Asymmetry in reflex responses of nasal muscles in anesthetized guinea pigs

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Sekizawa, Shin-Ichi, Teruhiko Ishikawa, and Giuseppe Sant'Ambrogio. Asymmetry in reflex responses of nasal muscles in anesthetized guinea pigs. J. Appl. Physiol. 85(1): 123–128, 1998.—Nasal reflexes elicited by mechanical or electrical stimulation of nasal afferents were studied in anesthetized guinea pigs. Probing the nasal cavity of one side evoked a greater activation of the contralateral than the ipsilateral nasal muscles and, occasionally, sneezing. Similarly, electrical stimulation of the ethmoidal nerve often caused sneezing, with a greater activation of the nasal muscles and a greater increase in resistance on the contralateral side. Asymmetrical activation of the nasal muscles in response to mechanical stimuli induces asymmetrical airflows, especially during sneezing, between the two sides of the nasal cavity. Most of the expired air is forcibly blown out through the ipsilateral nostril, thus improving the elimination of irritants from the nose.

electromyogram; mechanical irritation; sneeze; trigeminal nerve

NASAL RESISTANCE is a major constituent of total airway resistance during nose breathing (1, 15, 17) and results from two different mechanisms. The first operates at the level of the nasal mucosa and depends on the amount of blood present in the venous erectile tissue (6). The second, the so-called nasal valve, is modified by the activity of the nasal muscles (abductors and adductors) and may account for up to 70% of total nasal resistance (17a).

The nasal airway consists of two tracts, separated by a septum, arranged in parallel and innervated on either side by efferent and afferent nerve fibers. The two tracts may respond differently to unilateral stimuli. In fact, Dylewska et al. (5) showed that mechanical stimulation of the nasal mucosa on one side changed nasal vascular resistance of the contralateral side. However, no specific studies have been conducted on the function of nasal muscles, which are represented mainly by nasal dilators (ala nasi, etc.) and a sphincter-like muscle (the compressor naris) (18).

Sneezing, which frequently can be evoked by irritation of the nasal mucosa, removes irritants and clears the airways. This highly coordinated reflex requires the participation of pump and upper airway muscles that are activated with a characteristic pattern (9). The purpose of this study was to investigate the electrical activity of nasal muscles involved in sneezing, as well as the pressure changes in the two nasal cavities. For this purpose, the electromyogram (EMG) activity of nasal muscles of both sides was recorded during sneezing elicited by mechanical stimulation of the nasal mucosa of one side or by unilateral electrical stimulation of trigeminal afferents.

METHODS

Animals, anesthesia, surgical procedure, and recording. After our protocol was approved by the institutional Animal Care and Use Committee, experiments were carried out on 24 adult male guinea pigs (459 ± 8.1 g). The animals were anesthetized with Urethane (1.2 g/kg, ip) and placed in a supine position on an operating table. The superior and recurrent laryngeal nerves were bilaterally cut to minimize possible interferences from respiratory vagal reflexes caused by airway cannulations. The cervical trachea was exposed and cut at the 6th to 8th ring to insert a tracheal cannula through which the animal breathed spontaneously. A pneumotachograph incorporated in the tracheal cannula was connected to a differential pressure transducer (15196; Gould, Hato Rey, Puerto Rico) to record respiratory airflow (V). Two saline-filled catheters were placed into the intrathoracic esophagus and a carotid artery for recording intrathoracic pressure (esophageal pressure; Pes) and arterial blood pressure (BP), respectively. Two pairs of enameled electrodes, with their metal terminals exposed for 1–2 mm, were placed bilaterally to record the EMG of nasal muscle activity. After the eyeballs were removed, the ethmoidal nerves (EN) and infraorbital nerves (ION) were identified within the orbits. In some experiments (as described in protocol 2), two polyethylene catheters were inserted into the laryngeal lumen and through the oro- and nasopharynx, reaching up to the nostrils, with the catheter tips positioned 3–4 mm inside the nostrils. A constant airflow (1.0 l/min) was passed through these catheters in the expiratory direction to measure pressure in each nostril (Pns) (15196 and 16720, Gould-Puerto Rico). In this situation, Pns reflects changes in resistance, as determined by the nasal muscle activity. All the signals were simultaneously recorded by a thermal-array recorder (TAS5000, Gould, Valley View, OH) and stored every 0.265 ms on a computer system equipped with an analog-to-digital converter (WinDAQ/200, Dataq Instruments, Akron, OH).

Protocol 1. Mechanical stimulation of the nostril. A thin silk braid (1-0), the tip of which was inserted within 5–8 mm from the entrance of the nasal cavity, was used to gently probe either side of the nasal mucosa. A tip of the braid could reach only the anterior part of nasal cavity: the nasal vestibule and the entrance of upper and/or middle meatus which are innervated by both the EN and the ION. The stimulation was performed for 10 s. The outside of the nostril was also probed with the same silk braid as a sham maneuver. For static mechanical stimuli, a tiny homemade balloon (3-mm OD) was used and inflated into the nasal cavity for 20 s. To increase respiratory “drive,” the tracheal cannula was occluded at end expiration during five consecutive breathing efforts. Nasal stimuli and tracheal occlusions were done before and after bilateral sectioning of the EN and/or the ION.

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Protocol 2. Electrical stimulation of the nasal branches of the trigeminal nerve. Both orbits were filled with warmed mineral oil, and then both ENs and IONs were sectioned peripherally. The central cut end of the EN or ION was electrically stimulated for 20 s with a pair of platinum electrodes in series (SD9, Grass Instruments, Quincy, MA; 20 Hz, 25 V, 2.5-ms duration). This made possible the stimulation of either side of the nasal afferents at the same strength without interfering with the recording of Pns.

Data analysis. To quantify the raw EMG signals, they were rectified and integrated (IEMG) (time constant = 0.1 s) by a data analysis system (CODAS, Dataq Instruments, Akron, OH). Mean BP and heart rate were obtained from the arterial BP signals. Values were averaged before (control) and during stimulation and were expressed as means ± SE. Control values were obtained for the same duration or, in the case of stimulation and were expressed as means ± SE. Control values were obtained for the same duration or, in the case of tracheal occlusion, for five consecutive breaths. The IEMG and Pns were categorized in the case of unilateral mechanical or electrical stimulation as ipsilateral or contralateral response. In protocol 2, the data were analyzed during a control breath, a sneeze, and an augmented breath. To evaluate the relationships between these events (IEMG, Pns) and the phases of the breathing cycle, we have represented inspiration and expiration as a percentage of their total duration. To summarize the responses of IEMG and Pns during sneezing or augmented breath (see Fig. 5), their signals were normalized by dividing for the value during the first 0.1 s of a control inspiration.

Differences within a group were determined through Wilcoxon's signed rank sum test, and comparisons between groups were determined through two-way ANOVA for repeated measures. The Dunnett post hoc test was used for multiple comparisons. The level of significance was set at P < 0.05.

RESULTS

Mechanical stimulation of the nostril. The electrical activity of nasal muscles increased during probing, with the response of the contralateral side being greater than that of the ipsilateral side (an asymmetric response; Figs. 1 and 2). BP and heart rate consistently decreased during probing (Fig. 3), with no difference between right and left nostril stimulation (P > 0.05). These responses were, to some extent, diminished by cutting either the EN (n = 8) or the ION (n = 6) and were entirely abolished by the section of both nerves (Fig. 1B and Table 1). In 6 of 18 animals, a sneezing reflex was observed. In some cases, probing could elicit this reflex even after bilateral section of either the EN or the ION. Balloon inflation could also cause hypotension of BP (41.4 ± 3.2 to 38.6 ± 3.2 mmHg) and increase the nasal EMG activity to a greater extent on the contralateral side, but only at the "on" and "off" of the stimulation. Probing outside the nostril did not affect the EMG activity (Table 1) and the cardiovascular parameters. An increase of respiratory drive caused by tracheal occlusion (lack of volume feedback) increased...
the EMG activity bilaterally, and this response was still present after section of both the EN and the ION (Fig. 1C).

Electrical stimulation of the nasal branches of the trigeminal nerves. A sneeze was occasionally observed after electrical stimulation of the EN after a period of disruptive breathing (n = 10 of 43 trials), but the ION stimulation failed to induce sneezing. An augmented breath was occasionally evoked by the EN or the ION stimulation (n = 7 of 84 trials). During control breathing before stimulation, Pns of both ipsilateral and contralateral sides slightly, but significantly, decreased during inspiration (0.03 ± 0.01 and 0.03 ± 0.00 kPa decreases, respectively), and nasal EMG activity of both sides increased [0.48 ± 0.09 and 0.61 ± 0.09 arbitrary units (au) increases, respectively], indicating a prevailing abductor muscle activity. Changes in Pns and EMG activity were quite similar on both sides (P > 0.05). Figure 4 shows changes of Pns and nasal EMG activity during a control breath, a sneeze, and an augmented breath. Figure 5 shows the normalized and averaged responses of both Pns and nasal EMGs. As seen in Fig. 5, during sneezing, Pns of both ipsilateral and contralateral sides decreased similarly in inspiration just as in the case of a control breath. However, before the end of this phase, while the contralateral Pns abruptly increased, the ipsilateral Pns continued to decrease. Therefore, an asymmetric response of the nasal EMG emerges, as seen in Fig. 1. An increase in the baseline level of contralateral Pns was also recognized as an asymmetric response during sneezing (Fig. 5). On the contrary, similar changes in Pns and the EMG occurred in both sides during augmented breaths (Fig. 5). Peak and/or valley values of EMG, Pns, and V are shown in Table 2.

A sneeze was clearly distinguished from an augmented breath by showing a clear peak in Pns (2.9 ± 0.5 kPa) followed by a peak expiratory flow (1.2 ± 0.21/min) at 0.11 ± 0.00 s after the onset of expiration (Fig. 4). BP decreased significantly by 4.9 ± 0.6 mmHg (P < 0.001), and there was no difference between stimulation of either nerve.

DISCUSSION

The nose is composed of two separate tracts arranged in parallel, a unique feature of the upper airway. Little is known about the muscle activity associated with each one of the two nostrils and their respective flow-resistive changes. In this study, it was found that nasal stimulation, specifically a mechanical stimulation of either nostril, activated nasal muscles asymmetrically, with the response being greater in the contralateral than in the ipsilateral side. This response could be abolished by trigeminal denervation, even when the nasal muscles were activated in response to tracheal occlusion that increased drive to the facial motoneurons. This indicates that the asymmetrical response of the nasal muscles was reflexly mediated through trigeminal afferents. These afferents may be identified with rapidly adapting mechanoreceptors which could be stimulated by probing the nasal cavity and inducing a sneezing reflex. In fact, slowly adapting mechanoreceptors of the ION stimulated by intranasal balloon inflation (11) did not induce any clear asymmetrical response.

Changes in Pns in each nostril indicate changes in resistance of the segment of nasal airway downstream from the tip of the upper airway catheter, i.e., the only

Table 1. Changes of electrical activity of nasal muscles during stimulation

<table>
<thead>
<tr>
<th>Stimulus and Side</th>
<th>Probing</th>
<th>Balloon Inflation</th>
<th>Probing Outside</th>
<th>Tracheal Occlusion</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Ipsilateral</td>
<td>Contralateral</td>
<td>Ipsilateral</td>
<td>Contralateral</td>
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<tr>
<td>Intact (n = 18)</td>
<td></td>
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<tr>
<td>Control</td>
<td>2.43 ± 0.27</td>
<td>2.46 ± 0.28</td>
<td>2.34 ± 0.27</td>
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<td>ION cut (n = 6)</td>
<td></td>
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<tr>
<td>Control</td>
<td>3.14 ± 0.55†</td>
<td>7.13 ± 1.12‡</td>
<td>2.50 ± 0.29†</td>
<td>2.95 ± 0.34‡</td>
</tr>
<tr>
<td>Stimuli</td>
<td></td>
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<td></td>
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<tr>
<td>EN cut (n = 8)</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>1.93 ± 0.24</td>
<td>1.96 ± 0.24</td>
<td>1.93 ± 0.24</td>
<td>1.96 ± 0.25</td>
</tr>
<tr>
<td>Stimuli</td>
<td>2.15 ± 0.22†</td>
<td>2.69 ± 0.35‡</td>
<td>2.11 ± 0.24†</td>
<td>2.08 ± 0.23*‡</td>
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<td>ION cut (n = 6)</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>2.53 ± 0.35</td>
<td>2.56 ± 0.37</td>
<td>2.54 ± 0.37</td>
<td>2.67 ± 0.39</td>
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<td>Stimuli</td>
<td>2.89 ± 0.40 †</td>
<td>5.97 ± 1.20‡</td>
<td>2.80 ± 0.41‡</td>
<td>3.06 ± 0.45†</td>
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<tr>
<td>Denervated (n = 14)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Control</td>
<td>1.79 ± 0.14</td>
<td>1.79 ± 0.14</td>
<td>1.80 ± 0.14</td>
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</tr>
<tr>
<td>Stimuli</td>
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<td>1.79 ± 0.14</td>
<td>1.80 ± 0.14</td>
<td>1.81 ± 0.14</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of guinea pigs; ION, infraorbital nerve; EN, ethmoidal nerve. *P < 0.05, difference from control; †P < 0.05, difference from denervated group; §P < 0.05, difference from ipsilateral group; $P < 0.05, difference between groups.
tract of the upper airway that is not bypassed by the catheter and thus is still under the control of the nasal musculature. Figure 5 illustrates the average changes in Pns in the two nostrils during a sneeze elicited by electrical stimulation of the ethmoidal nerve. During most of the inspiratory phase, Pns decreases to a similar extent on both sides. However, toward the end of this phase, there is a rather abrupt increase in the contralateral Pns that is associated with a further decrease of the ipsilateral Pns. Figures 4 and 5 indicate that these changes are instead caused by the activation of different nasal muscles and the recruitment of the contralateral nasal constrictor only, which would thus behave as the laryngeal constrictor activated during the compressive phase of sneeze. Thus we may infer that, in “normal” and “intact” subjects, the difference in resistance between the two nostrils can induce an asymmetrical airflow, diverting expired air through the ipsilateral side. Therefore, this pattern of activity could be helpful in removing the irritant substances from the nose. On the contrary, augmented breaths (Fig. 5, right), which could also be induced by electrical stimulation of the EN or the ION, showed large changes in V, similar to sneezes, without any asymmetrical changes of Pns. Thus the appearance of greater changes in contralateral pressure during expiration could be assumed to be a specific feature of the sneeze reflex.

As seen in Fig. 5, the baseline Pns in the contralateral side during sneezing is higher than the Pns of the control breath. This phenomenon is also characterized as an asymmetric response and might be due to an activation of the compressor naris. Thus the EMG activity in the contralateral side may consist of abductors and adductors, especially in inspiration, and it is greater than that of the ipsilateral side.

Mechanical stimulation of either nostril was performed by gently probing with a silk braid or inflating a balloon within the nasal cavity. Probably this stimulation, especially probing, did not have the same magnitude in each trial. However, there was no difference in BP changes between the stimulation of the two sides (Fig. 3), which suggests that the stimulation might have been done with almost the same effectiveness.

Whereas mechanical stimulation to the nasal mucosa in artificially ventilated dogs induced hypertension (16), stimulation in spontaneously breathing dogs

Fig. 4. Records taken during electrical stimulation of right EN (A) and right ION (B) in 1 guinea pig. Stimulation could elicit a sneezing reflex and an augmented breath, respectively. Pns, pressure in nostril. Symbols and abbreviations as in Fig. 1. Dotted lines, time of onset of expiration. Left Pns (Pnsr, contralateral side) rapidly increases early in expiratory phase of sneezing (A). However, in augmented breath, pressure of both nostrils changes similarly. Scale of right Pns (Pnsl) is magnified.
caused hypotension (5). In the present study, BP decreased with mechanical stimuli, suggesting that nasal mechanoreceptors could induce hypotension. Moreover, nasal chemical stimulation, such as an administration of ammonia or capsaicin in guinea pigs, caused hypotension (10, 14), but the difference probably depends on the excitation of different primary afferents. In fact, in guinea pigs, nasal trigeminal mechanoreceptors have a scant chemosensitivity (11, 12), and chemoreceptors have a poor mechanosensitivity (13, 14).

It is known that sneezing can be suppressed by the application of pressure on the upper lip or the outside of the nose (2), both of which are innervated by the ION. This may support our findings that show that electrical stimulation of the ION could not induce sneezing. However, Wallois et al. (19) showed that electrical stimulation of the ION could not elicit sneezing, and this may be a reason for their different results.

Although Davies and Eccles (3) mentioned that pressure stimuli (puffs of air) to the nasal vestibule evoked an increase in ipsilateral nasal activity that was mediated through the EN and the ION, they did not consider contralateral responses and did not use probing, which is entirely different from pressure stimuli. Probing activates rapidly adapting receptors, and pressure stimuli activate slowly adapting receptors. In fact, they could not elicit sneezing, and this may be a reason for their different results.

It is known that, during sneezing, complete closure of glottis, which might help to intensify the expiration, is observed right after the inspiratory phase, as in case of coughing (2). However, the nasal narrowing represented as an increase in Pns in Fig. 4 was not observed in the initial phase of expiration but in the late or end phase of expulsive expiration. Considering that an increase in Pns was seen in only one side of the nasal cavity, a role of nasal adductors during sneezing might be entirely different from laryngeal ones.

Asymmetrical responses resulting in changes in airflow resistance of the nasal passages have been described as the so-called “nasal cycle” (6) and the “crutch reflex” (7, 8). Both are mediated by the autonomic nervous system and are due to changes in the perfusion of the nasal mucosa. The nasal cycle, i.e., an alternation of flow between the two nasal passages, seems to be dependent on the information arising from nasal mechanoreceptors. In fact, changes in the nasal resistance of one side cause a reciprocal change in that of the other (2). Moreover, it has been shown that mechanical stimulation of the nasal mucosa could increase contralateral vascular resistance with little ipsilateral change (5). Thus nasal mechanoreceptors of one side can affect not only the contralateral nasal muscles but also the contralateral vasculature. However, in our study, changes in resistance due to vascular changes of the nasal mucosa were not considered for Pns, because there was no mucosa between the tip of the catheter and the opening of the nostril.
In conclusion, asymmetrical activation of the nasal muscles in response to mechanical stimuli may induce asymmetrical changes in resistance and airflow, especially during sneezing, between the two sides of the nasal cavity. Expired air would mostly go through the ipsilateral nostril, thus improving the elimination of irritants from the nose.

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