Effects of airway occlusion at functional residual capacity in pentobarbital-anesthetized kittens

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Trippenbach, T., R. Zinman, and R. Mozes. Effects of airway occlusion at functional residual capacity in pentobarbital-anesthetized kittens. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 51(1): 143-147, 1981.—The effects of airway occlusion at the end of inspiration on timing parameters and the “integrated” phrenic activity were studied in 1- to 21-day-old kittens at two levels of pentobarbital anesthesia before and after bilateral vagotomy. In intact kittens during the first 2 wk of life, instead of the classical effects of airway occlusion at functional residual capacity (FRC), shortening of both inspiratory and expiratory times were observed. These effects were not altered by an additional dose of pentobarbital sodium. In kittens of all ages, airway occlusion performed after bilateral vagotomy had no significant effects on either timing and phrenic activity. Therefore, vagally mediated reflexes are probably responsible for the paradoxical effects of airway occlusion at FRC. However, a possible contribution of the chest wall receptors cannot be excluded.

Hering-Breuer inspiratory-inhibitory reflex; esophageal pressure; phrenic activity; intercostal muscles

In adult cats, the “integrated” phrenic activity increases on the same time course whether the afferent input from pulmonary stretch receptors is intact or withdrawn as occurs after vagotomy or during airway occlusion at functional residual capacity (FRC). The same rate of rise of integrated phrenic activity with and without vagal volume-related afferent input illustrates the restricted role of the Hering-Breuer reflex to inspiratory inhibition. The rate of rise of activity of the first occluded breath and inspiratory time, however, can be influenced by other inputs, e.g., irritant receptors (12), J-receptors (7), or intercostal muscle receptors (7, 10).

The end-expiratory occlusion technique has been used in infants by Olinsky et al. (8) and Kirkpatrick et al. (4) to evaluate postnatal development of the Hering-Breuer inspiratory inhibitory reflex. They described an occlusion-related inspiratory prolongation that was greater in preterm than term babies, suggesting that the strength of this reflex decreased with age.

There is little known about developmental changes in the Hering-Breuer reflex during the early postnatal period. Estimation of the postnatal variations in the strength of this reflex is complicated by the fact that, besides a classical effect of airway occlusion at FRC, a shortening of inspiratory time can be recorded both in babies (5, 6, 14) and in newborn rabbits (15). The origin of this reflex is unknown. There are several possibilities: 1) the pulmonary deflation receptors (6, 14), 2) the extra-thoracic airway stretch receptors (11), 3) the intercostal muscle receptors (10), 4) the costovertebral joint mechanoreceptors (13), and 5) a rapid increase in venous return due to a decrease in the intrathoracic pressure during airway occlusion (9).

The aim of this study was to define occlusion-related changes not only in inspiratory time but also in expiratory time and the integrated phrenic activity during the early developmental period. The experiments were done on kittens during the first 3 wk of life. Airway occlusions at the end-expiratory level were performed 1) at two levels of pentobarbital anesthesia to study the role of anesthesia in the paradoxical effects of airway occlusion and 2) before and after bilateral vagotomy to eliminate all vagally mediated reflexes as possible contributors to this reflex effect.

METHODS

Experiments were carried out on pentobarbital-anesthetized kittens (20 mg/kg ip Nembutal, Abbott) during their first 3 wk. Animals were divided into three experimental groups according to their age. The 11 kittens in the first group, 1-6 days, weighed 0.109 ± 0.036 kg. The 9 kittens in the second group, 8-13 days, weighed 0.198 ± 0.032 kg, and the 9 kittens in the third group, 15-20 days, weighed 0.285 ± 0.032 kg. Animals were supine on a heating pad and spontaneously breathed a mixture of warmed and humidified 50% O₂-50% N₂ to prevent hypoxia. Skin in the midline of the neck was cut under additional local anesthesia with lidocaine if necessary, and the trachea was cannulated close to the rib cage inlet. The phrenic activity was obtained from the C₃ root placed on the bipolar electrode under mineral oil. The nerve was usually left intact for entire diaphragmatic innervation, but sometimes it was cut for a better signal-to-noise ratio. The phrenic activity was amplified (Grass model P511-H), rectified, and integrated by a moving-average phrenic processor. To monitor respiratory movements that occur simultaneously with the inspiratory phrenic activity and to distinguish this activity from occasional electrode noise, esophageal pressure was recorded. For this purpose, a balloon catheter, 0.5 cm diam and 1 cm long, filled with air but with no tension on its walls was located in the lower one-third of the esophagus and connected to a differential-pressure transducer (Hewlett-Packard 270). Tracheal pressure was recorded.
from the side arm of the tracheal cannula connected through a short stiff plastic tube to a pressure transducer (Sanborn 267B). Both pressure signals were fed into carrier amplifiers (Hewlett-Packard 885A) for signal conditioning. Rectal temperature was monitored with a glass rectal thermometer, and care was taken to maintain body temperature at about 37°C.

The data were recorded on a six-channel pen recorder (Gould, Brush 260). Records were obtained about 30 min after the initial injection of 20 mg/kg pentobarbital, 15–20 min after an additional dose of 10 mg/kg pentobarbital, and 10–15 min after vagotomy. At each condition, several airway occlusions at FRC were performed for one to three breaths at intervals of 2–3 min. The effect of airway occlusion at FRC on inspiratory (Ti) and expiratory (Te) times, maximal phrenic amplitude (Phr max), and the rate of rise of phrenic activity measured at 0.2 s from the onset of inspiration (Phr 0.2) of the first occluded breath was evaluated as percent change of the same parameters of the preceding control breaths. Means ± SD for the relative changes in each parameter were calculated. Statistical significance of the results was tested by two-tailed t test.

RESULTS

In Fig. 1, examples of the integrated phrenic activity during control and airway occlusion at FRC from three kittens with different ages are shown at two levels of pentobarbital anesthesia. At the control level of anesthesia (20 mg/kg pentobarbital), airway occlusion resulted in classical Ti and Te prolongations and an increase in Phr max with no change in Phr 0.2. After an additional dose of 10 mg/kg pentobarbital (Fig. 1, right), the effects of the airway occlusions varied with age. In the 5-day-old kitten, Ti and Te were not affected, whereas Phr max and Phr 0.2 were slightly increased. In the 10-day-old kitten, Ti and Te were not affected, whereas Phr max and Phr 0.2 were slightly decreased. Finally, in the 19-day-old kitten, changes in Ti, Te, Phr max, and Phr 0.2 were similar to those observed in the adult cat: a pronounced increase in Ti was followed by an almost unchanged Te, Phr max increased, and Phr 0.2 was unaffected.

Besides the classical effects of airway occlusion, in kittens during their first 2 wk, most occlusions caused paradoxical changes in at least one of the parameters measured. In these kittens, as long as vags were intact, the most frequent effect was increased phrenic activity. Considerably increased phrenic activity was recorded during the first occluded breaths at two levels of pentobarbital anesthesia (Fig. 2). The biphasic time course of this activity is characteristic for an augmented breath (3). After vagotomy, the occlusion-related augmented breaths were never observed, but sometimes (see Fig. 2) a decrease in the Phr max was observed.

An additional dose of pentobarbital did not diminish variability in Ti, Te, and phrenic activity. In fact, in most kittens during their 2nd wk, paradoxical effects of airway occlusion on Phr max slightly increased. However, after vagotomy, changes in all parameters were definitely less pronounced and were recorded during only a few occlusions.

All the paradoxical effects of airway occlusions on Ti, Te, Phr max, and Phr 0.2 were seen in kittens during their first 17 days. However, in 15- to 17-day-old kittens, augmented breaths and shortening of Ti were seen only during a few occlusions, and shortening of Te was never observed. At both levels of anesthesia in 18- to 21-day-old kittens, airway occlusion resulted in the classical (for adult cats) anesthesia-dependent changes in Ti and Te and in an increase in Phr max on the same time course as with the vagal phasic feedback (vagi intact). A summary of the effects of airway occlusions on Phr max, Ti, and Te at both levels of anesthesia and after vagotomy, is presented in Figs. 3 and 4. Figure 3 illustrates the effects of airway occlusion on Phr max as a function of changes in Ti in all the kittens studied; the values are given as a percent change from the control unoccluded breaths. Each point represents one mean ± SD. After 20 mg/kg pentobarbital (Fig. 3A), 3-wk-old kittens had similar increases in both Ti (67.2 ± 25.9%) and Phr max (61.7 ± 19.0%). Both changes were significant (P < 0.025 and 0.01, respectively). From
After the additional dose of 10 mg/kg pentobarbital (Fig. 3B) in the 3-wk-old kittens, $\Delta T_i$ increased $81.0 \pm 32.8\%$ ($P < 0.005$) and $Phr_{max}$ increased $50.0 \pm 32.0\%$ ($P$ change in rate of rise of phrenic activity was sometimes observed. Airway occlusion in each panel is indicated by arrows. (Peak amplitude of phrenic activity of a breath after release of airway occlusion in upper panel is cut off by pen recorder.)

**FIG. 3.** Summary of effects of airway occlusion at functional residual capacity on integrated phrenic activity ($Phr_{max}$) and inspiratory time ($T_i$) expressed as percent change ($\pm SD$) from control values. Each point represents mean obtained for all kittens of 1st (●), 2nd (■), and 3rd (▲) wk of life observed at control level of pentobarbital anesthesia (A), after additional dose of 10 mg/kg of anesthetic (B), and after bilateral vagotomy (C).
> 0.05). These relatively high standard deviations were present because during airway occlusions at FRC in kittens younger than 18 days an apneustic pattern of breathing was not recorded. In kittens during their first 2 wk, mean changes in both $T_I$ and $Phr_{max}$ were not significant. As indicated by the large standard deviations, paradoxical effects of airway occlusion were unaffected by an additional dose of the pentobarbital. The results did not depend on the intact C5 root of phrenic nerve. However, the phrenic afferent fibers, although very scanty (16), may contribute to the recorded inspiratory activity.

The effects of airway occlusions on $T_E$ and $T_I$ are illustrated in Fig. 4 as percent change from the control of both parameters for all the kittens. After 20 mg/kg pentobarbital (Fig. 4A), 3-wk-old kittens had slightly greater changes in $T_I$ than in $T_E$ (48.1 ± 17.8%, $P < 0.01$). (Changes in $T_I$ are described above.) In contrast, in kittens during their first 2 wk, the increase in $T_E$ was insignificant. After the additional dose of pentobarbital (Fig. 4B) in 3-wk-old kittens, augmented changes in $T_I$ were followed by diminished changes in $T_E$ (31.5 ± 18.5%) compared to the results obtained at low anesthesia. Whereas $\Delta T_I$ was significant ($P < 0.025$), $\Delta T_E$ did not reach significance. In kittens during their first 2 wk, an additional dose of pentobarbital had no effect on changes in $T_E$.

In all animals, after vagotomy airway occlusions at FRC did not produce significant changes in $Phr_{max}$, $T_I$, and $T_E$, and the variability of all the responses was less. This is illustrated in Fig. 3C and Fig. 4C. However, during a few occlusions a slight increase or, less frequently, a slight decrease in $T_I$ and/or $T_E$ and a decrease in $Phr_{max}$ were still observed.

**DISCUSSION**

In kittens during the 3rd wk of life during both levels of anesthesia, the overall effects of airway occlusions at FRC on $T_I$, $T_E$, and phrenic activity were similar to those described in the adult cat (2). Without vagal phasic input, $T_I$, $T_E$, and $Phr_{max}$ increased significantly, and rate of rise of phrenic activity was unaffected. In kittens older than 17 days, occlusions performed at the deeper level of anesthesia often produced apneusis. On the other hand, effects of airway occlusion at FRC in kittens during their first 2 wk during both levels of anesthesia exhibited a great variability in all the parameters measured. At the control level of anesthesia in kittens of the first age group, the only significant change was that in $T_I$. This occlusion-related $T_I$ prolongation was no longer significant after an additional dose of pentobarbital. However, the paradoxical effects of airway occlusion at FRC in kittens during their first 2 wk during both levels of anesthesia exhibited a great variability in all the parameters measured. At the control level of anesthesia in kittens of the first age group, the only significant change was that in $T_I$. This occlusion-related $T_I$ prolongation was no longer significant after an additional dose of pentobarbital. However, the paradoxical effects of airway occlusion at FRC in kittens during their first 2 wk during both levels of anesthesia exhibited a great variability in all the parameters measured. At the control level of anesthesia in kittens of the first age group, the only significant change was that in $T_I$. This occlusion-related $T_I$ prolongation was no longer significant after an additional dose of pentobarbital. However, the paradoxical effects of airway occlusion at FRC in kittens during their first 2 wk during both levels of anesthesia exhibited a great variability in all the parameters measured. At the control level of anesthesia in kittens of the first age group, the only significant change was that in $T_I$. This occlusion-related $T_I$ prolongation was no longer significant after an additional dose of pentobarbital. However, the paradoxical effects of airway occlusion at FRC in kittens during their first 2 wk during both levels of anesthesia exhibited a great variability in all the parameters measured. At the control level of anesthesia in kittens of the first age group, the only significant change was that in $T_I$. This occlusion-related $T_I$ prolongation was no longer significant after an additional dose of pentobarbital. However, the paradoxical effects of airway occlusion at FRC in kittens during their first 2 wk during both levels of anesthesia exhibited a great variability in all the parameters measured. At the control level of anesthesia in kittens of the first age group, the only significant change was that in $T_I$. This occlusion-related $T_I$ prolongation was no longer significant after an additional dose of pentobarbital. However, the paradoxical effects of airway occlusion at FRC in kittens during their first 2 wk during both levels of anesthesia exhibited a great variability in all the parameters measured. At the control level of anesthesia in kittens of the first age group, the only significant change was that in $T_I$. This occlusion-related $T_I$ prolongation was no longer significant after an additional dose of pentobarbital. However, the paradoxical effects of airway occlusion at FRC in kittens during their first 2 wk during both levels of anesthesia exhibited a great variability in all the parameters measured. At the control level of anesthesia in kittens of the first age group, the only significant change was that in $T_I$. This occlusion-related $T_I$ prolongation was no longer significant after an additional dose of pentobarbital.
tors probably contribute to the observed phenomenon. This hypothesis could be supported by the recent results of Thach et al. (14) who described an occlusion-related T1 shortening in an infant with a complete spinal lesion at the C5 level. However, the effects of airway occlusion on timing parameters could be masked by the breath-to-breath irregularity, especially after vagotomy in the youngest kittens. Therefore, on the basis of our study, a contribution of chest receptors in this reflex cannot be excluded.

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