

MEASUREMENT OF CGRP RELEASE FROM MOUSE SAPHENOUS AND VAGUS NERVES FOLLOWING CHEMICAL AND ELECTRICAL STIMULATION

MATERIALS AND PROTOCOLS

- **Stimulation procedures:** The nerves were placed in a specialized recording chamber (Avere Solutions UG (former Axolent)) between two glass tubes, modified as described in (1). The bath was perfused with SIF (Synthetic interstitial fluid), bubbled continuously with carbogen, and temperature-regulated with a Peltier thermode, continuously throughout the experiment duration. For the electrical stimulation, the cut ends of the nerve were placed through a silicon membrane of the glass tubes. Silver wire electrodes in one of the glass tubes were used as the cathode and anode. The electrical stimulation protocol consisted of stimuli of 0.25 Hz, 3/6/9 Hz, and 0.25 Hz again (for recovery). The chemical stimulation consisted of the application of a 60 mM depolarizing potassium solution (prepared as described in ref) and a solution of 0.3 mM capsaicin (Sigma Aldrich, Germany) diluted in SIF. Each stimulus was only given once to reduce the desensitization of the nerves. The duration of the stimuli was 5min with a 5min pause between each stimuli for recovery.
- **ELISA assays:** CGRP levels during the electrical stimulation and chemical stimulation experiments were evaluated with the **CGRP rat ELISA kit** (A05482, Bertin Bioreagent, France) as described in (2). Briefly, the eluates of the CGRP release experiment were mixed 4:1 with 5-fold concentrated commercial CGRP-EIA buffer. The CGRP-EIA had to be run immediately after the release experiment to prevent the loss of neuropeptide. Because of the low levels of CGRP in the vagus nerve, CGRP release from the vagus (but not saphenous) nerve was measured from two nerves threaded together into the recording and stimulating suction electrodes, to obtain CGRP levels above the ELISA kit's detection threshold. The CGRP release results were divided by two in order to have the per-nerve release. The ELISA assays were run on 96 well-plates, which were photometrically determined using a microplate reader (Opsys MRTM, Dynex Technologies, Chantilly, VA). Results can be seen in **Figure 1**.

RESULTS

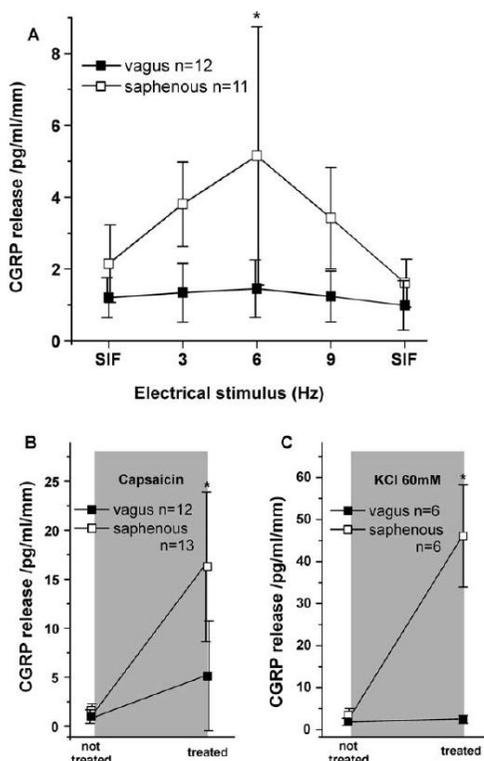


Figure 1: CGRP release from vagus and saphenous nerves. (From (1).)

The CGRP release is quantified as picogram neuropeptide per milliliter fluid released per millimeter nerve.

(a) CGRP release during electrical stimulation at different frequencies (3, 6, and 9 Hz). Saphenous (white squares) release is significantly increased with a maximum at 6 Hz. For the vagus nerve, only a minor nonsignificant increase is observed.

(b, c) Capsaicin (0.3 mM)- and KCl (60 mM)-induced release in saphenous (white squares) versus vagus (black squares) nerves. Capsaicin significantly increased CGRP release for the saphenous nerve but not for the vagus nerve. With the KCl depolarizing solution, a significant increase in the CGRP release was only observed in the saphenous nerve.

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CONCLUSION

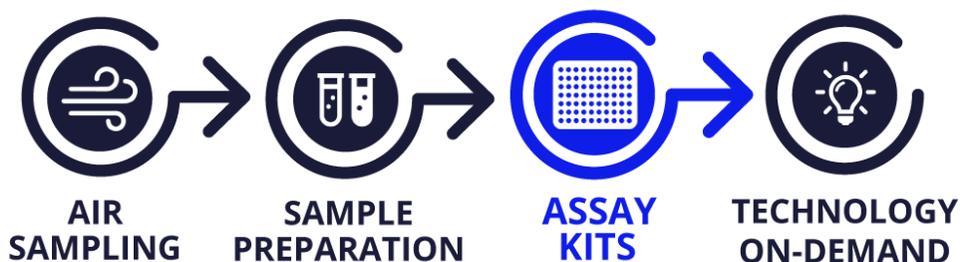
The findings of this study indicate that both electrical and chemical stimulation can induce CGRP release in unmyelinated segments of saphenous nerves, but not in unmyelinated segments of vagus nerves. These results, together with measurements of conduction velocity changes, show that vagus and saphenous nerves possess different activation and conduction properties, which could correlate with their distinct physiological roles. A better understanding of the mechanisms that modulate these conduction properties could help further our understanding of pathologies associated with cutaneous and visceral afferent nerves. The CGRP Rat ELISA kit from Bertin Bioreagent allows accurate measurements of CGRP in mouse nerves.

ACKNOWLEDGEMENTS & REFERENCES



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2. Averbeck, B., P. W. Reeh, and M. Michaelis. "Modulation of CGRP and PGE2 release from isolated rat skin by α -adrenoceptors and κ -opioid-receptors." *Neuroreport* 12.10 (2001): 2097-2100.

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