Effect of CO_2 on the metabolic and ventilatory responses to ambient temperature in conscious adult and newborn rats

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1. In newborn and adult rats, hypoxia decreases metabolic rate, especially at low ambient temperature (T_a). We examined whether a similar effect can occur during hypercapnia.

2. We measured metabolism (oxygen consumption, $\dot{V}_{O_2}$; open flow-through method), and expiratory ventilation ($\dot{V}_E$; barometric method (adults), airflow plethysmograph (newborns)) in air and 2% or 5% CO_2 in normoxia.

3. In adults, $\dot{V}_{O_2}$ was higher at $T_a = 10$ °C than 25 °C. At each T_a, CO_2 breathing did not change $\dot{V}_{O_2}$, but increased $\dot{V}_E$, less at 10 °C (up to +100%) than at 25 °C (+161%). Blood pressure was maintained at both values of T_a and CO_2, while pulse rate and body temperature were decreased in 5% CO_2 at 10 °C.

4. In newborns, the metabolic response to lowering T_a (from 40 to 20 °C) much depended on behavioural responses, being larger in groups of two or four pups than in individual animals. In no case did CO_2 influence the response. $\dot{V}_E$ increased during 5% CO_2 exposure, more so at $T_a = 33$ °C (+69%) than at 25 °C (+49%).

5. In both adults and newborns, hypoxia (10% O_2) always decreased metabolic rate.

6. We conclude that hypercapnia has no appreciable effects on metabolic rate in rats (both newborns and adults) even at low T_a, a result quite different from the hypometabolic response to hypoxia.

During acute hypoxia, a drop in metabolism and body temperature has been observed in various newborn and adult mammals. Although the mechanisms of hypoxic hypometabolism are not fully understood, in mammals the phenomenon seems to be mostly related to the inhibition of thermogenesis (Mortola & Gautier, 1995 for review).

Whether hypercapnia had similar hypometabolic effects has been less studied. This seems important not only for obtaining additional insights about the hypometabolic phenomenon, but also because, clinically, hypoxia can be accompanied by some CO_2 retention. The results presently available on adult mammals give contradictory results, since hypercapnia has been said to increase, have no effects, or decrease metabolic rate (Hales & Findlay, 1968; Stupfel, 1974; Jennings & Laupacis, 1982; Wagner, Matsushita & Horvath, 1983; Kaminski, Forster, Bisgard, Pan, Dorsey & Barber, 1985; Gautier, Bonora & Trinh, 1993; Sachdeva & Jennings, 1994). The very few reports on newborns also provide mixed information (Várnai, Farkas & Donhoffer, 1970; Várnai, Farkas & Donhoffer, 1971; Mortola & Matsuoka, 1993).

Aside from intrinsic species differences, numerous factors can contribute to the variability of the results. At high levels of inspired CO_2 metabolic rate is depressed (Stupfel, 1974), but at low CO_2 levels sympathetic reactions to the acute stress may prevail. The degree of hyperventilation may also play a role because of its energetic cost as well as the increased heat loss, which, by tending to decrease body temperature, would stimulate thermogenesis. These factors should also change with age, because of differences in ventilatory chemosensitivity and the larger heat dispersion per unit mass, of smaller and younger, compared with older and larger, animals.

With the present experiments we have re-examined the issue of the metabolic effect of hypercapnia, and attempted to separately consider some of the above factors. Firstly, mild hypercapnia (2% CO_2) and moderate hypercapnia (5% CO_2) have been examined at different ambient temperatures in conscious adult rats and the results interpreted with the help of parallel measurements of ventilation, body temperature, blood pressure and blood gases.

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In newborn rats, the interaction between ambient temperature and CO_2 has been examined in individual or grouped animals, therefore taking advantage of behavioural huddling as a means of varying the degree of heat dissipation. To assess the potential role of body size *versus* age, newborn dogs, which have approximately the body mass of adult rats, have also been studied. Finally, moderate hypoxia (10% O_2) has been tested for the purpose of comparison to hypercapnia.

METHODS

Experiments were performed on adult and newborn Sprague–Dawley rats; a few additional experiments were performed on newborn dogs. The study was approved by the Animal Ethics Committee of this Institution.

Adult rats

Ten male adult rats (232–283 g) were used. The basic experiment consisted of measurements of oxygen consumption ($\dot{V}_{O_2}$) and ventilatory pattern at two ambient temperatures (T_a), 25 and 10 °C; the former was 2–3 °C above the prevalent T_a of the laboratory and corresponded to the rat's thermoneutrality.

Early on the day of the experiment, a polyethylene catheter (PE-50, total volume 0·1 ml) was implanted in the tail artery under local anaesthesia (mepivacaine hydrochloride, 4–6 mg subcutaneous) for measurements of blood pressure and pulse rate, as well as for blood gas analysis (Saiki, Matsuoka & Mortola, 1994). Each sample was immediately analysed in a blood gas analyser (model 1302; Instrumentation Laboratory, Lexington, MA, USA; with repeated calibrations between measurements) for arterial O_2 pressure (P_{a,O_2}), arterial CO_2 pressure (P_{a,CO_2}) and arterial pH (pH_a), which were corrected to the body temperature (T_b) of the animal. The rat was returned to the cage after the operation, and appeared to have fully recovered in 2 h. Not less than 3 h later, the animal was placed into the metabolic chamber, with the tail catheter emerging outside and connected to a liquid-filled pressure transducer (model 1290C; Hewlett-Packard, Andover, MA, USA) for the measurement of blood pressure, via a three-way stopcock. The zero signal of the transducer corresponded to the level of the chest of the rat. The output was amplified, and the signal was recorded on a two-channel Gould pen recorder. The metabolic chamber consisted of a Plexiglass container surrounded by water, which was set to the designed temperature. A steady flow of air (1400 ml min^{-1} (STPD, standard temperature and pressure, dry)) was continuously delivered through the chamber and was controlled by a calibrated flowmeter. The rat was in the chamber for at least 30 min to get accustomed to the new environment before the measurements began. The different gas mixtures (2% CO_2 or 5% CO_2 with 21% O_2 in nitrogen) were delivered from calibrated pressurized tanks. The washout time of the animal chamber was 2 min and data collection started 15–30 min after the onset of each exposure; by this time $\dot{V}_{O_2}$ and the respiratory exchange ratio were stable (Saiki *et al.* 1994). $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were measured by the flow-through method (Frappell, Saiki & Mortola, 1991). The in-flowing and out-flowing concentrations of the gas, passed through a drying column (Drierite, Xenia, OH, USA), were monitored by a calibrated infrared CO_2 analyser (model LB-2; Beckman, Anaheim, CA, USA) and a polarographic O_2 analyser (model OM-11; Beckman). Gas concentrations were displayed on a computer monitor during on-line acquisition every 5 s. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were computed as the product of the in-flow–out-flow difference of the corresponding gas concentrations, averaged over 1 min in steady state, multiplied by the flow. Details and limitations of this methodology have been discussed previously (Frappell *et al.* 1991; Frappell, Dotta & Mortola, 1992). $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were converted to STPD and normalized by the weight of the animal in kilograms.

On termination of the metabolic measurements and after sampling for blood gas analysis, the inlet and outlet of the chamber were closed, and the breathing pattern was measured by the barometric technique. The oscillations in chamber pressure were monitored by a sensitive pressure transducer (model DP45 $\pm$ 5 cmH_2O; Validyne, Northridge, CA, USA) and recorded on paper at 10 mm s^{-1}; the signal was calibrated for volume by injecting the chamber with a known amount of air using a graduated syringe. The record was analysed with a graphics tablet connected to a minicomputer; fifty consecutive breaths were measured for each condition. Tidal volume (V_T) and expiratory ventilation ($\dot{V}_E$) were computed at BPTS (body pressure and temperature when saturated with water vapour) and normalized by the weight of the animal in kilograms. Colonic temperature (taken as representative of T_b) and T_a were monitored by a fine tungsten–constantan thermocouple (model DP30; Omega, Stamford, CT, USA). From the values of P_{a,CO_2} and $\dot{V}_{CO_2}$, alveolar ventilation ($\dot{V}_A$ (BTPS)) was calculated from the alveolar gas equation for CO_2:

$$\dot{V}_A\,(\text{STPD}) = \dot{V}_{CO_2}\,(\text{STPD})/((P_{A,CO_2}/P_b) - F_{I,CO_2}),$$

where P_b represents barometric pressure (dry), alveolar CO_2 pressure (P_{A,CO_2}) is considered equal to P_{a,CO_2}, and F_{I,CO_2} is the inspired CO_2 fraction.

Measurements were obtained during normoxia, 2% CO_2 and 5% CO_2 in normoxia, always in this order, with 30 min air breathing period between the two CO_2 exposures. Each exposure lasted 30 min. Experiments were performed at a T_a of 25 °C in the morning, and 10 °C in the afternoon. Between the morning and afternoon sessions, the animal was returned to its cage, with free access to water and chow. In four animals, after the 5% CO_2 test at 10 °C, additional measurements were obtained during hypoxic exposure (10% O_2 balanced with N_2, 30 min).

Newborn rats

Pups were born in the laboratory, where the adult pregnant females were housed in individual cages at a T_a of 20–25 °C and relative humidity of 50–53%, daily light–dark cycle of 12:12 h, with free access to rat chow and water. In total, seventy-two newborn rats, aged between 4 and 6 days (day of birth, day 0) and body weight between 7 and 14 g, were studied. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were measured by the flow-through method, as in adults. $\dot{V}_E$ was measured by airflow plethysmography. No blood gas analysis was performed. T_b was measured immediately before and after the measurements, and T_a was monitored throughout the whole experiment with thermocouples, as for the experiments on adults.

For measurements of metabolic rate at various T_a values, pups, either individually or in sets of two or four, were placed in chambers ranging in size between 20 and 200 ml. The flow of the gas passing through the chamber was controlled by a flowmeter and set at a rate ranging between 100 and 610 ml min^{-1} (STPD). T_a was continuously monitored and represented the average of the reading of three thermal probes placed at various locations in the chamber.

The effect of T_a on metabolic rate during air or hypercapnia was studied between day 4 and 6, beginning with measurements in air (day 4), then 2% CO_2 (day 5), and finally 5% CO_2 (day 6). To account for a possible effect of age on the metabolic response (Mortola & Dotta, 1992), a control group was also studied at each age during air breathing. Measurements were performed on sets of two pups each, and each experimental condition included six sets of animals. As described previously (Mortola & Dotta, 1992), pups were placed in the metabolic chamber preheated to approximately 33 °C by a heating lamp 13 cm in diameter. During the first 20 min, T_a was kept at 32–34 °C. Then, in normoxia or in hypercapnia, T_a was gradually increased to 40 °C over the next 10 min, followed by continuous measurements of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ as T_a was lowered from 40 to 20 °C at a constant rate of 0·5 °C min^{-1}, by adjusting the distance of the heating lamp and placing cold pads on the outer surface of the chamber.

In order to examine the effect of pups' huddling on the metabolic responses to CO_2 at different T_a values, the measurements described above were repeated on 6-day-old rats, breathing air (control, C) or 5% CO_2 (experimental, E). In this case, the pups were placed in the metabolic chamber either individually ($n = 5$, body weight 12·4 ± 0·3 g (C), 11·8 ± 0·5 g (E), mean ± S.E.M.), or in sets of two pups (7 sets, total body mass 21·8 ± 1·5 g (C), 24·7 ± 1·3 g (E)), or in sets of four pups (3 sets, total body mass 48·9 ± 3·0 g (C), 49·1 ± 3·4 g (E)).

Simultaneous measurements of metabolism and ventilation were performed on 6-day-old pups ($n = 10$, body weight 13·3 ± 0·5 g) at $T_a = 33$ and 25 °C, during breathing air (morning) or 5% CO_2 (afternoon). To this end, we slightly modified a set-up previously described (Saetta & Mortola, 1985; Saiki & Mortola, 1994). The rat was placed in a double chamber; the back chamber was used for measurements of breathing pattern by airflow plethysmography, and the front chamber to measure metabolism by the flow-through method (Fig. 1). The gas flow was controlled by a flowmeter at a level of 100 ml min^{-1} (STPD). First, measurements were collected during 15–30 min at $T_a = 33$ °C. Then, T_a was lowered to 25 °C at a speed of 1 °C min^{-1}; the pup remained at T_a of 24–26 °C for about 10 min, at which time $\dot{V}_E$ and $\dot{V}_{O_2}$ data were collected. At least 3 h elapsed between the morning and afternoon sessions, during which the pup was returned to the mother.

Newborn dogs

Six newborn dogs, 4–6 days of age, with body weight between 358 and 533 g (mean, 444 ± 28 g) were studied. Metabolic and ventilatory responses to 5% CO_2 at T_a of 28 °C were measured by the same procedure adopted for the experiments on adult rats. In three animals, after the 5% CO_2 test, hypoxia was also tested (10% O_2 balanced with N_2, 30 min).

Statistical analysis

All values are presented as means ± S.E.M. In the rats, the statistical comparisons of the data were performed by one-way repeated-measures analysis of variance, followed by *post hoc* contrasts with Bonferroni limitations (7 limitations in adults, 4 limitations in newborns), in order to compare the effect of CO_2 at a fixed T_a (25 °C and 10 °C in adults, 33 °C and 25 °C in newborns) and the effect of T_a with air, 2% or 5% CO_2. In newborn rats, the metabolic responses to lowering T_a from 40 to 20 °C were compared between C and E by repeated measures analysis of variance with one grouping factor (C *versus* E). Additional comparisons were performed by two-tailed paired t test, where appropriate. In all cases, the level of significance was considered at $P < 0.05$.

At the end of the experiments, all animals were killed by an overdose of sodium pentobarbitone.

RESULTS

Adult rats

In normoxia at T_a of 25 °C, $\dot{V}_{O_2}$ averaged 24·2 ± 0·7 ml min^{-1} kg^{-1}, and significantly increased at T_a of 10 °C (+70%). At either T_a, hypercapnia (2% and 5% F_{I,CO_2}) induced little changes in $\dot{V}_{O_2}$ (+3 to 7%). On the other hand, expiratory ventilation ($\dot{V}_E$) and alveolar ventilation ($\dot{V}_A$) increased markedly with hypercapnia (Fig. 2), and relatively more so at higher T_a (Fig. 3). The lower hypercapnic hyperventilation at T_a of 10 °C resulted in a slightly, yet significantly, higher arterial P_{a,CO_2} and acidemia in 5% CO_2 (values are presented in Fig. 2, panel *B*). With increased F_{I,CO_2}, T_b significantly decreased at T_a of 10 °C, but not at 25 °C (Table 1).

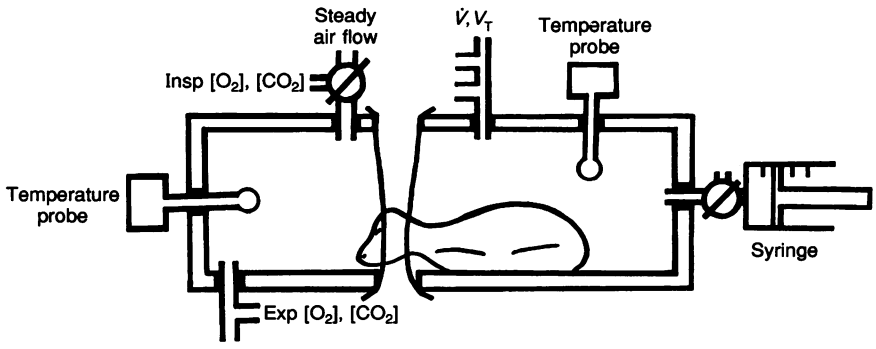

Figure 1. Schematic diagram of apparatus for metabolic and ventilatory measurements in newborn rats

See text for details. Insp and Exp [O_2], [CO_2], inflowing and outflowing O_2, CO_2 concentrations; $\dot{V}$, respiratory flow; V_T, tidal volume.

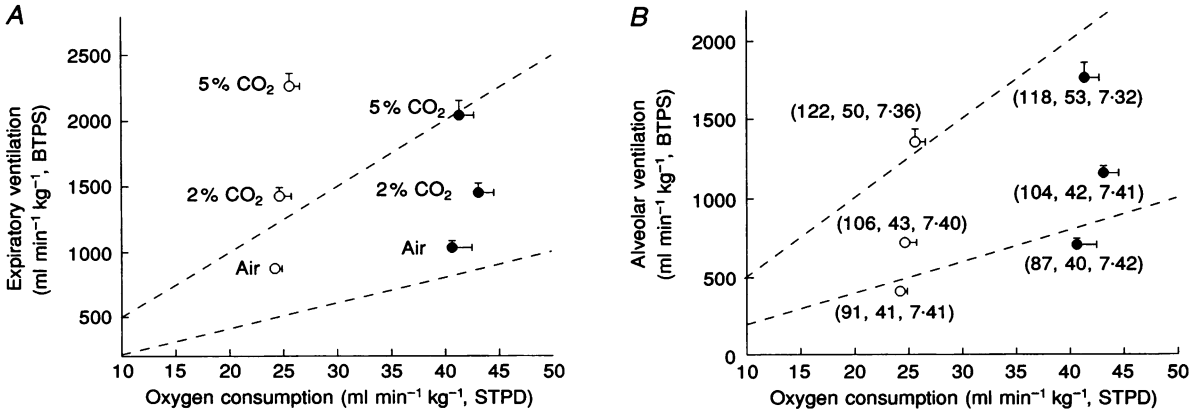

Figure 2. The effect of hypercapnia on ventilation–O_2 consumption relationships in adult rats

Each point represents the mean ± S.E.M. of 10 rats at 25 °C (○) and 10 °C (●). In parentheses, mean P_{a,O_2}, P_{a,CO_2} and pH_a, respectively. Dashed oblique lines are constant $\dot{V}_E:\dot{V}_{O_2}$ ratios. During hypercapnia, $\dot{V}_{O_2}$ changed very little, whereas $\dot{V}_E$ (*A*) and $\dot{V}_A$ (*B*) increased markedly. The ventilatory responses to hypercapnia (5% CO_2) were less at 10 °C than at 25 °C, as also indicated by the higher P_{a,CO_2} and lower pH_a at $T_a = 10$ °C.

In air, with the drop in T_a, both blood pressure and pulse rate increased. Hypercapnia slightly increased blood pressure at $T_a = 25$ °C, but had no effects at 10 °C. Pulse rate was unaffected by CO_2 at 25 °C, whereas it slightly dropped at 10 °C (Table 1).

Four rats were exposed to 10% O_2, soon after the CO_2 test was finished, at $T_a = 10$ °C. Hypoxia, in contrast to hypercapnia, decreased $\dot{V}_{O_2}$ and T_b to 26·1 ± 2·0 ml min^{-1} kg^{-1} (about −40% from air) and 32·4 ± 0·4 °C (−4·9 °C from air), respectively; $\dot{V}_E$ did not change significantly (from 1101 ± 92 to 1045 ± 99 ml min^{-1} kg^{-1}, −5%), whereas $\dot{V}_A$ dropped significantly (from 740 ± 60 to 588 ± 58 ml min^{-1} kg^{-1}, −20%). In hypoxia, P_{a,O_2} averaged 36 ± 2 mmHg, P_{a,CO_2} 29 ± 1 mmHg and pH_a 7·55 ± 0·01.

Newborn rats

In order to avoid the prolonged separation of the newborns from the dam more than once a day, the tests in air, 2% and 5% CO_2 were run, respectively, on postnatal day 4, 5 and 6. To account for the potentially confounding effect of postnatal age, on each day, control pups were also tested. It was apparent that the metabolic response to T_a was independent of the CO_2 level (Fig. 4). In fact, even the largest difference between pairs of data points (day 6, T_a of 20 °C, Fig. 4) was not statistically significant.

Changes in the number of pups per set had profound effects on the metabolic responses to T_a, the lowest response to cold occurring in the individual pups, and the highest when pups were in groups of four (Fig. 5); in no case did hypercapnia (5% CO_2) significantly modify the relationships. At

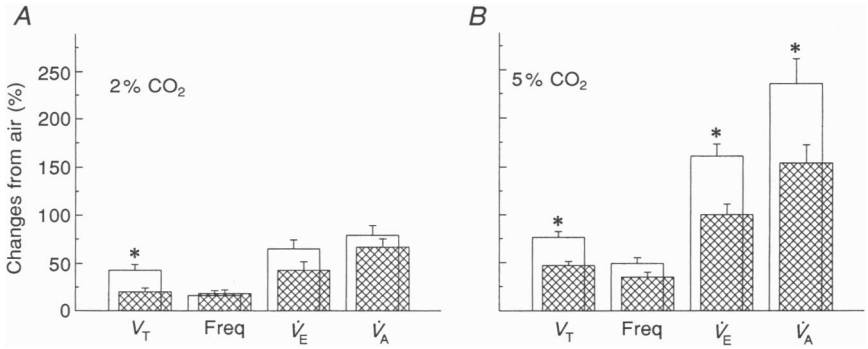

Figure 3. Influence of T_a on ventilatory responses to hypercapnia in adult rats

Bars represent means ± S.E.M. of 10 rats at $T_a = 25$ °C (□) and 10 °C (▩). * $P < 0·05$ between the two T_a values. The hypercapnic ventilatory responses were less at lower T_a. Freq, respiratory frequency.

Table 1. Body temperature, blood pressure and pulse rate of adult rats during the experimental period

	T_b (°C)	Blood pressure (mmHg)	Pulse rate (beats min^{-1})
T_a, 25 °C			
Air	38·1 ± 0·1	97 ± 2	409 ± 7
2% CO_2	38·2 ± 0·1	105 ± 2*	393 ± 10
5% CO_2	37·8 ± 0·1	108 ± 2*	415 ± 10
T_a, 10 °C			
Air	37·3 ± 0·1†	103 ± 1†	523 ± 9†
2% CO_2	37·0 ± 0·1†	103 ± 1	522 ± 8†
5% CO_2	36·1 ± 0·2*†	104 ± 1	466 ± 10*†

Values are means ± S.E.M. Statistical comparisons are air *vs.* 2% CO_2 or 5% CO_2 (*$P < 0·05$), and T_a of 10 *vs.* 25 °C (†$P < 0·05$); *post hoc* test with Bonferroni limitations after one way repeated analysis of variance. $n = 10$.

T_a of 20 °C, T_b invariably decreased below the nest value of 35–36 °C; the decrease was similar whether the newborn was in air or in 5% CO_2, and in either case, the drop was more marked for individual pups ($T_b = 29$ °C in both air and 5% CO_2), than in sets of two (31 °C in air, 28 °C in 5% CO_2), or sets of four (33 °C in air, 32 °C in 5% CO_2).

On the other hand, 10% O_2 breathing at $T_a = 20$–25 °C decreased both metabolic rate (Fig. 5, ▲) and T_b; the latter averaged 24·8 °C in the case of individual pups, 25·2 °C for the sets of two pups, and 28·3 °C for the sets of four pups.

Table 2 summarizes the data obtained in 6-day-old newborn rats, in which $\dot{V}_E$ was also measured. As in the experiments described above, CO_2 breathing did not alter $\dot{V}_{O_2}$ whereas it increased $\dot{V}_E$, and, as in adults, significantly more so at the higher T_a.

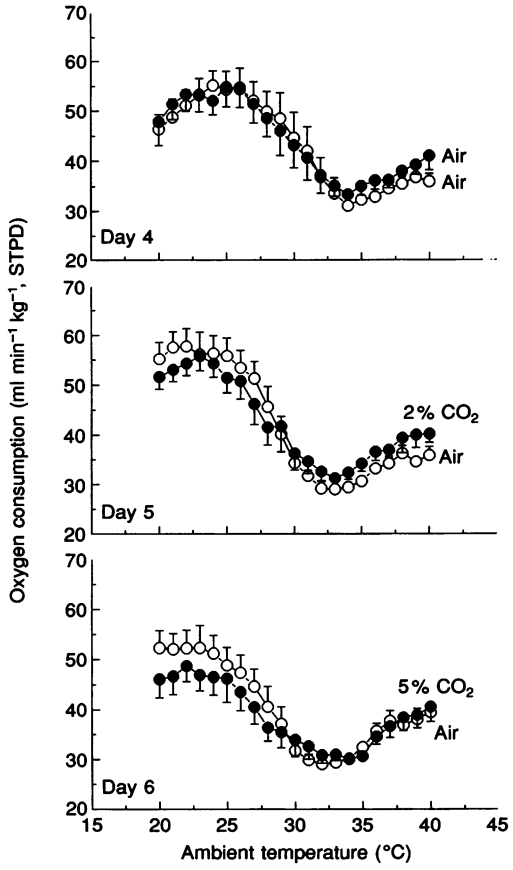

Figure 4. Oxygen consumption ($\dot{V}_{O_2}$) of 4- to 6-day-old newborn rats over the 40–20 °C range in T_a

Each point represents mean ± S.E.M. of 6 sets of 2 pups each. Experimental group (●) was exposed to air (day 4, top), to 2% CO_2 (day 5, middle), and to 5% CO_2 (day 6, bottom), whereas control group (○) was exposed to air on all days. The hypercapnia did not change the metabolic response to T_a.

Table 2. Metabolic and respiratory responses to hypercapnia in 6-day-old newborn rats

	T_a (°C)	$\dot{V}_{O_2}$ (ml min^{-1} kg^{-1})	V_T (ml kg^{-1})	Respiratory frequency (breaths min^{-1})	$\dot{V}_E$ (ml min^{-1} kg^{-1})	$\dot{V}_E/\dot{V}_{O_2}$	T_b (°C)
Air	33	31 ± 1	7·2 ± 0·3	138 ± 7	949 ± 32	31 ± 1	35·9 ± 0·4
	25	49 ± 2*	7·4 ± 0·3	178 ± 10*	1289 ± 52*	27 ± 1	32·6 ± 0·3*
5% CO_2	33	34 ± 1	10·9 ± 0·5†	152 ± 5	1588 ± 36†	46 ± 1†	35·9 ± 0·4
		[113 ± 4%]	[152 ± 5%]	[111 ± 4%]	[169 ± 6%]	[151 ± 6%]	
	25	46 ± 2*	10·5 ± 0·3†	185 ± 7*	1900 ± 59*†	42 ± 2†	32·7 ± 0·3*
		[96 ± 3%‡]	[142 ± 5%]	[106 ± 5%]	[149 ± 7%‡]	[157 ± 9%]	

Values are means ± S.E.M. Percentage of air is indicated in the square brackets. Statistical comparisons of the absolute values between $T_a = 33$ °C *vs.* 25 °C (* $P < 0.05$), and between air *vs.* 5% CO_2 († $P < 0.05$); *post hoc* test with Bonferroni limitations after one-way repeated analysis of variance. Percentage changes between $T_a = 33$ °C *vs.* 25 °C (‡ $P < 0.05$; Student's paired t test). $n = 10$. V_T, tidal volume.

Newborn dogs

Similar results were observed in newborn dogs; 5% CO_2 did not change $\dot{V}_{O_2}$, whereas it increased $\dot{V}_E$ and slightly decreased T_b at $T_a = 28$ °C (Table 3). The effect of 10% O_2 breathing was tested in three of these animals; $\dot{V}_{O_2}$ and T_b decreased to 14·8 ± 0·7 ml min^{-1} kg^{-1} and 36·9 ± 0·1 °C, respectively.

DISCUSSION

$\dot{V}_{O_2}$ in hypercapnia: previous data in other species

In humans, during hypercapnia, several investigators observed an increase in both $\dot{V}_{O_2}$ and $\dot{V}_E$, the former being commonly attributed to the energetic cost of the hyperventilation (Shepard, 1955, for references). Similar results were obtained in the ox and the pony (Hales & Findlay, 1968; Kaminski *et al.* 1985). Some exceptions, however,

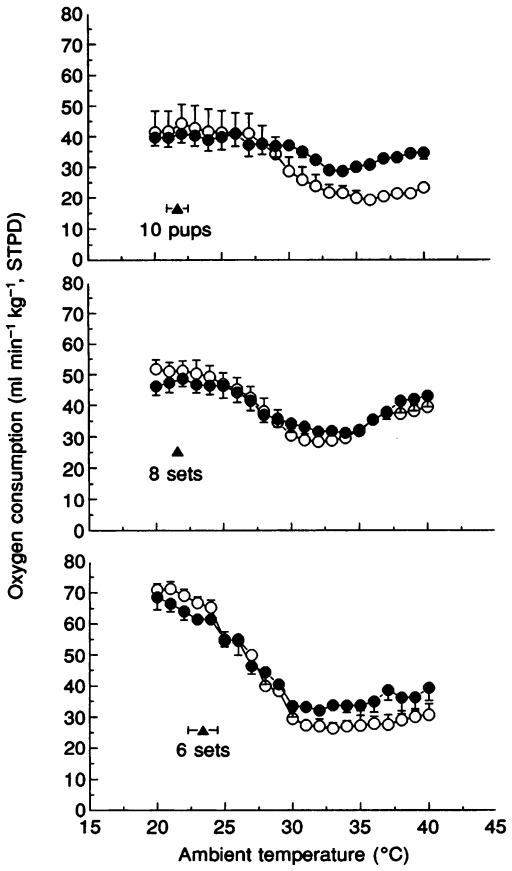

Figure 5. Effect of huddling on the metabolic responses to T_a in 6-day-old newborn rats

Each point represents the mean ± S.E.M. of individual animals (5 pups, top), 2 pups per set (7 sets, middle), and 4 pups per set (3 sets, bottom). Control group (○) and experimental group (●) were exposed to air and 5% CO_2, respectively, over the 40–20 °C range in T_a. Metabolic responses to lower T_a were increased by huddling, and were similar between control and experimental groups. Some animals from both groups (▲) were then exposed to 10% O_2 at 25–20 °C. In all cases, hypoxia abolished the metabolic response to cold.

Table 3. Metabolic and respiratory responses to hypercapnia in 4- to 6-day-old newborn dogs

	$\dot{V}_{O_2}$ (ml min^{-1} kg^{-1})	V_T (ml kg^{-1})	Respiratory frequency (breaths min^{-1})	$\dot{V}_E$ (ml min^{-1} kg^{-1})	$\dot{V}_E/\dot{V}_{O_2}$	T_b (°C)
Air	18·4 ± 0·2	18·0 ± 0·8	47 ± 3	831 ± 74	45 ± 5	37·7 ± 0·2
5% CO_2	18·0 ± 0·6	35·8 ± 1·1*	48 ± 3	1736 ± 153*	97 ± 8*	37·5 ± 0·1*

Values are means ± S.E.M. Statistical comparisons between air and 5% CO_2 (*$P < 0·05$; Student's paired t test). $n = 6$.

have been reported. For example, Wagner *et al.* (1983), in human subjects breathing 4% CO_2, found that $\dot{V}_{O_2}$ did not differ from the normocapnic value in either warm or cold conditions. No changes in $\dot{V}_{O_2}$ were observed in dogs breathing 5–8% CO_2 (Jennings & Laupacis, 1982). Very high CO_2 concentrations (20%) in rabbits decreased thermogenesis and body temperatures (Stupfel, 1974), but no changes occurred with 6% CO_2 (Maskrey & Nicol, 1979). A large drop in $\dot{V}_{O_2}$ (−40%) has recently been observed in cats during 4% CO_2 breathing at room temperature (Sachdeva & Jennings, 1994). At a first glance, the above results on different mammalian species, taken globally, may suggest a tendency for CO_2 to increase $\dot{V}_{O_2}$ in the largest species, and perhaps to decrease it in the smallest. In hypoxia (10% inspired oxygen), the hypometabolic response was previously found to be body mass dependent, being larger in smaller mammalian species (Frappell, Lanthier, Baudinette & Mortola, 1992). If this was the case also for hypercapnia, one would expect that in the rat CO_2 breathing should induce a clear hypometabolic response.

Metabolic effects of hypercapnia in rats

With high inspired CO_2 concentrations (~9–10%), the rat's thermogenic response to cold was abolished (Stupfel, 1974), and the preoptic thermosensitive neurons maintained their elevated activity irrespective of the temperature (Tamaki, Nakayama & Matsumura, 1986). A major drop in $\dot{V}_{O_2}$ was observed even at thermoneutrality, but with extraordinarily high inspired CO_2 (19·5–32·5%; Stupfel, 1974). On the other hand, with inspired CO_2 concentrations between 4 and 7%, the $\dot{V}_{O_2}$ value of the rat increased (Pappenheimer, 1977; Lai, Lamm & Hildebrandt, 1981; Gautier *et al.* 1993); a minor drop (−7%) was only reported to occur in the cold (Gautier *et al.* 1993). In the adult rat, we could detect only a small (3–7%), and not significant, trend for $\dot{V}_{O_2}$ to increase with 2 and 5% inspired CO_2 concentrations. In the newborn rat, the $\dot{V}_{O_2}$–T_a relationship depended a great deal upon behavioural mechanisms (huddling), and was not affected by hypercapnia.

Hence, even in a small mammal like the rat, the metabolic response to moderate hypercapnia is qualitatively different from hypoxia, which consistently reduces $\dot{V}_{O_2}$ (Mortola & Gautier, 1995). The question arises about whether these differences could be attributed to factors indirectly involved in the metabolic response to hypercapnia.

The cost of hyperpnoea. Both hypoxia and hypercapnia induce hyperventilation, but the absolute increase in $\dot{V}_E$ (hyperpnoea) can be substantially different. This is particularly the case in newborns, where $\dot{V}_E$ in hypoxia can be similar to or even less than the values in normoxia (Saetta & Mortola, 1987; Mortola, Rezzonico & Lanthier, 1989). Hence, it could be asked whether the O_2 cost of the larger hyperpnoeic response to CO_2 may contribute to the metabolic stability in hypercapnia. Several considerations suggest that this is a minor factor. From the values of respiratory system compliance and resistance of newborn (Mortola, 1983) and adult rats (Mortola, Matsuoka, Saiki & Naso, 1994), and the recorded breathing patterns during air, hypoxia or hypercapnia, it is possible to estimate the work of breathing; from this, assuming that the respiratory muscles operate with an efficiency of 10%, one can compute the respiratory cost (Otis, Fenn & Rahn, 1950). We calculated that the respiratory cost would be within 4% of the corresponding $\dot{V}_{O_2}$, in either 5% CO_2 or 10% O_2, for both newborns and adults, in agreement with previous calculations (Mortola *et al.* 1994). Therefore, notwithstanding the approximation of such estimates, it seems that the cost of the hyperpnoeic work in the rat breathing 10% O_2, or 5% CO_2, can hardly be considered an issue in determining the metabolic level.

The thermogenic stimulus. A decrease in T_b during hypercapnia has often been observed in several species, including the rat (Stupfel, 1974; Gautier *et al.* 1993). In rats, even when $\dot{V}_{O_2}$ increased during hypercapnia (Lai *et al.* 1981; Gautier *et al.* 1993), T_b consistently decreased, by 1–1·5 °C, a phenomenon which probably reflects the heat loss of the hyperpnoea and peripheral vasodilatation, and is therefore likely to be related to the severity and duration of the hypercapnia. From the data of Lai *et al.* (1981) on chronic hypercapnic rats it can be seen that the time course of $\dot{V}_{O_2}$ (an early increase followed by a gradual return to baseline within a few days) matched the time course of the changes in T_b. Hence, it is conceivable that during hypercapnia a drop in T_b represents a thermogenic stimulus; in such a case, the insignificant metabolic rise in the rats of

the present study, compared with the increase observed in some previous studies (Pappenheimer, 1977; Lai *et al.* 1981; Gautier *et al.* 1993), could be attributed to their very small (−0·3 °C) drop in T_b.

In the newborn, huddling, by varying heat loss, represents an important behavioural component of its defence mechanisms against cold (Alberts, 1978). The newborn rat, when alone in the cold, approximately doubled $\dot{V}_{O_2}$, whereas, when in groups of four pups, it increased it more than threefold, and better maintained T_b. Yet, in all cases, the metabolic curve was the same in normocapnia and hypercapnia, indicating, first, that hypercapnia did not alter behavioural thermogenesis and, second, that differences in the T_a–T_b relationship due to huddling did not interfere with the metabolic response to CO_2.

In contrast with the adult, hypercapnia did not decrease T_b below the normocapnic value either in the newborn rat, or in the newborn dog, which had a body mass similar to that of the adult rat. It is possible that, in the newborn, the heat-dissipative effects of CO_2 are less than in adults. In addition, in a very small animal like the newborn rat, the effect of T_a on T_b is likely to have overwhelming importance compared with the CO_2-associated heat dissipation. At any rate, the fact that in the newborn hypercapnia did not modify the T_b response to T_a is an additional indication that the maintenance of the metabolic level in CO_2 cannot be accounted for by a thermogenic stimulus.

In summary, from these data in newborn and adult rats, it seems possible to conclude that the similarity of the $\dot{V}_{O_2}$–T_a relationship between normocapnia and moderate levels of hypercapnia is not the fortuitous result of the thermogenic effect of a lowering in T_b; in fact, during CO_2 breathing, T_b does not necessarily decrease, and, when it does, it stimulates metabolic rate above the normocapnic value. The latter represents a major difference from the thermoregulatory consequences of hypoxia, during which the drop in T_b is primarily caused by the hypoxic hypometabolism (Mortola & Gautier, 1995). Hence, in contrast with hypoxia, moderate hypercapnia would interfere with thermoregulation largely as a result of the increase in heat dissipation, whereas the effect of CO_2 on heat production would be indirect, via the decrease in T_b. This view agrees with data collected in other adult species. For example, in rats, CO_2 breathing did not modify the shivering response to cooling (Gautier *et al.* 1993), and in human subjects, $\dot{V}_{O_2}$ was unaltered by breathing 4% CO_2, whether in warm or cold conditions (Wagner *et al.* 1983).

Sympathetic stimulation. The fact that blood pressure was maintained at a constant value with unchanged or reduced pulse rate suggests a minimal role of sympathetic stimulation and arginine vasopressin (Walker, 1987). It could be argued that the sympatho-adrenal activation was offset by the acidemia (Tenney, 1956); however, the degree of acidemia (from pH 7·42 to 7·32 during 5% CO_2 breathing) was too small a change for appreciable effects on catecholamines (Nahas, Ligou & Mehlman, 1960).

Hypercapnic ventilatory response at low temperature

During hypercapnia, the hyperpnoeic response, examined as the percentage change from normocapnia, was less at lower T_a in both adults and newborns. This agrees with previous observations in adults, and it is qualitatively similar to that observed during hypoxia (Maskrey, 1990; Gautier & Bonora, 1992; Gautier *et al.* 1993; Saiki *et al.* 1994). In hypoxia, the lower degree of hyperpnoea in cold, compared with warm, conditions could be attributed to the larger drop in metabolic rate, since the magnitude of the hyperventilation, reflected by the drop in arterial P_{CO_2}, was independent of T_a (Saiki & Mortola, 1994). During CO_2 breathing, on the other hand, $\dot{V}_{O_2}$ did not decrease, and arterial P_{CO_2} during 5% CO_2 breathing, with respect to normocapnia, increased slightly more in cold (+13 mmHg) than in warm conditions (+9 mmHg). It is possible that hypothermia negatively interacts with the CO_2 ventilatory drive at various sites of the chemosensitive loop (Cherniack, von Euler, Homma & Kao, 1979; Maskrey, 1990). In addition, the fact that, in newborns, the ventilatory values attained during 5% CO_2 breathing in the cold were close to values previously measured during 10% CO_2 (Saetta & Mortola, 1985; Rezzonico & Mortola, 1989), also raise the possibility that the rats were breathing close to their maximal ventilation, and therefore mechanical limitations may have contributed to the magnitude of the hypercapnic ventilatory responses in cold conditions.

In conclusion, we found that breathing moderate concentrations of CO_2 (2–5%) had negligible effects on the whole-body $\dot{V}_{O_2}$ of rats, whether newborns or adults, irrespective of T_a and of the degree of behavioural thermogenesis. This is very different from the hypometabolic effect of moderate hypoxia. Several considerations suggest that during moderate degrees of hypercapnia various factors associated with increased CO_2, including the cost of hyperpnoea, increased heat loss, and hormone-induced hypermetabolism, should not be of major importance in determining the metabolic level.

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Acknowledgements

We thank Lina Naso for technical assistance. The study was supported by the Medical Research Council of Canada.

Received 23 March 1995; accepted 8 September 1995.