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Determining the Optimal Normalization Factor of Different Target Arteries for ex vivo Vascular Function Experiments: A New Standardized Procedure

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Keywords

Wire myography · IC_1 · IC_{100} · Isolated arteries · Vascular function · Factor k

Abstract

The standardization of resistance vessel preparation is crucial to compare physiologic vascular reactivity under different experimental conditions. Here, we describe a generalizable experimental setup for ex vivo vascular function experiments and their mathematical basis. Porcine basilar arteries and chicken common carotid arteries were isolated post mortem via standardized surgical approaches. The inner circumference of these vessels with a passive wall tension corresponding to 100 mm Hg (IC_{100}) as well as the circumference at which the active force production of the vessel is maximal (IC_1) were determined systematically. The IC_1/IC_{100} ratio (also referred to as factor k), a value that is believed to be constant for a defined vessel type in one species, was calculated by a novel mathematical approach. Here, we present an easy-to-use toolbox for the systematic and computer-based calculation of factor k and simplified optimal pre-stretching of any vascular segments for wire myography experiments.

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Introduction

Measurement of isometric contraction force by wire myography is a widely used method to study the response of isolated arteries to vasoactive agonists or antagonists ex vivo. Arteries from a variety of vascular beds of different species can be isolated for this purpose [1–8]. In order to reproducibly study vascular tone regulation ex vivo, the individual pretension of vessel segments for wire myography critically determines the potential to obtain maximum vascular reactivity without provoking vasomotion (spontaneous oscillatory activity) due to immoderate distension [9–11]. The inner circumference (IC) of passively stretched vessels to a transmural pressure of 100 mm Hg (IC_{100}) multiplied by a species- and vessel-specific factor (factor k) is commonly referred to as IC_1 . Pre-stretching of vessels to IC_1 yields the maximal active force production upon contractile stimuli [4, 12, 13]. However, a generalizable protocol for the systematic determination of factor k of different arteries is currently lacking in the literature. Although current software can offer some

Michael Hugelshofer and Jeremy Deuel contributed equally to this work.

computational support, factor k or IC_1 are often a matter of empirical guessing or approximately estimated on the basis of the active tension graphical concept [14]. A mathematical approach to exactly calculate factor k or IC_1 avoids data interpretation bias and would enrich and refine the technique used until now and promote the quality and reproducibility of vascular physiology studies.

Methods

Surgical Approach and Tissue Preparation

Basilar arteries were isolated from fresh heads of slaughtered pigs from the local slaughterhouse (SBZ, Zurich, Switzerland). Brainstems were isolated and transferred to pre-cooled (4 °C) Krebs-Henseleit buffer (KHB). Using a dissection microscope, the vertebral arteries were identified and cut 2 mm proximal to the vertebrobasilar junction. The artery segment between the vertebrobasilar junction and the caudal cerebral artery was used to prepare up to 6 vascular rings (at a length of 2 mm per ring). For illustration, see Figure 1a. Additionally, common carotid arteries were isolated from freshly slaughtered chicken obtained from a local chicken farm. Up to 12 vascular rings (at a length of 2 mm per ring) per animal were prepared under a dissection microscope. For a schematic illustration, see Figure 1b. The time between the death of the animal and the transfer of the dissected vessels into the KHB was kept below 90 min.

Buffers and Chemicals

Krebs-Henseleit Buffer

KHB was prepared in batches of 5 L containing (in mM): NaCl 118, KCl 4.7, MgSO₄ 1.2, CaCl₂ 2.5, KH₂PO₄ 1.2, and NaHCO₃ 25. High-potassium KHB (highK-KHB) contained the same salt concentrations (in mM) as KHB, except for: NaCl 72.7 and KCl 50. The solutions were bubbled with a 95% O₂ and 5% CO₂ mixture and adjusted to pH 7.4. Glucose was added immediately before use to a concentration of 2 g/L (11.1 mM). The final theoretical osmolarity for both solutions was 318.7 mosm/L.

Wire Myography

The vascular rings were mounted on two 0.2-mm diameter pins of a Multi-Channel Myograph System 620M (Danish Myo Technology, Aarhus, Denmark), immersed in temperature-controlled (37 °C) and continuously aerated (95% O₂ and 5% CO₂ gas mixture) organ baths containing 5 mL KHB. The two pins were adjusted as closely as possible to record an initial force reading of zero (baseline). After an equilibration period of 60 min, the normalization procedure for the IC_1/IC_{100} determination was carried out as described elsewhere [12, 14]. In short, passive stretch and potassium-mediated active contraction were performed in KHB and highK-KHB, respectively. All force readings were recorded after the contractions reached a plateau, and washing between the steps was performed by three KHB exchanges over 15 min until the passive force reading reached the level of the preceding passive stretch. Thereafter, the sequence was repeated with the next passive stretch to obtain data from at least 8 different vessel circumferences.

For the applied experimental setup, the active contraction and washing steps were omitted, and IC_{100} was calculated from at least

5 measured data points of passive stretch using the linear fitting model described below. For illustration of the experimental procedure, see Figure 2. An exemplary calculation is integrated in online supplementary File 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000505729).

Data and Statistical Analysis

Determination of Factor k

Data analysis for the IC_1/IC_{100} ratio was based on the quasi-randomly recorded sets of various ICs and corresponding force readings. A total of 19 vascular rings from 4 pigs and 12 vascular rings from 2 chicken were used with force recordings at 8–11 different circumferences per vessel segment. Passive wall tension (WT_p) was fitted to an exponential function

$$WT_p(IC) = m_p \times e^{IC} + q_p, \quad (1)$$

while the active wall tension (WT_a) was fitted by a linear regression model

$$WT_a(IC) = m_a \times IC + q_a. \quad (2)$$

Therefore, the change in wall tension (WT_Δ) upon isometric activation achieved by KCl-induced vasoconstriction was calculated by

$$WT_\Delta(IC) = WT_a(IC) - WT_p(IC). \quad (3)$$

By inserting equations 1 and 2 into equation 3, we obtain the difference in wall tension between the active and passive function, thus, the maximal dynamic range of wall tension the vessel can actively contract and relax within.

$$WT_\Delta(IC) = m_a \times IC - m_p \times e^{IC} + q_a - q_p. \quad (4)$$

The maximum of the function or the apex of the curve, respectively, determines IC_1 and was calculated by finding the zero of the first derivative of equation 4:

$$IC_1 = \ln \left(\frac{m_a}{m_p} \right). \quad (5)$$

The intersection of the passive tension curve with the linear isobar for 100 mm Hg or 13.3 kPa defines IC_{100} . The isobar can be calculated by

$$WT_{isobar}(IC) = \frac{13.3 \text{ kPa}}{2\pi} \times IC \quad (6)$$

and the passive wall tension at the intersection point, and it is the intersection of an exponential 1 and a linear function 6. This point cannot be calculated exactly. A computational approximation can be made by linear interpolation or by using Lambert's W function (product logarithm, see also [15]):

$$IC_{100} = q_p - \frac{13.3 \text{ kPa}}{2\pi} \times \frac{W \left(\frac{-m_p \times e^{\frac{q_p}{m_p}}}{m_p} \right)}{m_p}. \quad (7)$$

Finally, the factor k specific for the given artery segment was calculated by

a

b

(For legend see next page.)

$$\text{Factor } k = \frac{IC_1}{IC_{100}} \quad (8)$$

R statistical software version 3.4.2 was used for calculations, statistical analyses, and illustrations (<http://www.r-project.org>). Other formulas and terms used for the vascular function experiments are as follows: IC of the vessel

$$IC = \text{pin radius} \times (2 + 2\pi) + 2 \times \text{gap distance between pins}, \quad (9)$$

wall tension (WT) calculation considering the vessel length

$$WT = \frac{F}{2 \times \text{vessel length}} \quad (10)$$

whereas F is the raw force measured by the transducer, and effective transmural pressure

$$P_i = \frac{2\pi \times WT}{IC} \quad (11)$$

Symbols used in the equations are explained in Table 1.

The IC_1/IC_{100} results showed a normal distribution in a Q-Q plot as well as by Shapiro-Wilk's test for normality, $p = 0.2836$. Each vessel was counted as an individual experiment, since no significant interindividual differences for the animals could be found by ANOVA (F value 1.469, p for difference 0.263 for the 4 pigs, not performed for the 2 chicken). The active wall tension was fitted by a linear regression model. To test if an exponential fit would yield a better regression model, we performed a χ^2 statistic

Table 1. Explanation of the symbols used in the equations

WT	wall tension
WT_p	passive wall tension (prior to contraction)
WT_a	active wall tension (after contraction)
WT_{Δ}	difference between the active and passive wall tension, thus the tension the artery can actively build up
WT_{isobar}	wall tension at the 13.3-kPa (100 mm Hg) isobar
m_p	slope of the linear regression curve for the passive wall tension
q_p	y-intercept of the linear regression curve for the passive wall tension
m_a	slope of the linear regression curve for the active wall tension
q_a	y-intercept of the linear regression curve for the active wall tension
e^{IC}	Euler's number to the power of the inner circumference (exponential function)
P_i	transmural pressure

Fig. 1. a Illustrative example of porcine basilar artery isolation from freshly slaughtered pigs through a transclival approach. A total of 5–6 vascular rings per animal (at a length of 2 mm) were prepared under a dissection microscope while avoiding extensive mechanical vessel manipulation. **b** Scheme of chicken carotid artery isolation. Up to 12 vascular rings (at a length of 2 mm) were isolated per animal.

of an ANOVA comparison between a linear and an exponential regression for each experiment. The median of the p values (adjusted for multiple testing error) was 0.148, thus excluding a significant difference between the linear and the exponential regression. We subsequently favored the simpler linear regression model for active wall tension.

Discussion

Passive pre-stretching of vascular rings to an IC with optimal contractile response to vasoactive stimuli (IC_1) is critical to standardize experimental conditions in wire myography [16]. Several authors have published values for factor k or optimal initial tension of different species and target arteries [2–8, 12]. However, these values were determined empirically using the in-built normalization tool of the corresponding wire myography machine for the determination of IC_1 without a systematic mathematical approach to calculate factor k for the specific target arteries. In the often-used active tension graphical concept, for example, which is described in the DMT Manual [14], the active tension graph is obtained by subtracting the measured passive tension from the value obtained during an active contraction. This approach allows to estimate a range of passive tension in which an acceptable IC_1 value can be selected. This estimation is inherently unprecise and prone to subjective interpretation. However, our novel mathematical approach presented in this study combines an exponential fitting and linear regression model in conjunction with Lambert's W function to exactly calculate factor k or IC_1 of different target vessels systematically. The mathematical basis of our approach avoids possible bias introduced by the approximate estimation of factor k and thereby critically improves the reliability and reproducibility of vascular function experiments across different species and target vessels. As expected, factor k was constant for a specific target vessel and species in individual experiments, whereas it differed markedly between porcine basilar and chicken carotid arteries (Fig. 3, 4).

The overall mean factor k (IC_1/IC_{100}) of all 19 porcine basilar artery segments was found to be 0.877 ($SD \pm 0.087$) (Fig. 3). The overall mean factor k of all 12 chicken common carotid artery segments was found to be 1.016 ($SD \pm 0.050$) (Fig. 4) with the limitation that the chicken experiments were performed only in two animals. To simplify the use of the described method for any target vessel, an easy-to-use software toolbox was assembled and is provided as a supplement to this publication (online suppl. File 1).

Fig. 2. Flow-chart with step-by-step description of pre-stretching and calculation of IC_1 and IC_{100} . For simplification, the colors of the boxes are color matched to the panels in the provided calculation tool (online suppl. File 1). * A value for the factor k is calculated after input of 3 measurements. A good fit and a reliable determination of factor k require at least 8 different measurements. ** Values for IC_1 and IC_{100} are calculated after input of 2 measurements. For a good fit and reliable determination, a minimum of 5 different measurements is required.

Fig. 3. a Representative experiment to determine IC_1/IC_{100} of porcine basilar arteries. Wall tension is shown against the inner circumference of the tested vessel. Passive stretching (blue) and contractive force readings (orange) are plotted as filled circles, while the exponential and linear regression fittings are shown as continuous lines. The calculated changes in wall tensions ($WT_a - WT_p$) are shown by circles and the dashed line in red. The vertex (red asterisk) determines the maximum additional wall tension (IC_1). The intersection of the isobar for 13.3 kPa (grey line) and the fitted passive stretching curve (blue line) is marked by a grey asterisk and determines IC_{100} . Thus, factor $k = IC_1/IC_{100}$ can be calculated, which is 0.877 for the porcine basilar artery in this example. **b** Box plot of the IC_1/IC_{100} ratio of porcine basilar arteries ($n = 19$). Mean 0.877 (SD ± 0.087).

Fig. 4. a Representative experiment to determine IC_1/IC_{100} of chicken common carotid arteries. Wall tension is shown against the inner circumference of the tested vessel. Passive stretching (blue) and contractive force readings (orange) are plotted as filled circles, while the exponential and linear regression fittings are shown as continuous lines. The calculated changes in wall tensions ($WT_a - WT_p$) are shown by circles and the dashed line in red. The vertex (red asterisk) determines the maximum additional wall tension (IC_1). The intersection of the isobar for 13.3 kPa (grey line) and the fitted passive stretching curve (blue line) is marked by the grey asterisk and determines IC_{100} . Thus, factor $k = IC_1/IC_{100}$ can be calculated, which is 1.019 for the chicken carotid arteries in this example. **b** Box plot of the IC_1/IC_{100} ratio of chicken carotid arteries ($n = 12$). Mean 1.016 (SD ± 0.050).

In summary, this study provides a generalizable mathematical approach to determine factor k for different target vessels and species, including a toolbox to simplify normalization procedure for vascular function experiments.

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Statement of Ethics

Fresh cadaver heads from animals slaughtered for meat production were supplied by the Schlachtbetriebe Zurich AG. No ethical approval was needed for this study.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

M.H., C.A.S., and D.J.S. conceptualized this study. M.H., J.D., C.A.S., R.B., and R.H. performed the experimental work. J.D. and R.B. performed data analysis. M.H., J.D., D.J.S., C.A.S. and R.B. undertook manuscript writing and preparation of figures. All authors reviewed and edited the final manuscript.

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