THE ROLE OF TIMING AND MAGNITUDE OF THE VAGAL INPUT IN CONTROLLING THE PHRENIC OUTPUT IN RABBITS AND BABOONS

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Abstract. The effects of a short train of electrical impulses applied to the central stump of a cut vagus nerve at various moments of the central respiratory cycle were studied in 28 rabbits and 3 baboons. The animals were anaesthetized (halothane), vagotomized, paralyzed and artificially ventilated. Stimulation in inspiration elicited always an inhibitory effect (latency 8–9 ms) the magnitude of which increased towards the end of inspiration. The amplitude and duration of inhibition increased also with the frequency of impulses and/or the duration of the volley. Stimulation in expiration shortened this phase after latency being shorter towards the end of expiratory pause. It is suggested that excitation of thin myelinated vagal fibres has a facilitatory effect on the inhibitory response to information being conducted along thick myelinated fibres during inspiration.

INTRODUCTION

In 1976 when some results of the present study were published (12, 13, 16) and their mathematical interpretation was proposed (14), the view that the rate of rise of inspiratory activity could be controlled “on-line” by the vagal input was not widely accepted (see 1, 4, 7). The present paper is not intended, however, to provide new data extending earlier observations; it is merely demonstrating some effects of a short-lasting stimulus on the central mechanisms that control frequency and amplitude of breathing (see also 12, 13).
METHODS

The experiments were performed with 28 rabbits of either sex. The experimental protocol was described elsewhere (12, 13, 17), the difference being that in the present study one impulse (or a short volley of impulses) was triggered at a preset moment of the central respiratory cycle and the effect was analysed from averaged integrated activity of the phrenic nerve. Three additional experiments were performed with 3 baboons under similar conditions, the premedication with NLA being replaced by ketamine (Ketalar–Parke–Davis, 10 mg kg$^{-1}$, i.m.).

RESULTS

In previous papers it was shown that activity conducted by thick myelinated vagal fibres is computed by the respiratory controller after spatial and temporal summation (17, 18). Time constant of this process is comparable with the duration of the inspiratory phase. It was also found that activity of thick myelinated fibres in expiration prolongs this phase proportionally to the frequency of stimulating impulses. The threshold frequency for $T_E$ changes ranged from 10 to 30 imp/s. Stimulation applied in the present work was regarded as threshold when one impulse affected the central respiratory rhythm. Through analysis of obtained responses (see also 17, 18), including compound action potential, a conclusion is reached that such a stimulation excites simultaneously both thick and thin myelinated fibres of the vagus nerve. During stimulation (whose intensity we shall call “alfa + delta”) at low frequencies (i.e. from one to 60 imp/s) the respiratory cycle is always reduced and this response does not depend upon the timing of stimulation (Fig. 1). Only during this type of stimulation one observes slow changes in respiratory rhythm which we have termed “short-term memory” (16). This phenomenon has a long time constant (several tens of seconds) and is dependent upon excitation of thin myelinated vagal fibres (17, 18). Hence, one impulse applied to the vagus nerve in the present experiments excited simultaneously both thick and thin myelinated fibres, the effects at the output being a net result of different phenomena.

The experiments were performed in different subgroups in which the central end of the vagus nerve was stimulated 1) with one impulse (or a short volley of impulses) at various moments of the respiratory cycle; 2) with volleys of different frequencies of impulses; 3) with volleys of different duration (always shorter, however, than $T_I$). Figure 2
Fig. 1. Sequential histogram of respiratory cycle duration (T). Effects of stimulation of A-alfa and A-delta vagal fibres (f = 50 imp/s). From left to right: control, stimulation “alfa + delta” in inspiration, stim. “alfa” in inspiration (repeated twice), then in expiration (T prolonged) and “alfa + delta” in expiration. T, time in s; N, number of consecutive cycle. Temp. = 38°C; End-tidal CO₂%= = 5.0, A.B.P. = 100 Torr.

Fig. 2. Stimulation triggered 25 ms after the onset of inspiration (f imp. = = 100/s). Phrenic nerve activity integrated and averaged — $K_8$, $G_8$, $G_2$, number of subsequent inspirations, control — and during stimulation, respectively. Below: stimulus marker (retouched). The record presents the first 51.2 ms of inspiratory discharge.

shows that there is no effect of a short volley of impulses triggered 25 ms after the start of inspiration. When one impulse is triggered with a delay of 50–100 ms from the onset of inspiration, the effect is very subtle (Fig. 3A). It does, however, increase when a short volley is
Fig. 3A: Stimulation with 1 impulse triggered 50 ms after the onset of inspiration. B: Stimulation with a short volley of impulses (f imp. = 100/s) triggered 102.5 ms after the onset of inspiration. Denotations as in Fig. 2.

Fig. 4A and B: Stimulation triggered 205 ms after the onset of inspiration. Denotations as in Fig. 2.

Fig. 5. Effects of stimulation with one impulse triggered 410 ms after the onset of inspiration on its pattern and duration. K16, control (averaged 16 times); G2, G4, G8 and G16, stimulation over 2, 4, 8 and 16 subsequent inspirations, respectively.
applied instead of a single impulse (Fig. 3B). On the other hand, stimulation triggered about 205 ms from the onset of inspiration elicits a clear-cut inhibition of phrenic discharge, the effect increasing with the frequency of stimulation (Fig. 4A and B). Stimulation applied towards the end of inspiration usually cuts inspiration short (Fig. 5). Stimulation of the same intensity (i.e. "alfa + delta") applied in expiration cuts short this phase, the effect being the stronger, the latter in expiration the stimulus is applied (Fig. 6A). A stimulus applied at the end of the expiratory pause immediately triggers the next inspiration (Fig. 6B).

![Fig. 6A and B: Stimulation with 1 impulse triggered at different moments of expiration. Dashed line, control record; solid lines, respiratory cycle during stimulation. Lower traces, stimulus marker (impulse retouched). Other denotations as in Fig. 2. Note that the second inspirations (A) are distorted in the process of averaging because of irregular effects of stimulation on the duration of expiratory pause. Further explanations in the text.](image)

Experiments performed with baboons yielded similar results, differences being largely quantitative (Fig. 7A and B). Frequencies that stimulate respiratory rhythm were lower in monkeys than in rabbits and amounted from 1 to 20 imp/s. Higher frequencies were already prolonging the duration of respiratory cycle when applied in expiration.
Fig. 7A. Stimulation in a baboon, with one impulse (370 mV) triggered at different moments of the respiratory cycle. Records in each subsequent (1–7) trace: top, integrated phrenic n. activity; bottom, arterial blood pressure. Stimulation marked by dots. M, monkey; VC, vagi cut; st.v, vagal stimulation.
Fig. 7B. Stimulation in a baboon with volleys of impulses (frequencies and intensities at the bottom of figure) applied in inspiration. Records as in A.
Fig. 7C. As above, stimulation in expiration.
(Fig. 7C). This was one of the reasons why only "alfa + delta" intensity was tested in this small group of animals. It should be pointed out that even when a short volley of impulses is applied in expiration, the first response consists in an inspiratory excitation (Fig. 7C). Similar effects were seen in rabbits with short volleys 100 imp/s (13). In monkeys one impulse applied at the beginning of expiration sometimes an abortive inspiratory effort (see Fig. 7A).

DISCUSSION

The interpretation of our results is not at all simple, because several groups of vagal fibres were being excited by the stimulus used by us (15). Different fibres conduct different information which is processed in different ways and gives rise to different effects (17, 18). We are fully aware that a clear understanding of our results will require a new technical approach; we think, however, that a few conclusions might already be proposed. The results indicate that the same stimulus applied at various moments of the respiratory cycle can produce a different (even qualitatively) response. It seems that neither frequency, nor duration, nor timing of stimulation are important separately, but all these parameters taken together. This apparently results from the number of action potentials being conducted by the vagus nerve in a unit of time and the mechanisms processing this information. Depending upon the actual central excitatory state, the same information might elicit inhibitory or excitatory effects in inspiration and expiration, respectively. It is worth noting that the same effect can be observed on a single respiratory neurone (13). The inhibitory effects in inspiration depend upon timing, the effect being stronger in the late part of inspiration. The duration of inhibition after one stimulus grows from 7 to 11.5 ms as inspiration progresses (13).

Similar effects of duration, frequency and timing of stimulation on the parameters of respiratory pattern were observed by Boyd and Maaske (5), Fallert and Spillmann (10) and Bradley (3). The effects of timing and amplitude of inflation were also indicated by Clark and Euler (6), Romaniuk et al. (22), Feldman and Gautier (11), Younes et al. (24) and Kubin and Lipski (19), although the findings were interpreted in a different way. We think, however, that Fallert and Spillmann (10) stimulated also thick and thin myelinated vagal fibres.

In the preceding paper (17) we have pointed out that it is not clear whether the inhibitory effect of "alfa + delta" stimulation is due to the large number of thick (alfa) fibres excited or to a combined action of large and thin (delta) fibres. The latter has a time constant longer
than one respiratory cycle, and its magnitude is increasing breath-by-breath during stimulation (Fig. 6). These results could imply that the activity of thin myelinated fibres might have a facilitatory effect on the inhibitory response to activities conducted via large fibres. The inhibitory effect is stronger towards the end of inspiration i.e. closer to the threshold of the inspiratory “off-switch”. It is known that this threshold decreases with time from the onset of inspiration (8, 9). This phenomenon can be easily detected when the effects of “alfa + delta” stimulation are observed on a single phrenic motoneurone (21). The later in inspiration the given motoneurone starts its firing, the more sensitive it is to the vagal input (see also the preceding paper). The view that there is a facilitatory component of thin myelinated fibre activity for activities from pulmonary stretch receptors is supported by experiments with thermal block of conduction in the vagus nerve (23). Further evaluation of our material might be based upon the hypothesis of Berger (2) suggesting that I alfa neurones are inhibited by pulmonary stretch receptors, whereas I beta neurones are excited by lung irritant receptors. The validity of this hypothesis, however, has to be subjected to further tests (20).

It is worth noting that the inhibitory effects observed are present in normocapnic and normothermic conditions. With the central respiratory drive decreased (e.g. by hypocapnia), and in expiration, excitatory effects start to dominate. The problem of inspiratory facilitation by vagal activity is further discussed in the next paper (18).

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