and chronaxie were determined by means of the implanted stimulator. Measurements were managed by a laptop PC, which communicated with the implanted stimulator via a radiofrequency link. At each setting the denervated TA was palpated manually for a response.

The pulse duration used to determine the rheobase was 100 ms. The amplitude was resolved to 0.04 mA in a 3-stage protocol. The pulse amplitude was then set at 2x rheobase, and the chronaxie resolved to 0.5ms in a 2-stage protocol. At each step 10 bipolar constant-current pulses were delivered while the muscle was palpated.

Immediately after completion of the surgical procedure, while the animals were recovering, measurements were taken to give a baseline reading. The measurements were then repeated at weekly intervals over the 24-week denervation period. The animals were lightly restrained during the measurements, but were fully conscious.

At the end of the denervation period physiological tests were carried out under terminal general anaesthesia (continuous intravenous Hypnorm infusion). In the course of these measurements a full strength-duration curve was obtained, from which the rheobase and chronaxie could be determined.

Data analysis

Data shown are mean \pm SEM, $n = 6$. Alterations in the values over the period of denervation were assessed for significance by ANOVA. Statistical significance was set at $p < 0.05$.

Results

Rheobase

During the first few days of denervation the rheobase increased significantly ($p < 0.05$) to 0.80 ± 0.13 mA (Fig. 2). This increase was transient and values then decreased and normalised for the remainder of the experimental period.

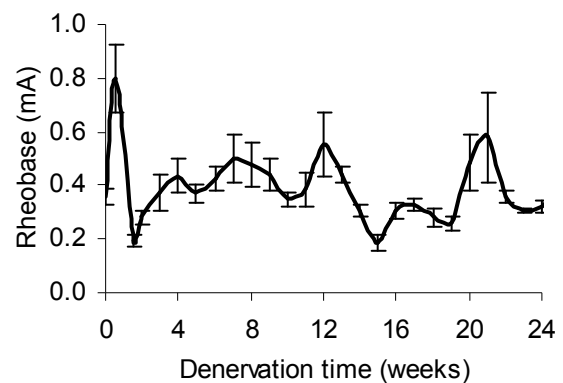


Figure 2: Rheobase values obtained from conscious rabbits over a 24-week period of denervation. Data shown are mean \pm SEM for $n = 6$ animals.

Chronaxie

Chronaxie increased 3-fold from 4.5 ± 1.1 ms to 14.1 ± 1.1 ms ($p < 0.01$) over the initial 2 weeks of the denervation period (Fig. 3), and remained at this elevated level throughout the 24-week experimental period.

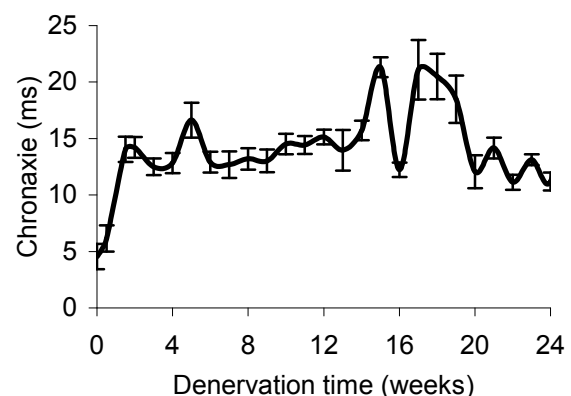


Figure 3: Chronaxie values obtained from conscious animals over the 24-week denervation period. Data shown are mean \pm SEM for $n = 6$ animals.

To see if there was any relationship between the rheobase and chronaxie values the data was compared by time point within individual animals (Fig. 4). There was a tendency for chronaxie to decrease when rheobase increased, and vice versa.

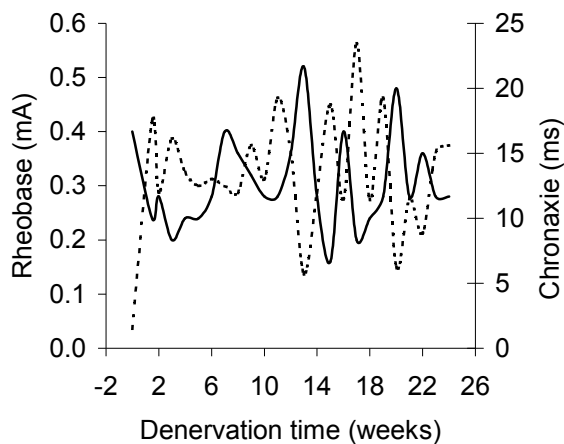


Figure 4: Rheobase (solid line) and chronaxie (dotted line) values obtained from an individual conscious rabbit over the 24-week denervation period.

Validation of conscious measurements

Comparison of the rheobase and chronaxie measurements from the conscious animals and measurements obtained during physiological assessment showed a clear correlation. Neither the rheobase (Fig. 5) nor the chronaxie (Fig. 6) were significantly different between the last measurement in the conscious animals and the values derived from the strength-duration curve measured under terminal anaesthesia.

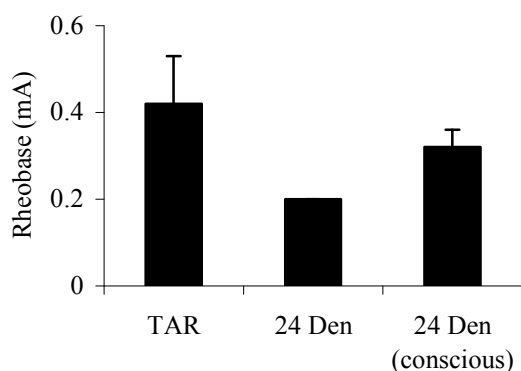


Figure 5: Rheobase values obtained from innervated contralateral control muscles (TAR) and 24-week denervated muscles (24 Den) under general anaesthesia, and values from 24-week denervated muscles in conscious animals (24 Den conscious). Data are shown as mean \pm SEM for $n = 3$ animals.

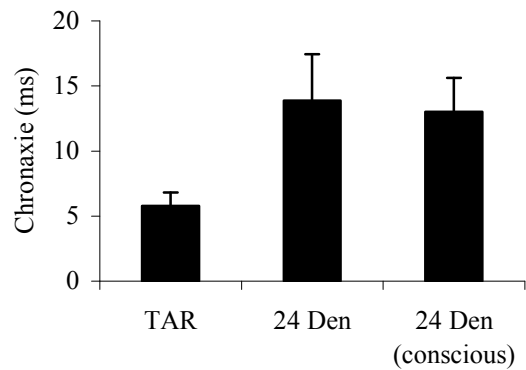


Figure 6: Chronaxie values obtained from innervated contralateral control muscles (TAR) and 24-week denervated muscles (24 Den) under general anaesthesia, and values from 24-week denervated muscles in conscious animals (24 Den conscious). Data are shown as mean \pm SEM for $n = 3$ animals.

Discussion

This is, to the best of our knowledge, the first study to provide a detailed time course of changes in the excitability of denervated skeletal muscle over an extended period within individual animals.

The initial baseline measurements were essentially those for normal innervated muscle, and they were very similar to those obtained from the innervated contralateral control TA muscle. The values for chronaxie were higher than expected from the literature [2] (3.7 ± 1.0 ms, compared to 0.43 ± 0.02 ms). This may be an effect of anaesthesia resulting in a prolongation of the measured chronaxie.

The transient increase in rheobase in the first 3 days following denervation may be the result of post-operative oedema. This could have produced some shunting of the stimulation current around the fibres, and may also have made the muscle response less easy to palpate. The rheobase has returned to normal values within 1-2 weeks.

The significant 3-fold increase in chronaxie occurred between 7 and 10 days after denervation, with a small increase already seen after 3 days. Degeneration of the motor endplates takes between 30 – 70 hours [1]. Once the endplates are no longer functional the sarcolemma has to be stimulated directly, to elicit a twitch response with electrical stimulation.

A relationship was observed between the rheobase and chronaxie: if the rheobase was lower the chronaxie tended to be higher. This is to be expected since the measurement of chronaxie

depends on the prior determination of rheobase. A slight overestimation of the rheobase value will tend to reduce the measured chronaxie. The measurements were made throughout by the same team in the same way to minimize subjective variation in assessing the response. Nevertheless, inter-week variability was observed in the rheobase values, and this was probably attributable to variation in the sensitivity with which the experimenter was able to palpate the twitch response. Some variation may also have resulted from small differences in the location of the twitch response or the animal's limb position.

This study was conducted with a custom-made implanted electronic stimulator that is capable of repeatedly measuring the excitability of a denervated muscle over an extended period of denervation (24 weeks). These data allow changes in excitability to be tracked within individuals, improving the scientific value of the data and reducing the number of animals used.

References

- [1] Gutmann E, Zelena J: Morphological changes in the denervated muscle. The denervated muscle, Chapter 2 pg 57-102. Ed: Gutmann E. 1962 Czechoslovak Academy of Sciences
- [2] Kiernan MC, Burke D, Andersen KV, Bostock H: Multiple measures of axonal excitability: A new approach to clinical testing, *Muscle & Nerve*, 23(3): 399-409, 2000.

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Author's Address

Dr Zoe Ashley
Muscle Research Group
Department of Human Anatomy & Cell Biology
University of Liverpool, L69 3GE, UK
e-mail: zoeash@liverpool.ac.uk