Independence of hypoxic death of inspiratory PCO₂ in rats and fossorial mole rats

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other hand, Wilson and Kilgore (2) regarded CO₂ concentration as the sole lethal determinant in computations of gas diffusion in mammalian burrows. A need to sacrifice animals by restriction of their external gas exchange during a study of the adaptations to fossoriality of the mole rat (Spalax ehrenbergi) (3) posed a question on the validity of both assertions, which have not been verified experimentally. In the present study we addressed ourselves to the question of whether, and how, CO₂ does affect death in a confined atmosphere. A convenient laboratory mammal, the white rat, with hypoxic and hypercapnic tolerance typical of mammals, and the fossorial mole rat, which is extremely adapted to limited hypoxia and hypercapnia (3–6), were chosen as the animal subjects.

METHODS

We simulated the common situation of gradual hypoxia developing at a rate dependent on O₂ depletion by the animal’s metabolism, which also dictated the maximal CO₂ buildup rate. Environmental temperature, known to affect hypoxic survival, was controlled; however, rectal temperature (T₂) changes associated with severe hypoxia were monitored but allowed to occur. Other physiological variables, such as VO₂, VCO₂, heart rate, and respiratory rate, were continuously measured in order to get better insight into possible contributing mechanisms. The electroencephalogram was also recorded, in the hope of establishing an earlier end point (7, 8) that may be more meaningful in regards to resuscitation attempts.

Animals and surgical preparations

Thirty male white Charles River rats, 234 ± 29 (SD) g, and seven mole rats of both sexes (Spalax ehrenbergi), 171 ± 27 (SD) g, were used. Electroencephalographic (EEG) and electrocardiographic (EKG) electrodes were implanted in the rats under general (sodium Pentothal) anesthesia 3 to 5 days before the experiment. The EEG electrodes were stainless steel screws penetrating the skull in the parietal region. Transthoracic subcutaneous wires served as EKG electrodes. All electrodes were soldered through insulated wires to a female Amphenol miniconnector (Amphenol Connector Div., Bunker-Ramo Corp., Broadview, IL) fastened to the skull with dental cement. No attempt was made to implant electrodes on, or to otherwise wire, the mole rats, which would usually quickly tear off such connections.

Experimental system

A spherical glass chamber of 5-liter capacity, immersed in a water bath thermostated to 30ºC, provided the confined atmosphere. An ambient temperature of 30ºC was chosen because it is in the upper thermoneutral zone of both strains (9), thereby insuring similar initial normoxic conditions, minimal thermogenesis, and restrained hypothermia when VO₂ started to decline.

An external air pump (Bodine Electrical Co., Chicago, IL) circulated the chamber’s atmosphere in a closed circuit, either through or bypassing a CO₂-absorbing (soda lime) column. A fraction of the flow was diverted into a parallel closed loop via a desiccant chamber and through an oxygen analyzer (Servomex DA580, Taylor & Servomex Ltd., Crowborough, Sussex, U.K.) and an infrared CO₂ analyzer (Mijnhardt UG51). Penetrations for a N₂ leak, a male miniconnector and cable, and a temperature probe (Yellow Springs Instrument Co., Yellow Springs, OH) were provided in the chamber.
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**Procedure**

A rectal thermistor was inserted and taped to the tail; the miniconnectors were mated and the rat placed in the chamber. The chamber was then air- and water-tightened and totally immersed in the thermoregulated bath. An adaptation period of up to 30 min with the pump working in an open system was used to calibrate the analyzers and transducers and to assure stable readings of monitored variables. When the chamber was switched to the closed system, the animal’s metabolism gradually reduced the $O_2$ concentration and elevated the $CO_2$ concentration.

The very fine entrance port of the $N_2$ leak eliminated the slow pressure drop associated with the animal’s gas exchange and with $CO_2$ absorption, yet it allowed rapid pressure fluctuations caused by the animal’s breathing to be recorded with a Validyne pressure transducer (Validyne Engineering Corp., Northridge, CA). A continuous record of respiration, EKG, EEG, body temperature ($T_b$), and gas concentrations was obtained with a Dynograph recorder (Beckman Instruments, Inc., Schiller Park, PA).

At the cessation of breathing the EEG curve flattened out. Therefore the EEG flattening and the last gasp were used as the end point at which the chamber’s atmosphere was sampled with a syringe for a more accurate determination of gas composition on the MicroScholander (Scientific Instruments, Rutledge, PA) gas analyzer.

In the first series of experiments a range of final $CO_2$ concentrations was produced by initiating $CO_2$ absorption at different time periods from the start of closed-circuit breathing, followed by a period of no $CO_2$-absorption. The correlation of the final $P_{O_2}$ to the final $P_{CO_2}$ could then be tested on 20 rats. In the second series, 10 rats were divided into two equal groups. In the first (no-$CO_2$ group), $CO_2$ was absorbed; and in the second ($CO_2$ group), $CO_2$ was left to accumulate throughout the entire experiment. In this series, we could compare $O_2$ consumption and $CO_2$ production (both from chamber volume and from gas composition), along with the other variables, throughout the exposure time for the two extreme conditions.

No monitoring of physiological variables was attempted on the mole rats. They were put into the chamber with varying lengths of $CO_2$-absorbing periods, and gas composition was determined at the end point.

**Statistical analysis**

The relation of lethal $P_{O_2}$ to the final $P_{CO_2}$ was tested using linear regression where the significance of the difference of the slope from zero was tested using $t = b/SE(b)$.

The effect of $P_{CO_2}$ and $P_{O_2}$ on physiological parameters (oxygen consumption, body temperature, breathing frequency, and heart rate) was analyzed by the analysis of variance using "two-factor experiment with repeated measures" (10). The effect of $P_{CO_2}$ was analyzed both for the entire $P_{O_2}$ range and for each level of $P_{O_2}$ (10 Torr decrements). For the comparison of lethal $P_{O_2}$ and the survival time for both $CO_2$ and no-$CO_2$ groups, we used the $t$ test.

**RESULTS**

The final $P_{O_2}$ for all animals, plotted as a function of the final $P_{CO_2}$, is shown in Fig. 1. There was no correlation between lethal $P_{O_2}$ and environmental $P_{CO_2}$, over a range of 0–117 Torr,
either in the rat or in the mole rat, for which the mean lethal Po$_2$ was 38.0 ± 8.4 (SD) Torr and 20.9 ± 3.5 (SD) Torr respectively; the linear regression of lethal Po$_2$ on Pco$_2$ (Fig. 1) yielded slopes of 0.004 and −0.006 for rats and mole rats respectively. Both values are statistically not different from zero slope: $t = −0.005$ and $t = −0.003$, respectively. The mean time that elapsed from closing the chamber to the final gasp was 145 ± 34 (SD) min for all the rats and 245 ± 65 (SD) min for the mole rats and was independent of Pco$_2$ [142 ± 25 (SD) min and 166 ± 26 (SD) min for CO$_2$ and no-CO$_2$ groups respectively]. The mean lethal Po$_2$ of the 5 white rats from the no-CO$_2$ group (second series), 35.3 ± 2.9 (SD) Torr, was not statistically different from that of the CO$_2$ group, 37.8 ± 2.3 (SD) Torr, although the latter died at a mean chamber Pco$_2$ of 92.0 ± 7.8 (SD) Torr. The initial mean rectal temperature ($T_r$) of all white rats, 38.8 ± 0.9 (SD) °C, dropped during the exposure to a final mean $T_r$ of 36.5 ± 0.9 (SD) °C. One white rat from the first series, which had wet its fur prior to the experiment, had a final $T_r$ of 35.0°C and a lethal Po$_2$ of 26.7 Torr. It was excluded from the analysis.

The effects of zero vs. maximal CO$_2$ accumulation on variables measured during the course of hypoxia and gleaned from the second series are expressed in Figs. 2–5, which depict changes in oxygen consumption, rectal temperature, breathing frequency, and heart rate, in that order, all as a function of ambient oxygen partial pressure. The lethal Po$_2$ of only one animal from the CO$_2$ group was below 35 Torr; thus, although the final Po$_2$ was not significantly different, no mean is depicted for the CO$_2$ group at 35 Torr O$_2$. Thus there is a sudden, steep decline in $V_O_2$ as Po$_2$ falls below 65 Torr (Fig. 2). There was no statistical difference between $V_O_2$ of the CO$_2$ and no-CO$_2$ groups. However, the mean $V_O_2$ of the CO$_2$ group dropped faster, so that below 58 Torr it was lower than that of the no-CO$_2$ group.

The mean rectal temperature of both groups was not significantly different over the range of Po$_2$ (Fig. 3). Mean rectal temperature started to fall below a Po$_2$ of 95 Torr for the no-CO$_2$ group and below a Po$_2$ of 85 Torr for the CO$_2$ group. The CO$_2$ group demonstrated a lower $T_r$,
at 45 Torr, including a final T, of 35.6 ± 0.8 (SD) °C as compared to 36.8 ± 0.8 (SD) °C for the no-CO₂ group (P < 0.05).

The breathing frequency response to pure hypoxia is shown in the no-CO₂ curve of Fig. 4. The mean breathing frequency increased gradually to twice its control value from a PO₂ of 120 Torr to 55 Torr, before dropping sharply to below the control level in the last 10 Torr. The CO₂ group showed an immediate rise in mean breathing frequency, which reached the same maximal value as the no-CO₂ group but at a PO₂ of only 115 Torr. It then plateaued until a final drop at a PO₂ when the mean breathing frequency of the no-CO₂ group was still elevated. The breathing frequency of the CO₂ group in the PO₂ range of 125–105 Torr was significantly higher (P < 0.005) than the no-CO₂ group and at 45 Torr O₂ is significantly (P < 0.005) lower than that of the no-CO₂ group.

A gradual increase in mean heart rate occurred in the PO₂ range of 115–55 in the no-CO₂ group (Fig. 5) but not in the CO₂ group (P < 0.05). Both groups showed a dramatic bradycardia at severe hypoxia: the CO₂ group at a PO₂ of 55 Torr and the no-CO₂ group at a PO₂ of 45 Torr (P < 0.005).

No slowing of the EEG was seen prior to cessation of breathing nor the appropriate “loss of consciousness” in the animals' behavior. This may be partly related to the recording equipment, and further experiments to elucidate the response of EEG are planned.
DISCUSSION

The experimental protocol chosen (chamber volume relative to body mass) simulated confinement with a time course of a few hours. This period is intermediate in comparison to some short-term acute asphyxie and hypoxic experimental insults with time courses of minutes and to longer-term chronic exposures of days. On the whole, the body has to rely on short-range physiological defenses before succumbing to general system failure and irreversible damage.

The main finding of this study is the independence of the lethal Po₂ from environmental Pco₂. That both the well-adapted fossorial mole rat (9) and the laboratory rat (which, considering body mass, conforms to characteristic mammalian physiological traits) share this finding would justify its generalization to other mammals. Of the short-range responses of gas-transport mechanisms to which the animal can resort in order to maintain Vo₂ in the face of gradual hypoxia, the most obvious is hyperventilation, which could elevate Paco₂ and thus CaO₂. A decrease in the demand for oxygen is another possible response to the hypoxic danger. A recent study by Adams et al. (11) showed a critical oxygen-transport (Q × CaO₂) rate of 23 ml · kg⁻¹ · min⁻¹ in the anesthetized rat, below which acutely hypoxic animals could no longer maintain Vo₂. When Vo₂ decreased, survival could be prolonged by selective perfusion and O₂ delivery to the heart and the brain.

How does hypercapnia intervene in the above responses?
Hyperventilation

Any additional hyperventilation above that induced by hypoxia, although it is not aimed at reducing $P_{A\text{CO}_2}$ and after a while is ineffective in preventing its gradual rise, would help to maintain a higher $P_{A\text{O}_2}$. This is a distinct advantage, because in hypoxia where alveolar $P_{O_2}$ falls on the steeper portion of the $O_2$-dissociation curve, even relatively small $P_{A\text{O}_2}$ increases would substantially increase $O_2$ loading. Actually, combined progressive hypoxia and hypercapnia result initially in a higher phrenic nerve activity than that associated with any individual insult (12), but later on decline earlier at a higher $P_{O_2}$ or at a lower $P_{CO_2}$, respectively. Although total ventilation was not measured, it is known to be frequency dominated in the rat (5). The ventilation-frequency patterns of hypoxia and asphyxia in our rats agree in form with those of Cherniack et al. (12), which were observed in anesthetized dogs over a much shorter time course. When comparing the nonabsorbed to the absorbed $CO_2$ situation, one finds that, assuming $VO_2$ is initially similar, $F_{I\text{CO}_2}$ and $P_{O_2}$ are equal at any time for the two states. Therefore the hyperventilating $CO_2$ group maintains a higher $P_{A\text{O}_2}$. At extreme hypoxia, higher ventilation in the no-$CO_2$ group should maintain a higher $P_{A\text{O}_2}$ than in the $CO_2$ group at the final stage. The depressed ventilation in severe hypercapnia is known to be augmented by hypoxia (12).
Fig. 5. Heart rate as a function of ambient $P_{O_2}$. For explanation, see Fig. 2.

**Cardiac output**

Carbon dioxide affects the cardiovascular system in a complex manner. The final outcome is dependent on a balance of mainly depressing local effects on cardiac muscle and vascular smooth muscle and of central autonomic sympathomimetic effects. In man and large laboratory mammals a general reduction in peripheral resistance and an increase in cardiac output are believed to exist at intermediate (5%–10%) $CO_2$ levels before acidosis is severe enough to inhibit the sympathetic response and cause overall depression (13). However, the known depressing effect of $CO_2$ on the heart rate agrees with other findings (14, 15). The relation of cardiac output at the final stage to the $CO_2$ level is as yet unclear.

**Shifts of the oxygen dissociation curve**

It is now accepted that rightward shifts of the oxygen dissociation curve (ODC), or a high $P_{O_2}$, may aid tissue transport of $O_2$ and extraction during rest and exercise at normoxic or mildly hypoxic conditions, whereas a leftward shift, or a low $P_{O_2}$, may be advantageous in severe
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hypoxia (16, 17). Thus, high-altitude mammals and burrowing animals have low \( P_{O₂} \) readings and the degree of the leftward shift of the ODC curve correlates well with the lethal \( P_{O₂} \) (18). Carbon dioxide would, therefore, be expected to aggravate matters by its Bohr-effect rightward shift, although part of this action may be offset by \( CO₂ \) inhibition of 2,3-diphosphoglycerate production (19). This production in the rat can occur fast enough to be appreciable even with our rather short hypoxic time course.

At the final stage, the depressed ventilation in the no-\( CO₂ \) group may cause \( CO₂ \) accumulation in the body fluids, thus shifting the ODC curve to the right. High production of lactic acid (4 mM/liter and \( pH \), of 7.0 in dogs) during initial asphyxia (20, 21) may obscure the \( CO₂ \) effect by shifting the ODC curve in both groups to the right and may possibly reduce the difference in ODC due to \( CO₂ \) between both groups.

Selective perfusion

Any local peripheral vasodilative effect of \( CO₂ \) is masked by the rapidly ensuing general constriction induced by the sympathomimetic action of the gas. The exception is the cerebral vasculature, where vasodilation dominates. Thus \( CO₂ \) would be expected to enhance selective perfusion of the brain at the expense of the periphery and may even prolong consciousness from the time \( VO₂ \) decreases. This effect may not express itself at very high \( CO₂ \) concentrations when \( CO₂ \) becomes narcotic.

Reduction in oxygen demand

A reduction in oxygen demand defends against hypoxia in both normal animals (22) or in cooled animals (23). The earlier decrease of body temperature in the \( CO₂ \) group is probably due to increased peripheral perfusion and inhibition of nonshivering thermogenesis (24, 25). Whether the small decrease in \( T_b \) of the \( CO₂ \) group is advantageous in a hypoxic environment is unknown.

Carbon dioxide interacts with the hypoxic insult in a complex and unpredictable manner. The fact that \( CO₂ \) did not affect the final \( P_{O₂} \) or the survival time in a confined atmosphere could be the coincidental result of conflicting effects.

When one considers the course of hypoxic changes in the measured variables, it can be seen that both cardiac frequency and breathing frequency decline earlier in the presence of \( CO₂ \) and are preceded by the decrease in oxygen consumption.

Although a prelethal end point was not successfully established for cerebral dysfunction, \( CO₂ \) did not appear to significantly protect the brain, in accord with the finding that the level of \( P_{O₂} \), which caused EEG modification, was independent of \( P_{CO₂} \) in the asphyxiated dog (7) and the harbor seal (8).

The lethal \( P_{O₂} \) of the fossorial mole rat is lower than that of the white rat, 21 Torr vs. 38 Torr, respectively, and is very low when compared to mammals of similar metabolic rate (9, 22). However, a strict comparison of our lethal \( P_{O₂} \) for the mole rate and white rat, to other findings (rat’s lethal \( P_{O₂} \), value of 32) (18, 22) is not possible, because of differences in rebreathing time and body temperature. Although the body temperature of the normoxic mole rat (35.5°C) (9), is lower than the rat’s (38.8°C) the \( T_b \), of the mole rat did not decline in similar hypoxic exposures up to \( P_{O₂} \) of 32 Torr (5). The low lethal \( P_{O₂} \) of the mole rat can be explained by its physiological adaptations to hypoxic hypercapnia, very short diffusion distances (3), and probably facilitated diffusion through myoglobin and left-shifted ODC (9). Therefore, the mole rat is able to keep its normoxic metabolic rate at a very low \( P_{O₂} \) and can also increase its
heart rate and ventilation at low $P_\text{O}_2$ (5, 6, 9). Miller and Miller (23) could successfully resuscitate mice cooled in hypoxia only if $CO_2$ was present in the atmosphere; this fact, combined with our finding that lethal $P_\text{O}_2$ is not affected by inspired $P_\text{CO}_2$, may open a new approach to survival in confined atmospheres.

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Arieli R, Kerem D. Indépendance de la $P_\text{CO}_2$ inspiratoire dans la mort par hypoxie chez les rats et les rats-taupes. Undersea Biomed Res 1984; 11(3):275-285.—Des rats blancs de laboratoire et des rats-taupes (Spalax ehrenbergi) furent soumis à l’hypoxie progressive en les renfermant dans un atmosphère restreint et thermo-contrôlé. Des niveaux variables de $CO_2$ dans le milieu ambiant furent obtenus en contrôlant la durée d’absorption de $CO_2$. Les rats avaient été implantés avec des électrodes permettant l’enregistrement de l’électroencéphalogramme (EEG) et de l’électrocardiogramme (EKG) ainsi qu’avec une sonde de température rectale. Les animaux furent observés jusqu’à leur dernier soupir et l’aplatissement de l’EEG, au moment duquel l’atmosphère de la chambre fut analysée. Le rat-taupé démontre un niveau terminal de $P_\text{O}_2$ significativement plus bas (20.9 ± 3.5 (ES) vs. 38.0 ± 8.4 (ES) torses); cependant, chez les deux animaux, la $P_\text{O}_2$ finale était indépendante de la $P_\text{CO}_2$ dans les limites entre 0–117 torses. Les rats montrèrent une diminution progressive de la température rectale à partir d’une $P_\text{O}_2$ de 80 torses, totalisant 2.3°C à la fin de l’expérience. La consommation d’oxygène ($V\text{O}_2$) des rats fut maintenue jusqu’à une $P_\text{O}_2$ de 65 torses et diminua par la suite. Un groupe de rats avec une accumulation maximale de $CO_2$ montra une plus grande diminution de la température rectale et une chute plus prononcée de la $V\text{O}_2$ par rapport à la $P_\text{O}_2$, comparativement à un groupe sans accumulation de $CO_2$. Le résultat principal était inattendu, à propos du synergisme théorique des effets adverses de l’hypoxie et hypercapnie, et devrait réorienter les idées courantes concernant la survie et la ressuscitation dans des espaces restreints.

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