Pflügers Arch (1992) 421:280–282

Pflügers Archiv
European Journal of Physiology

Short communication

Effects of local nerve cooling on conduction in vagal fibres shed light upon respiratory reflexes in the rabbit

Wiebe R. Patberg [1], Arie Nijmeijer [1], Jan K. Schut [1], Adrian Versprille [2], Jacob P. Zock [1], and Willem G. Zijlstra [1]

[1] Department of Physiology, University of Groningen, Bloemsingel 10, NL-9712 KZ Groningen, The Netherlands
[2] Pathophysiological Laboratory, Department of Pulmonary Diseases, Erasmus University, Dr. Molewaterplein 40, NL-3015 GD Rotterdam, The Netherlands

Received March 17, 1992/Accepted May 6, 1992

ABSTRACT

In ten vagus nerves the effect of local cooling on the compound action potential was studied in the temperature range of 34 to 0 °C in spontaneously breathing, anaesthetized rabbits. The mean temperature at which the myelinated (A) fibres were completely blocked, was 10.2 ± 2.4 °C (mean ± S.D.). In nine nerves, local vagus cooling to 0 °C failed to block all non-myelinated (C) fibres. In one nerve, total blocking occurred at 2.0 °C. We conclude that in the rabbit, the earlier found increase in tonic activity of the diaphragm following lung inflation or deflation during bilateral local vagus cooling to a temperature between 8 and 0 °C is due to afferent impulses in vagal C fibres.

Key words: Vagal A and C fibres, local nerve cooling, conduction block, compound action potential, respiratory reflexes, rabbit.

INTRODUCTION

Hess & Wyss [3] demonstrated that the activity of the diaphragm reflexly decreased through lung inflation and increased through lung deflation. Vagotomy abolished these effects. Previous studies in the rabbit [6, 10] showed that bilateral local vagus cooling to 8 °C blocked the Hering-Breuer reflex and increased the tonic activity of the diaphragm, that is the level of activity to which the diaphragm returns during expiration. The tonic activity of the diaphragm could be increased further by both lung inflation [2, 10] and deflation [10]. The influence of local vagus cooling on conduction in myelinated (A) and non-myelinated (C) vagal fibres has been studied in the cat [1, 7] and the dog [4, 5]. In general, the blocking temperature of the vagal A fibres was 7-8 °C; conduction in vagal C fibres was blocked between 4.6 and 0.3 °C.

We considered the above-mentioned effect on the diaphragm after bilateral local vagus cooling to 8 °C to be the result of afferent C fibre impulses. In order to confirm this, we studied the effect of cooling on the compound action potential (CAP) of the rabbit vagus nerve.

Offprint requests to: W. R. Patberg

METHODS

In 7 Chinchilla rabbits of either sex (2.5-3.5 kg), 10 vagus nerves (6 right and 4 left) were studied. The animals were anaesthetized with urethane (1.5 g kg^{-1}, subcutaneously) and placed in supine position. Rectal temperature was kept at 38 °C. Arterial blood pressure was monitored using a catheter in the femoral artery connected to a Statham P23AC pressure transducer. The vagus nerve was exposed in the neck over a length of about 5 cm and transsected about 1 cm distal from the nodose ganglion. The peripheral trunk was placed in the nerve groove (12 mm long, 0.8 mm wide) of a cooling thermode as described previously (11). Pairs of silver stimulating and recording electrodes were placed caudally and cranially to the thermode, respectively. Immersion in a pool of oxygenated paraffin oil prevented the nerve from drying.

After a 100 μs pulse, supramaximal for the A fibres, a 2-5 ms pulse was given which was supramaximal for the C fibres. The pulses were delivered 75 ms after each other by two separate stimulus isolation units (*Neurolog NL 800, Digitimer, Welwyn Garden, UK*) connected in parallel with each other to the stimulating electrodes. Differentially recorded CAPs were preamplified (*Neurolog NL 103*) using the cooling plate of the thermode as ground electrode. The band-passed (0.1-1 kHz) (*Neurolog NL 115*) signal was amplified and DC-offset (*Neurolog NL 106*) to fit it within the range of a 12-bit AD converter (*AD574AJD, Analog Devices, Norwood, MA, USA*) with sample/hold circuit. AD conversion of the recorded signal was started 2.5 ms prior to the first stimulus, and continued for 50 ms (sample interval 50 μs). For the AD conversion that started prior to the second pulse, these periods were ten times longer. The digitized data were stored on disk for off-line analysis.

Starting from 34 °C, the thermode temperature was lowered stepwise by 1 °C each 90 s (in experiment nr. 1: 2 °C each 60 s) down to 0 °C. Thereafter, the thermode was rewarmed to 34 °C with the same step size. The stepwise change in the thermode temperature never took more than 5 s. Just before each change in temperature, the two stimuli for the

activation of vagal fibres were given and the CAP was recorded.

Triggering of stimuli, AD-conversion of the recorded signals, selection and logging of the thermode temperature and data storage was controlled by a program running on a microprocessor (Motorola 68B09) system. In three rabbits, the experiment was repeated on the left vagus nerve after completion of the experimental protocol on the right one (Exp. nrs. 2, 3 and 5). The digitized CAPs, stored on disk, were analyzed using the *LabVIEW* software package (*version 1.2, National Instruments, Austin, TX, USA*) running on an *Apple Macintosh II* computer.

We considered the conduction in the A and C fibres to be blocked when the corresponding component of the CAP had disappeared into the noise. The highest thermode temperature at which this occurred was taken as blocking temperature. The positive and negative deflection of each component was measured with respect to the baseline. The sum of both deflections was taken as the peak-to-peak amplitude of the corresponding component. Finally, these amplitudes were calculated as a percentage of the first one, obtained at a thermode temperature of 34 °C.

RESULTS

In all vagus nerves studied, the CAP consisted of two components: the A wave, conducted by the myelinated fibres and the C wave of the non-myelinated fibres. The averaged conduction velocity at a nerve temperature of 34 °C was 29.1 m/s for the A wave and 1.0 m/s for the C wave.

Figure 1 shows the changes of the CAP during local vagus cooling and rewarming in experiment nr. 3b. The effects of cooling to 0 °C were reversible in all experiments except nr. 2b, in which the C fibres poorly recovered.

The increase in latency between the stimulus and the response showed that the conduction velocity in both A and C fibres decreased with decreasing nerve temperature (Fig. 1). The concomitant broadening of the response indicated the increasing dispersion of the conduction velocities of the individual fibres. The decrease in amplitude of the A and C waves resulted in part from this dispersion but also, as cooling progressed, from blocking of fibre conduction.

During cooling to 0 °C the amplitude of the C wave was greater than the amplitude of the noise, except for one nerve. So, in all but one vagus nerves the temperature for total C fibre blocking was below 0 °C. The results are consolidated in Table 1.

Table 1. Temperatures below which vagal A and C waves were blocked completely

Exp. nr.	Vagus nerve	Blocking temperature (°C)	
		A wave	C wave
1	right	14.0	< 0
2a	right	10.0	< 0
2b	left	10.2	2.0 [a]
3a	right	9.0	< 0
3b	left	10.0	< 0
4	left	10.2	< 0
5a	right	7.0	< 0
5b	left	6.8	< 0
6	right	11.0	< 0
7	right	13.8	< 0

[a] During rewarming the C wave did not fully recover.

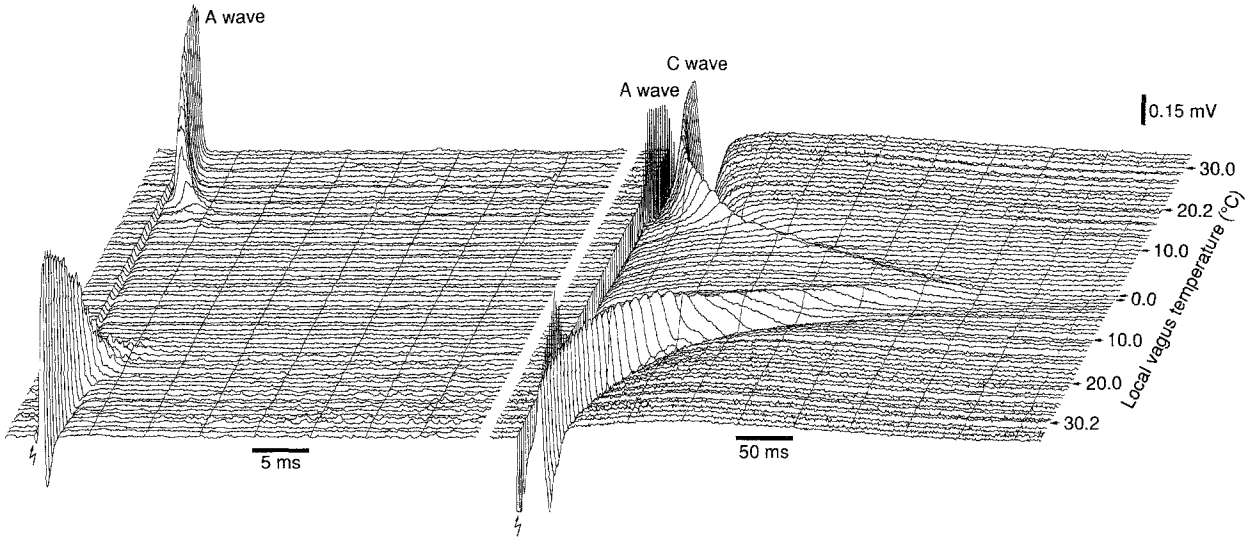

Fig. 1. Plot of A and C wave of the CAP against local vagus temperature during cooling and rewarming (exp. nr. 3b). The curves were obtained from bottom to top and plotted with increasing vertical and horizontal offset. Plotting of parts of a curve below previous ones was prevented. Apart from a gap of a few milliseconds, the curves on the left and the right are continuous, but the time scales are different. Stimuli: 100 μs/74 μA (first), and 4 ms/0.7 mA (second). At 10.0 °C the A wave was blocked completely. The C wave is clearly discernible at all temperatures. Note that the recorded spontaneous activity (visible as more disturbance in the upper and lower quarters) disappeared and reappeared simultaneously with the A wave.

In order to get an impression of the effect of local cooling as a routine method for differentially blocking of vagal A and C fibres in the rabbit, we have averaged the results of all experiments (Fig. 2). On average, differential blocking was achieved below 10.2 °C; in all vagus nerves studied, below 6.8 °C. In the temperature range of 6.8 to 2.0 °C, a considerable part of the C fibres was still able to conduct impulses.

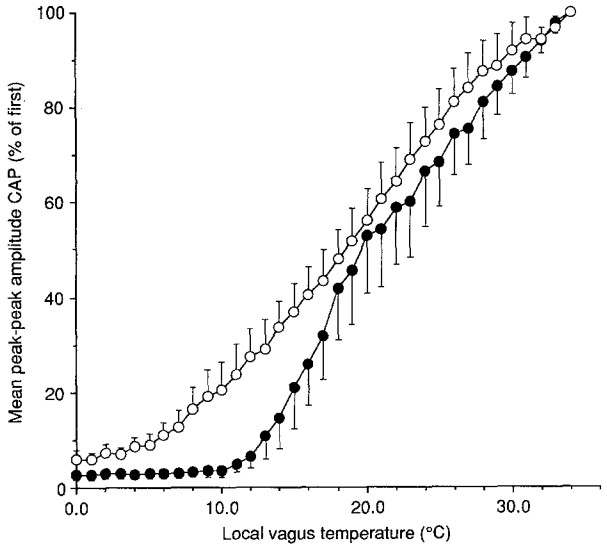

Fig. 2. Plot of the averaged peak-to-peak amplitude of the A (•) and C wave (o) of the compound action potential against local vagus temperature (10 experiments). Responses obtained at nearly the same thermode temperature (deviations <0.4 °C) were averaged. Blocking of conduction in A fibres generally occurred below 10.2 °C. Bars indicate S.E.M.

DISCUSSION

The present study originated from an earlier one [10] in which lung inflation as well as deflation reflexly increased the tonic activity of the diaphragm after bilateral local vagus cooling to 8 °C. We hypothesized that the vagal A fibres were completely blocked at 8 °C, and that the C fibres conducted the reflex stimuli. The present results are consistent with this hypothesis since in eight nerves the A fibres were completely blocked at 8 °C; in the other two this occurred at 7.0 and 6.8 °C (Table 1). In all nerves, C fibres were still conducting at these temperatures. The observation that in most experiments the blocking temperature found for the A wave was above 7 °C may have partly been the result of methodological factors. As Paintal [7] pointed out, pressure on the nerve will raise the blocking temperature. Although the nerve was carefully handled throughout the experiment, some compression will have been present since the nerves fitted snugly in the nerve groove for good thermal contact. In addition to the biological variation,we consider variation in compression, along with slight differences in the nerve preparation from experiment to experiment, to be a major reason of the differences in the blocking temperature between the nerves.

The temperature that blocks the conduction of spontaneous (repetitive) impulses is somewhat higher than that required for the blocking of single impulses [8]. This supports our view that the increase in tonic activity of the diaphragm after cooling of the vagi to a temperature below 8 °C, can only be due to afferent impulses in C fibres since all A fibres are blocked. The origin of these impulses is unknown but they might well be generated by the pulmonary J receptors [9].

From our earlier and present studies we conclude that a change in pulmonary volume beyond either side of the normal range stimulates vagal afferent C fibres, whose impulses increase the tonic (end-expiratory) activity of the diaphragm. However, with intact vagi this effect is fully masked during inflations, due to the inhibitory influence of the pulmonary stretch receptors. On the other hand, during deflations this vagal stimulation by C fibres enhances the desinhibition of the activity of the diaphragm that is caused by the lowered pulmonary stretch receptor activity.

REFERENCES

1. Douglas WW, Malcolm JL (1955) The effect of localized cooling on conduction in cat nerves. J Physiol (Lond) 130:53-71
2. Head H (1889) On the regulation of respiration. Part. 1. Experimental. J Physiol (Lond) 10:1-70
3. Hess WR, Wyss OAM (1936) Die Analyse der physikalischen Atmungsregulierung an Hand der Aktionsstrombilder des Phrenicus. Pflügers Arch 237:761-770
4. Jonzon A, Pisarri TE, Roberts AM, Coleridge JCG, Coleridge HM (1988) Attenuation of pulmonary afferent input by vagal cooling in dogs. Respir Physiol 72:19-34
5. Linden RJ, Mary DASG, Weatherill D (1981) The effect of cooling on transmission of impulses in vagal nerve fibres attached to atrial receptors in the dog. Quart J of Exp Physiol 66:321-332
6. Mühlemann R, Fallert M (1971) Der Hering-Breuer-Reflex bei künstlicher Beatmung des Kaninchens. II. Die Reflexunterbrechung durch schrittweise Vaguskühlung. Pflügers Arch 33:175-188
7. Paintal AS (1965) Block of conduction in mammalian myelinated nerve fibres by low temperatures. J Physiol (Lond) 180:1-19
8. Paintal AS (1966) Re-evaluation of respiratory reflexes. Quart J Exp Physiol 51:151-163
9. Paintal AS (1973) Vagal sensory receptors and their reflex effects. Physiological Reviews 53:159-227
10. Patberg WR (1983) Effect of graded vagal blockade and pulmonary volume on tonic inspiratory activity in rabbits. Pflügers Archiv 398:88-92
11. Patberg WR, Melchior HJ, Mast JG (1984) Blocking of impulse conduction in peripheral nerves by local cooling as a routine in animal experimentation. J Neurosc Methods 10:267-275