Localization of CO$_2$ Sensor Related to the Inhibition of the Bullfrog Respiration

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Abstract CO$_2$ sensitivity in the airways and the general skin surface of the bullfrog under urethane anesthesia or without anesthesia was investigated. Pressure in the buccal cavity as well as blood pressure in the sciatic artery were measured with a differential or a strain-gauge transducer. Air containing 2–14% CO$_2$ was introduced into the regions as given below. (1) The nose and the body surface, both regions were separated from each other and independently exposed to CO$_2$. (2) The larynx-lungs, the buccal cavity-lungs, the naso-buccal cavity separated from the airways at the glottis, and the internal or external nares, respectively. By analyzing the CO$_2$ sensitivity of the respective regions mentioned above, both the nasal mucosa and the skin surface were found to be responsible for the respiratory inhibition by CO$_2$. Sectioning both the olfactory and the trigeminal nerves abolished the CO$_2$-induced inhibition mediated by the nasal mucosa and electrical stimulation of the proximal cut end of these nerves inhibited respiration. These findings suggested the existence of afferent reflex pathways from the nasal mucosa by these cranial nerves. Significance of this CO$_2$-induced reflex was discussed.

CO$_2$ administered to bullfrogs elicits respiratory inhibition although increased blood $P_{CO_2}$ with decreased pH ultimately augments ventilation (SAKAKIBARA and AKIYAMA, 1977). SMYTH (1939) presumed that this respiratory inhibition by CO$_2$ is induced by a reflex mechanism and the receptors are located in the glottis and the larynx. However, there has so far been no investigation confirming his assumption. In this study, it was intended that the following problems be solved. (1) Are both the skin surface and the airways involved in the CO$_2$ inhibition response? (2) Are the CO$_2$ sensors actually located in the larynx and the glottis as assumed by Smyth? (3) What sensory nerves in the airways are concerned with this inhibitory reflex?

MATERIALS AND METHODS

Bullfrogs (Rana catesbeiana) captured in Saitama or Chiba prefecture during

Received for publication December 15, 1977
winter to summer were supplied by Nippon Bio-Materials Supplying Center Co., Ltd. They were kept in a humidified cage at ambient temperature and with light regimen for several days or weeks till experimental usage.

Thirty-three bullfrogs were used for the experiments. They were strained on an animal board and prepared under ether anesthesia. CO₂ tests were performed 2 to 3 hr after preparation. In some cases experiments were carried out under urethane anesthesia induced by immersing the whole frog in 1–2% urethane solution. The design of experiments was shown in Table 1 and Fig. 1. The ex-

Table 1. Experimental designs for the series of experiments.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Areas explored</th>
<th>Types of stimuli</th>
<th>No. of buccal cannulae</th>
<th>No. of lung cannulae</th>
<th>Nose mask</th>
<th>In or out of chamber (exp. conducted)</th>
<th>Blood pressure measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Skin and airway</td>
<td>CO₂</td>
<td>1</td>
<td>0</td>
<td>+</td>
<td>In</td>
<td>-</td>
</tr>
<tr>
<td>II-1)</td>
<td>Larynx-lungs</td>
<td>CO₂</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>In</td>
<td>-</td>
</tr>
<tr>
<td>2)</td>
<td>Buccal-lungs</td>
<td>CO₂</td>
<td>2</td>
<td>0</td>
<td>-</td>
<td>In</td>
<td>-</td>
</tr>
<tr>
<td>3)</td>
<td>Nose</td>
<td>CO₂</td>
<td>2</td>
<td>0</td>
<td>-</td>
<td>In</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>1st and Vth cranial nerves</td>
<td>CO₂</td>
<td>1</td>
<td>0</td>
<td>+</td>
<td>In</td>
<td>-</td>
</tr>
<tr>
<td>IV</td>
<td>Same as above</td>
<td>electric stimulation</td>
<td>1</td>
<td>0</td>
<td>-</td>
<td>Out</td>
<td>+</td>
</tr>
</tbody>
</table>

Fig. 1. Experimental set up. The frog was connected to a nose mask and placed in the respiratory chamber. To the nose mask or the chamber, air or various kinds of gas mixtures were separately supplied by an air pump or gas cylinders through humidifying bottles. CO₂ concentration in the chamber was adjusted by using a water manometer as well as an infrared CO₂ analyser. The temperature in the nose mask, pressure in the buccal cavity and in the sciatic artery were measured.

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Experimental procedures were as follows.

1) Respiratory movement: A cannula (2 mm in diameter) was inserted into the buccal cavity through the cranium behind the eye which could be conducted with little bleeding. Its outer end was connected to a differential pressure transducer (DLPU-0.05 Nihon Kohden) to record the respiratory pressure change in the buccal cavity.

2) Arterial blood pressure and heart rate: A polyethylene cannula (0.6 mm in diameter) was introduced into the sciatic artery and connected with a strain gauge transducer (LPU-0.1-350-II, Nihon Kohden). Heart rate was counted from the blood pressure recordings.

3) Nose mask: A rubber tube (5 mm in diameter, 50 mm in length) was adequately clipped and a small opening was made with scissors at the middle in the tube so that it could be fitted to the apex of the head around the nose; it was called a "nose mask." This was sewed up with the skin around the nose by ligating eight points using fine threads. In some cases α-cyano acrylate monomer (aron alpha) and dental cement were used to make a mask fit tightly to the skin. These adhesive agents, however, did not always secure a success, since secretions from the skin sometimes prevented tight adhesion. Various kinds of test gases and room air were supplied with a flow rate of 90–100 ml/min through the nose mask to explore the existence of the CO₂ sensor in the airways. Latency of the inhibitory response to CO₂ stimulation was also observed.

4) Respiratory chamber: In Experiment I, II, and III (Table 1) frogs were placed in a respiratory chamber (150 × 280 × 200 mm) made of transparent synthetic resin. The cannulae were either connected to the transducer for pressure measurement or used to supply gas mixtures. Through a bifurcated or a single tubing inserted into the chamber wall, various gas mixtures humidified through a water bottle were supplied into the cannulae or into the nose mask. A three-way stopcock located outside the chamber in the gas mixture line was used to switch inflowing gas from cylinders over an air pump (Fig. 1). With another branch of the bifurcated cannula the pressure or the temperatures of the inflowing gases were monitored by a differential pressure transducer or thermister thermometer (MAG-III, Nihon Kohden). From another inlet of the chamber air was constantly supplied at a rate of 1–1.5 liters/min through a humidifying bottle by an air pump. The pressure of the inflowing gas was monitored by a water manometer. The level of the pressure was used as a rough indicator for the rate of airflow. Similar amounts of air were withdrawn from the chamber with another air pump to maintain the chamber pressure constant, at the same level as the atmospheric pressure.

In order to study the CO₂ sensitivity of the general skin surface, CO₂ was continuously added to the air in the chamber with constant velocity, being monitored by the water manometer, up to 4–5% which was measured by an infrared CO₂ analyser (LIA-2B, Horiba Co., Ltd.).

5) Test gases: Preliminary study showed that CO₂ less than 3% had no
distinct effect, thus more concentrated CO₂ mainly was used in the experiments. Four to 5% CO₂ in air was applied to the general skin surface as described earlier. On the other hand 5.7% CO₂ with 23% O₂ in N₂ was usually supplied into the airways or the nose mask. Fourteen percent CO₂ in O₂, 15% CO₂ in O₂, 2.1% CO₂ with 23.5% O₂ in N₂, or pure CO₂ were also used. Pure O₂ and N₂ were also used for the control.

**Protocols of experiments**

**I. Identification of the sensor in the airways and the skin.** The frog wearing the nose mask was placed in the respiratory chamber. Air was separately and continuously supplied to the chamber as well as the nose mask. Test gas (CO₂ 5.7% and O₂ 23% in N₂) was supplied into the nose mask to examine CO₂ sensitivity of the frog airways. Sometimes air, which was flowing into the nose mask during pre-CO₂ loading, was stopped by turning off the three-way stopcock in order to examine whether a mild asphyxic condition in the nose mask elicited respiratory inhibition. Examination was followed to detect whether CO₂ sensitivity of the frog skin existed with constant air flow in the nose mask.

**II. Localization of CO₂ sensor in the airways.**

1) Perception of the larynx-lungs preparation: The airway was separated into two compartments, the larynx-lungs and the rest by sewing the glottis at its rostral end. Bilateral lung cannulae (2 mm in diameter) were inserted according to Jones (1970) under ether anesthesia. The frog was then placed into the respiratory chamber. Air was supplied to the lungs as well as the chamber. The lungs could not ventilate through their own buccal pump, but by artificial aeration through the cannulae the animal still showed buccal ventilation movements. Its pressure change was still a good indicator for respiratory activities.

Through lung cannulae 5.7% CO₂ was given to the lungs in order to detect CO₂ sensitivity of the larynx-lungs at the same rate as the air flow, and thereafter, CO₂ sensitivity of the whole body except the larynx-lungs compartments was reexamined by the same method as in protocol I.

2) Perception of the buccal cavity-lungs preparation: The internal nares were closed by two small vinyl sticks with aron alpha or two pieces of paper with starch or vaseline. Thus, the buccal cavity-lungs area was separated from the rest of the airways. Two cannulae were then inserted into the buccal cavity (Table 1), and 5.7% or 14% CO₂ was separately and alternatively given for 30 sec to 4 min. After this examination, CO₂ sensitivity of the whole body was also tested as described above.

3) Perception of the nasal cavity: After the above study, the stoppers of the internal nares were removed and the external nares were closed by various means: vinyl sticks with aron alpha, sewing skin around the external nares, or paper with starch. Through the two cannulae inserted into the buccal cavity, 2.1, 5.7 and 14% CO₂ were administered into the nasal cavities for 30 sec to 4 min.

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After this, examination of the CO₂ sensitivity for the whole body was repeated.

**III. The effect of denervation of the Ist and Vth cranial nerves.** The frog was first sham-operated instead of by denervation of the 1st and the Vth cranial nerves, where the nasal bone, cartilage or other connective tissues were resected. CO₂ sensitivity was then examined by administering the test gases through the nose mask. After denervation of either the Ist cranial nerves or the rami frontalis of the trigeminal nerves had been conducted, CO₂ sensitivity was examined to see whether or not it remained. Thereafter, the remaining Vth or Isth cranial nerves in each frog were denervated and the same examination was repeated. In another case both nerves were denervated at the same time and then CO₂ sensitivity was examined.

Usually the CO₂ concentration used was 5.7%. However, after denervation of both the cranial nerves, 14% CO₂ particularly was used to detect if any residual CO₂ sensitivity remained. The test for CO₂ sensitivity in these animals was repeated for several days after the denervation.

In some experiments, after CO₂ sensitivity in the airways had disappeared completely, the frog was tested to determine if the respiration could be inhibited in response to whole body CO₂ administration in the respiratory chamber.

**IV. The effect of stimulation of the Ist and Vth cranial nerves on respiration and circulation.** Under urethane anesthesia, the 1st and Vth cranial nerves were exposed and the surrounding connective tissues were carefully removed. Each nerve was cut at the periphery, and its proximal stump was stimulated electrically (1 msec of duration, 2-7 V in intensity, 1-200 Hz in frequency). Stimulation was repeated three times with a proper intermission during which respiration returned to normal.

## RESULTS

**I. Effect of CO₂ in the nose mask or in the respiratory chamber**

The humidified 5.7% CO₂ supplied through the nose mask inhibited respiration in all 3 animals. Pulmonary ventilations were almost replaced by buccal ventilations or both types of ventilations were so severely depressed that apnea was induced. An example is shown in the second row in Fig. 2. When the air flowing in the nose mask was turned off, respiration was slightly inhibited as well. This was seen in every trial conducted more than 10 times (shown also in the 2nd row in Fig. 2).

When 5.7% CO₂ was applied the latency was between 2 and 13 sec and mostly about 4 to 8 sec (15 trials in 3 frogs). The latency tended to be shortened with rising CO₂ concentration (Fig. 3). When CO₂ loading was terminated and converted to room air breathing normal respiration was resumed after a short delay. When completely suppressed, respiration needed a longer duration for its restoration than the partial inhibition. Next, while room air was given continuously through the nose mask, CO₂ was added into the respiratory chamber up to 4 to 5%
Fig. 2. Effects of CO₂ in the respiratory chamber and the nose mask on respiration. Increasing the CO₂ concentration to 5% in the chamber, stopping flow or inducing 5.7% CO₂ in the nose mask resulted in respiratory inhibition.

Fig. 3. Effects of varying the concentration of CO₂ given through the nose mask on the respiratory inhibition. With increasing CO₂ concentration, the latency of induced inhibition was shortened.
in CO₂ concentration. Figure 2 indicates that CO₂ thus induced in the chamber inhibited respiration. Pulmonary ventilations were partially replaced by buccal ventilations. The CO₂ inhibition induced solely via the skin, however, was variable in its extent or in latency.

II. The effects of CO₂ administered into different compartments of the airways on respiration

1) The effect of 5.7% CO₂ given into the lung compartment. The frogs with larynx-lung preparation were tested. Five point seven percent CO₂ administered into the lungs compartment through the bilateral cannulae inserted did not inhibit respiration but augmented it. When 5.7% CO₂ was replaced by humidified pure O₂, the respiratory frequency decreased. When CO₂ concentration in the chamber was raised up to about 4%, respiration was prominently inhibited.

2) The effect of flowing CO₂ into the buccal and lung compartment. Six frogs with the buccal cavity-lungs preparation were examined. When 5.7% CO₂ or 14% CO₂ was given intermittently into the buccal cavity, respiration was unchanged in most cases. Sometimes, however, respiration increased in amplitude without any change in frequency (Fig. 4).

![Diagram showing the effects of CO₂ on respiration](image)

Fig. 4. Separation of the airway and effects of CO₂ given to the different cavities. On the middle row, 14% CO₂ introduced into the buccal and lung compartment through the double buccal cannulae did not inhibit respiration. In the upper and lowest rows, 14% CO₂ given into the buccal and nasal cavities markedly inhibited ventilation. The lowest test was conducted after the test of the second row.

3) The effect of flowing CO₂ into the buccal and the nasal cavities. Four
frogs with closed external nares were kept breathing through the double buccal cannulae. When 5.7% CO$_2$ was introduced into the buccal cavity through the cannulae, more than 80% of pulmonary ventilations disappeared after a short delay. This inhibitory influence was augmented by rising CO$_2$ concentration (Fig. 5). It was often observed that all pulmonary and buccal ventilations were completely suppressed for 30–60 sec. When CO$_2$ administration was prolonged, respiration began to increase after the initial inhibition. On the other hand, neither N$_2$ nor O$_2$ influenced respiration. After this observation, the internal nares of most frogs again closed and the foregoing Experiment II-2) was repeated. Sometimes Experiment II-2) was preceded by Experiment II-3). Without reference to the sequence of the experimental procedure the results were the same (Figs. 4 and 5).

The latency from commencement of loading CO$_2$ to the onset of respiratory inhibition was generally similar to the case of loading CO$_2$ externally, i.e., from the nose mask. Sometimes pulmonary ventilations were altered to buccal ones on turning the air to 5.7% CO$_2$, then suppression of ventilation followed. In such cases the delay time was not clearly investigated.

In these experiments the pressure of flowing gases at the inlet of the buccal cannula was kept at the same level throughout the experiment. The temperature

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change measured in the nose mask was within 0.2°C throughout a given experimental series. Usually the temperature of each test gas was equal to that of the preceding room air flow in the nose mask. Such a small temperature variation was considered to have little influence on ventilation.

Although, as just mentioned, ventilatory responses were varied by introducing CO₂ in different airway cavities, all the frogs observed in this section II considerably decreased their ventilations in response to CO₂ introduction in the respiratory chamber. Therefore, perception of CO₂ in the skin surface was considered to have been preserved.

**III. The effect of denervation of the Ist and nasal branch of the Vth cranial nerves**

As demonstrated in the foregoing results, perception of CO₂ in the airway compartment was estimated to localize in the nasal epithelium, the role of the cranial nerves innervated there were investigated.

Seven frogs were sham-operated and the nose mask mounted. Respiration

![Fig. 6. CO₂ sensitivity after surgical procedures for the Ist and the Vth cranial nerves. The inhibitory effect of 5.7% CO₂ supplied through the nose mask was apparent in the sham-operated animal (upper row I), and after bilateral sectioning the ramus frontalis of the trigeminal nerves (middle row II). It was abolished after bilateral sectioning of the Ist and the Vth cranial nerves (the lowest row III). O₂ and N₂ had no effect on this response.](image)

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in the 5 frogs was inhibited in response to inhalation of humidified 5.7% CO₂. The latency from the commencement of CO₂ inflowing, to the respiratory inhibition was 7.3±2.1 sec (mean±SE, 9 cases in 4 animals). The other 2 frogs were not affected by 5.7% CO₂. Although the reason why they did not respond to CO₂ was not certain, it may have been due to the fact that drastic operational procedures exceedingly irritated the sensory elements from the surface of the nasal epithelium. It is known that mechanical stimulation of the nasal mucosa leads to excitation of medullary expiratory neurons in cats (Price and Batzel, 1970), although it is not known whether such a mechanical stimulation increases respiration and/or inhibits the nasal CO₂ responsiveness in frogs. Two of 5, in whom an inhibitory response by 5.7% CO₂ was demonstrated, were not available for the full course of the nerve dissection experiment, because their response was either weak or fluctuated on repeating the CO₂ test for 7 days. In the other 3 frogs the olfactory nerves could be denervated as well as the rami frontalis of the Vth cranial nerves. They were repeatedly examined for the effects of 5.7% CO₂ or 14% CO₂ from immediately till 4 days after the denervation. Figure 6 indicates the results of this examination. Their respiration was no longer inhibited by 5.7% CO₂ or even by 14% CO₂. These denervated frogs still maintained a CO₂ inhibitory response mediated by the skin surface. Figure 7 shows such an example where ventilation decreased as CO₂ increased in the respiratory chamber.

![Figure 7](image_url)

Fig. 7. CO₂ sensitivity of the skin surface in the frog which lost CO₂ sensitivity in the airways. The frog, which was denervated and lost CO₂ sensitivity in the airways as demonstrated in Fig. 6, still decreased ventilation when CO₂ was given over the skin surface. The upper and lower figures are successive recordings in one experimental run. (a) CO₂ concentration in the respiratory chamber. (b) Respiratory movements.
IV. The effects of electrical stimulation

1) The effects of electrical stimulation of the olfactory nerves on the cardiopulmonary function of frogs. Ten of 16 frogs apparently gave decreased respiratory movements after electrical stimulation of the proximal end of the innermost medial branch of the olfactory nerves. The intensities of stimulation used were 2 to 7 V with 1 msec duration. Respiration always decreased to a degree during electrical stimulation of the frequencies used in this experiment. Figure 8 demonstrates the results of one frog, No. 16. This animal gave decreased respiratory frequency and magnitude. The longest duration between one pulmonary ventilation and the next during stimulation was compared with the mean duration in the control. The former was divided by the latter. Figure 9 indicates that at 20 Hz electric stimulation this ratio was as high as 19 times the control respiratory

Fig. 8. Inhibitory effect of electrical stimulation of the olfactory nerves on respiration and blood pressure of frog No. 16.

Fig. 9. Extent of inhibitory effect of electrical stimulation of the olfactory nerves on respiration and circulation. Abscissa: frequency of electric stimulation in Hz. Ordinate: ratio of the observed values in the respiratory period and heart rate (blood pressure) obtained by electric stimulation to their respective control values. Marked inhibition by 20 Hz was noticed in ventilation. On the other hand, heart rate and blood pressure were hardly influenced. The ratio of the blood pressure was similar to that of the heart rate. Closed circle, respiratory period; cross, heart rate (blood pressure).
period (closed circle). Generally, stimulation in the range of 20 to 50 Hz maximally inhibited the respiration. Outside this range, both at higher and lower stimulation frequencies, respiration was less affected except one frog, No. 14. This animal showed higher respiratory frequencies during stimulation with 2 Hz than that of the control, while the reverse was seen with a stimulation frequency higher than 10 Hz. Thus diphasic respiratory responses to electric stimulation were observed. The heart rate and blood pressure were also slightly depressed during electrical stimulation. The highest responses during stimulation were compared with the mean value of each control (Fig. 9). The degree of inhibition was much weaker than that seen in respiration and sometimes no suppression was observed.

2) The effects of electrical stimulation of the nasal branch of the trigeminal nerves. Three frogs were electrically stimulated at the proximal end of the ramus frontalis of the Vth cranial nerves for several seconds or minutes. These experiments were performed after electrical stimulation of the 1st cranial nerves. The intensities of electrical stimulation were similar to the preceding section and the frequencies were 1 to 30 Hz. All frogs exhibited tachypnea followed by apnea to an extent, as well as slight tachycardia and prominent high blood pressure for 20 to 140 sec after stimulation (Fig. 10).

![Fig. 10. Effects of trigeminal nerve stimulation on blood pressure and respiration. On response to stimulation, blood pressure was increased and ventilation also increased progressively at first and was followed by apnea.](image)

The latency for induction of such responses was shortened with increasing stimulus frequencies. Most tachypneic patterns transiently observed were of breathing with progressively increasing buccal pressure, and were similar to those seen during hypoxic stimulation (in toads: BOUTILIER and TOEWS, 1977; in bullfrogs: in preliminary experiments), or in hypercapnia (MACINTYRE and TOEWS, 1976).

**DISCUSSION**

From results we obtained, it seems that the nasal mucosa mediated the CO₂.
inhibitory reflex in respiration.

Smyth (1939) presumed that the epithelium in the larynx or the glottis of the frog might perceive CO₂. However, we could not confirm his assumption when either 5.7% CO₂ or 14% CO₂ were introduced into the larynx and the glottis which were separated from the internal nares.

Recently, the chemoreceptors responsible for CO₂ in the airways or the lungs were demonstrated in mammals and birds (Bartoli et al., 1974; Boushey and Richardson, 1973; Fedde and Peterson, 1970; Burger et al., 1976; Osborne et al., 1977). However, in contrast to the CO₂ sensor in the nasal cavities of frogs in the present experiments, their CO₂ responses generally introduced tachypnea or a greater amplitude of respiration. On the other hand, in cats (Boushey and Richardson, 1973), it is reported to elicit slowing in breathing with small and inconsistent changes in tidal volume. Fourteen percent CO₂ (Fig. 4) as well as 5.7% CO₂ introduced into the buccal cavity immediately induced a greater amplitude in respiration. Sometimes gases other than CO₂ for example, N₂ or O₂ elicited a similar result. Therefore, at present in frogs, the presence of such a specific CO₂ sensor that induces respiratory increment as in other species remains uncertain. In these experiments temperature, pressure of flowing gas, or the degree of humidifying the gases being flown into the cavity apparently did not affect this response.

As is generally accepted, the nasal mucosa is innervated by both the trigeminal and the olfactory nerves, and not by other nerves such as the vagus. Both nerves may be afferent nerves concerned with this reflex; in fact, after denervation of both nerves the reflex was abolished (Fig. 6). In this experiment they were stimulated electrically at their proximal end, resulting in depression of respiration or apnea. These results meet the presumption that the afferent nerves from the nasal CO₂ sensor are conveyed by these cranial nerves.

It is apparent that the nature of the cardiopulmonary responses induced by CO₂ loading to the whole body was similar to the effect of electric stimulation of the olfactory nerves but not that of electric stimulation of the trigeminal nerves (Sakakibara and Akiyama, 1977). When applying electric stimulation of the trigeminal nerves, apnea was preceded by a transient ‘filling’-like respiration. This response was clearly distinguished from that of the olfactory nerves. One may have to distinguish carefully the role of the trigeminal nerves from that of the olfactory nerves for the nasal mucosa-mediated inhibitory reflex.

It may also be worth referring to the results shown in Fig. 2, where only stopping air flow into the nose mask was shown to induce respiratory inhibition within 10 sec. Such observations were repeatedly confirmed in many frogs, and the possibility that gases other than CO₂, i.e., N₂ or O₂ are involved may be excluded because only endogenous CO₂ should have accumulated in this situation. Owing to the long latency for the onset of respiratory inhibition, mechanical conditions, such as changing flow rate, also may be excluded from the factors responsible
for this observation. The above results suggest that the nasal CO₂ sensor serves to inhibit pulmonary ventilation in response to CO₂ in the range of all expired CO₂. In fact, 1.2% CO₂ given via the nose mask, as well as 0.5% CO₂ introduced over the surface of the whole animal were found sufficiently to inhibit respiration (in preliminary experiments). However, it is not known how it affects the ventilation by way of the nasal CO₂ sensor in the normal acid-base state or in its extremities.

The latency for respiratory inhibition caused by applying 5.7% CO₂ to the buccal cavities, introduced either externally or internally, was as long as 4–8 sec. Such latencies were intended to become prolonged with decreasing CO₂ concentration applied. Since 4–8 sec is longer than the duration of the normal respiratory cycle, breath by breath inhibition by the expired CO₂ is supposed tenuous to be accomplished.

When CO₂ was given to the frog in a respiratory chamber while its nose was separately being supplied with air through the nose mask, respiration decreased, but its extent was less than that when the whole body including the nasal region was exposed to CO₂. More conclusive evidence for the existence of CO₂ perception in the skin is shown in Fig. 7 which shows that a frog without an airway CO₂ sensor by surgical procedures still depressed its respiration in response to CO₂ in the respiratory chamber. This observation agrees with the results of experiment using toads (KATO, 1951).

I wish to thank Dr. Yoshiyuki Honda for his advice and criticism of the manuscript.

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