TECHNICAL NOTE

Evaluation of vagal nerve blockade with epineural lignocaine application

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Summary
The effect of vagal nerve blockade by epineural application of lignocaine was studied in the rat. Vagal nerve conduction was assessed by subdiaphragmatic esophageal electromyogram (EMG) response evoked by stimulation of the cervical vagus nerve. It was found that epineural application of lignocaine completely blocked the evoked EMG response within one minute. After washing away the anesthetic, recovery of nerve conduction was gradual, taking approximately 60 min. Our results have implications for the use of local anesthetic blockade to interrupt of vagal transmission in experimental designs.

Introduction
The cervical vagus nerve, being the tenth cranial nerve, is a major route for parasympathetic efferents and visceral afferents exiting and entering the brainstem, respectively. Blockade of vagal nerve transmission has been frequently employed as a mean for studying neural regulation involving these efferents and afferents. Recently it has been proposed that vagal efferents play an important role in mediating anti-inflammatory responses, inclu-ding the response produced by acupuncture (*Tracey, 2002*). In order to investigate the contribution of vagal efferents in mediating acupuncture anti-inflammation, we require an experimental model that provides reversible blockade of vagal transmission for a period of 30-45 min, during which time acupuncture is applied. Local anesthetic application and cooling are two commonly used techniques for reversible vagal blockade (*Fink & Carins, 1983; Patberg et al., 1984; Jaffe & Rowe, 1996)*. However, these two methods have different time characteristics. Local anesthetic blockade lasts for hours *(Fink & Carins, 1983; Jaffe & Rowe, 1996)*, whereas cooling is only suitable for a much shorter duration *(Davis et al., 1993; Zhang & Rowe, 1997),* due to the possibility of permanent cold damage *(Kennett & Gilliantt, 1991)*. Furthermore, a stable bilateral cool-block of the vagus nerve would be technically demanding in small animals, although it has been achieved in larger animals such as the dog (*Lee et al., 1987; Rudnicki et al., 1991)*. Thus, application of local anesthetics remains to be the only feasible option for reversible blockade of the vagus nerve for a substantial period of time in small animals.

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In the rat, which has been used most extensively in studying the nervous system, the time course of the effect of local anesthetic blockade on the vagus trunk *in vivo* has not been determined, although such information is available for individual fibers *in vitro (Fink & Carins, 1983)*. The purpose of this study was to evaluate the recovery time of vagal transmission in intact vagus nerve *in vivo*, by assessing the subdiaphragmatic esophageal EMG response evoked by electrical stimulation of the cervical vagus nerve.

Materials and Methods
The experimental procedures were approved by the Department of Health of Hong Kong under the Animals (Control of Experiments) Ordinance. Experiments were performed on Sprague-Dawley rats weighing 260-300 grams from the animal house of the Chinese University of Hong Kong. Animals were anaesthetized with chloral hydrate (i.p. 400 mg/kg, with supplemental dose of 40 mg periodically).
A midline incision was made above the sternum and the trachea was cannulated. The cervical vagus

nerves on both sides were dissected free from the overlying carotid artery for a length of 10-12 mm (Fig. 1). A bipolar platinum hook electrode (FHC, USA), insulated from the adjacent tissue by a thin plastic film, was placed under the most rostral end of the exposed nerve. The exposed vagus nerve was then covered with warm liquid paraffin. An incision was made 2 cm left from the midline below the rib cage, and the esogastric junction was exposed by deflecting the liver. Electromyographic (EMG) activity was recorded from the smooth muscle of the esogastric junction using sewn wire electrodes constructed from coated copper wire. The signals were differentially amplified (X-cell 3, FHC) and filtered (0.1-2kHz bandpass), then displayed on an oscilloscope and a computer chart recorder (Powerlab, ADInstruments, Australia). The first positive or negative deflection of the EMG signal exceeding the baseline noise following the stimulation was used for measurements of latency and threshold.

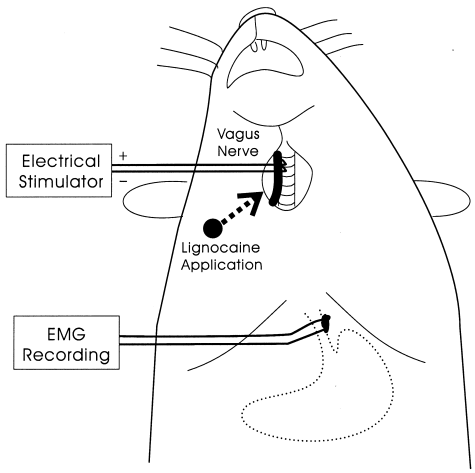

Figure 1

Schematic drawing of the experimental setup. A cotton ball containing 0.1 ml of 1% lignocaine (indicated by the arrow) was placed around the distal end of the exposed vagus nerve to achieve epineural blockade. The effect of blockade was evaluated by stimulating the cervical vagus nerve and recording the evoked electromyographic (EMG) response from the esogastric junction.

To achieve local anesthetic blockade, a cotton ball (20-25 mg) containing 0.1 ml of lignocaine (1%, Zuellig) was rubbed around the distal end of the exposed vagus nerve and then left in place. After 30 or 45 min, the cotton ball was removed, then the cervical vagus nerve was washed 3 times by applying cotton balls soaked with 0.9% saline, and again covered with warm paraffin. Electrical stimulation (A-M systems, USA), consisting of a single biphasic 50 μs pulse at various intensities up to 10 mA, was carried out prior to and during the epineural application of lignocaine, as well as at 10-minute intervals up to 60 min after washing. At the end of the experiments, animals were killed by an overdose of pentobarbital.

Statistical analysis

The threshold and onset of esophageal EMG activities evoked by stimulation of the vagus nerve were measured. Data were presented as mean and standard errors of the mean (S.E.M.). Values from post-anesthetic blockade were compared with the corresponding baseline value for each nerve using the paired *t*-test. The difference between the means was considered significant when $p<0.05$.

Results

Data were obtained from 11 vagus nerves in six rats. The lignocaine-containing cotton ball was left in contact with the vagus nerve for 30 min for five nerves and 45 min for the other six. Data from these 11 nerves were considered together, as similar changes were observed in latency and threshold of evoked EMG responses between the two groups of nerves. Fig. 2 illustrates a typical example of esophageal EMG changes evoked by suprathreshold stimulation of the cervical vagus nerve during control, lignocaine blockade, and post-wash period. It can be seen that upon epineural application of lignocaine, the EMG response disappeared within 1 min. No EMG response could be evoked while the lignocaine-containing cotton ball was in place. The esophageal EMG response returned immediately after the cotton ball was removed and the nerve washed with saline. However, the threshold of evoked EMG response remained elevated for up to 60 min. The threshold changes for the 11 nerves are summarized in Fig. 3. It can be seen that prior to application of lignocaine, the mean threshold of

electrical stimulation for evoking esophageal EMG response was 0.30±0.02 mA (n=11), with little variation between different nerves as indicated by the small standard errors of the mean. Immediately after saline wash, the mean threshold was much higher than the baseline. Then the threshold decreased gradually, reaching a plateau at 50-60 min. The mean threshold at 60 min was 0.48±0.03 mA, not significantly different from the pre-lignocaine threshold (P>0.05). Four nerves were tested for a longer period of 180 min, but no further decrease in thresholds was observed after 60 min. There were also increases in the latency of EMG response to suprathreshold stimulation after lignocaine blockade (Fig. 2 & 3). Maximal recovery in latency was reached at about 50 min (Fig. 3). However, the latency for some nerves did not return to the control level. As a result, the latency at 60 min post-lignocaine was still longer then that of the pre-lignocaine control (11.25±0.55 s vs 10.69±0.65 s, n=11, p<0.05).

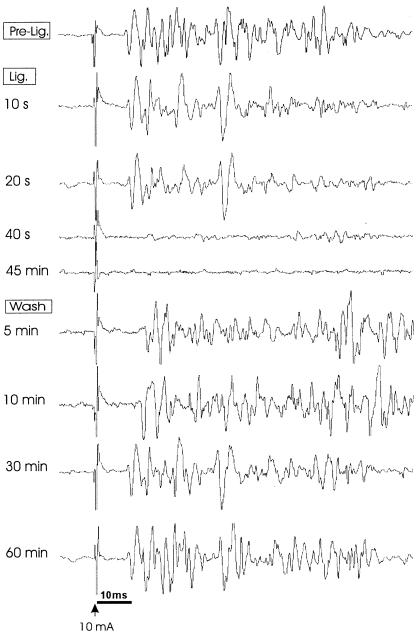

Figure 2

Computer chart records showing esophageal EMG responses evoked by simulation of the cervical vagus nerve. Pre-Lig. indicates the response evoked prior to lignocaine (Lig) blockade. The 4 time points (10s, 20s, 40s, and 45 min) below Lig. indicate the EMG responses taken at the corresponding time at which the vagus nerve was blocked by epineural application of lignocaine. The 4 time points below Wash indicate EMG responses at the corresponding time after the vagus nerve had been washed by saline.

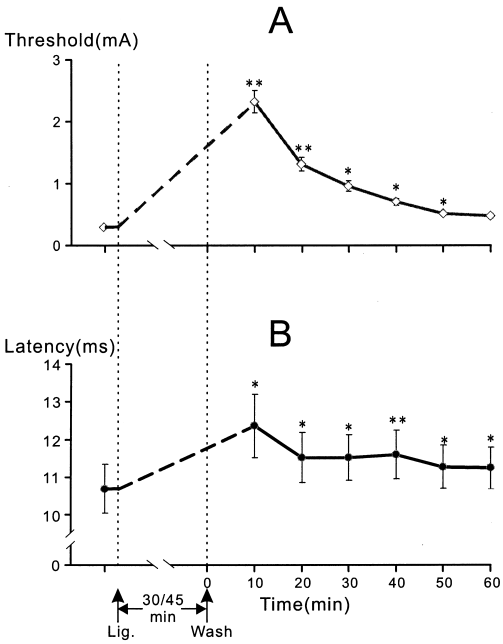

Figure 3

Line graphs showing the means and standard errors (S.E.M.) of the esophageal EMG threshold (A) and latency (B) evoked by vagal nerve stimulation at diferent time points. The latencies were determined by suprathreshold stimulation (10 mA, 50μs). The diamond in A and the dot in B closest to the left vertical axis indicate the mean baseline treshold and latency, respectively. The vagus nerve was completely blocked for the duration of epineural lignocaine application for 30 (n=5) or 45 (n=6) min, indicated by Lig. and Wash. The thresholds and latencies during the recovery (post Wash) period were compared with the respective baselines (*<0.05, **P<0.01, t-test).

Discussion

This is the first study assessing the time course of anesthetic blockade and recovery of the vagus nerve *in vivo*. The motor component of the vagal branch supplying the esophagus and the stomach, which was responsible for the evoked esophageal EMG response, contains a wide range of fiber sizes, comparable with that of the whole vagus trunk (*Gidda & Goyal, 1984; Prechtl & Powley, 1990).* Moreover, similar susceptibilities to local anesthetic blockade have been observed in vagal fibers of different sizes (*Fink & Carins, 1983; Jaffe & Rowe, 1996)*. For these reasons, our current findings should be applicable to the whole vagus trunk, although our data only concerned the vagal motor fibers supplying the smooth muscle of the esogastric junction. Our data showed that epineural application of 1% lignocaine produced immediate and sustained vagal nerve blockade, and a 50-60 min post-wash period was required for maximal recoveries in both threshold and latency. In comparison, a previous *in vitro* study showed that the latency of response of individual fibers reached maximal recovery in 20 min (*Fink & Cairns, 1983)*. The finding that neither the threshold nor latency of evoked EMG response returned fully to the control level is consistent with the previous study showing that the latency of individual fibers increased and some of the fibers lost their response after lignocaine blockade.

The current technique of local anesthetic blockade is simple and the recovery time is predictable. The amount of anesthetic used is small, and the wash helps to remove the residual anesthetics, minimizing any possible systemic effect of the local anesthetic. However, the 60 min recovery time after washing, probably reflecting the time course of action of the residual lignocaine, is still somewhat unsatisfactory for many kinds of experiment. In this regard, the current method of vagal conduction test, which involves recording of esophageal EMG evoked by stimulation of the cervical vagus nerve, is a simple and effective technique that can be used to evaluate the recovery time of other new short-acting anesthetics in the future.

To summarize, this study described a simple method for assessing the conduction blockade of the vagus nerve. Using this method, it was found that a predictable period of vagal nerve blockade could be achieved by epineural lignocaine application.

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