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Lactate as a modulator of hypoxia-induced hyperventilation

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Abstract

In the present study, we tested the hypothesis that lactate, which is a classic companion of hypoxic stress in mammals, is a modulator of hypoxia-induced hyperventilation. To this end, pulmonary ventilation (V_E) of male Wistar rats was measured by whole body plethysmograph, and dichloroacetate (DCA, 100 mg/kg) was used to inhibit lactate production. Plasma lactate levels, arterial pH (pHa), arterial carbon dioxide partial pressure ($PaCO_2$), arterial oxygen partial pressure (PaO_2), plasma bicarbonate (HCO_3^-) and oxygen consumption (VO_2) were determined as well. In normoxia, intraperitoneal DCA elicited a decrease only in plasma lactate levels. Hypoxia caused an increase in V_E, pHa and plasma lactate levels and parallel to decreases in $PaCO_2$, PaO_2 and VO_2 in the control group. DCA administration markedly reduced the ventilatory response to hypoxia by acting on tidal volume (V_T). This reduced ventilatory response caused by DCA was independent of VO_2. In conclusion, the present study indicates that lactate contributes to the initiation and maintenance of hypoxia-induced hyperventilation in rats, modulating the adjustments in V_T.

Keywords: Control of breathing; hypoxia; Hypoxiaventilatory response; lactic acid; Lactic acidventilation; Mammalsrat; Pharmacological agents; dichloroacetate

1. Introduction

Hypoxia elicits several compensatory responses in mammals, among which is increased pulmonary ventilation (V_E; Heymans et al., 1930). It is currently accepted that the hyperventilation induced by hypoxia results from activation of peripheral chemoreceptors and subsequent processing of this information by the central nervous system (Taylor et al., 1999), a pathway that is under the control of several mediators and/or modulators. Among them are nitric oxide (nitric oxide inhibitors administrated systemically, Haxhiu et al., 1995; administered centrally, Fabris et al., 1999), dopamine (blocker administrated systemically, Long and Anthonisen, 1995), glutamate (antagonist administrated systemically, Soto-Arape et al., 1995; administered centrally, Ang et al., 1992), and adenosine (antagonist admini-

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strated systemically, Long and Anthonisen, 1995; administered centrally, Barros and Branco, 2000).

Lactate has also been suggested as a modulator of hypoxia-induced hyperventilation. In support, infusion of the lactate anion, which is produced and released by hypoxic tissues (Gladden, 2001), causes an increased V_E in rats (Hardarson et al., 1998). In addition, lactic acid has been shown to increase chemoreceptor discharge in anesthetized cats (Linton et al., 1995). However, this latter observation may be at least in part mediated by the acidosis elicited by lactic acid.

Recent studies in humans (Georgopoulos et al., 1990) and goats (Aaron et al., 1996) have used the inhibitor of lactate production dichloroacetate (DCA) to directly verify the involvement of lactate in hypoxia-induced hyperventilation, but, at the dose of 50 mg/kg, DCA had little or no effect on the ventilatory response to hypoxia (Georgopoulos et al., 1990; Aaron et al., 1996). However, in these studies, the effectiveness of DCA in blocking lactate production was not assessed. We then hypothesized that the lack of effect of DCA on hypoxia-induced hyperventilation may have been due to an insufficient inhibition of lactate production. Accordingly, the maximum effect of DCA in inhibiting lactate production occurs at tissue concentrations of 1 mM, corresponding to the dose of approximately 133 mg/kg (Peeling et al., 1996).

Therefore, in the present study, we tested the hypothesis that lactate modulates hypoxia-induced hyperventilation by using a higher dose of DCA (100 mg/kg). This dose is close to the maximally effective dose and has been shown to prevent lactate accumulation following brain ischemia (Peeling et al., 1996). Moreover, the biological activity of DCA was determined by measuring plasma lactate levels.

2. Methods

2.1. Animals and surgical preparation

Experiments were performed on adult male Wistar rats (200–270 g) obtained from the animal facility of the University of Sao Paulo, campus of Ribeirao Preto, Brazil. The animals had free access to water and food and were housed at an ambient temperature of 25.0 ± 1.0 °C, with a daily 12:12 h light:dark cycle (lights on at 6:00 a.m.). All experimental protocols were performed according to the guidelines of the local ethical committee of the University of Sao Paulo (Comissao de Etica no uso de Animais-CEUA).

The animals were anesthetized with an intraperitoneal (i.p.) 2,2,2-tribromoethanol (250 mg/kg; Aldrich, WI) and a biotelemetry probe capsule (model: ER-3000 temperature; Mini-Mitter Co., Inc., Sunriver, OR) was implanted into the abdominal cavity. The wound was then closed with skin sutures and the implanted capsule was used to measure body core temperature (T_c). Another group of animals was anesthetized in the same way and a polyethylene catheter filled with 25 U/ml of heparinized saline was inserted into the femoral artery for blood sampling. The catheter was composed of a segment of PE-10 tubing (4.5 cm) heat-bonded to a 15 cm long PE-50 catheter. The catheter was secured in position with thread and PE-50 segment was passed under the skin to be finally extruded at the dorsum of the animals. After surgery, animals received an intramuscular injection of 100,000 IU of benzylpenicillin and recovered for 2 days before experimentation. The arterial catheters were flushed daily with heparinized saline.

2.2. Determination of ventilation

Measurements of ventilation (V_E) were performed by body plethysmograph method (Bartlett and Tenney, 1970). Freely moving rats were kept in a 5-L chamber ventilated with room air or with a humidified hypoxic gas mixture containing 7% of oxygen and balanced with nitrogen (AGA, Sertaozinho, SP, Brazil). During V_E measurements, the flow was interrupted and the chamber sealed for short periods of time (~ 1 min), and the pressure oscillations caused by breathing were monitored with the use of a differential pressure transducer (model MP45-14-871; Validyne, Northridge, CA). The signals were fed into a differential pressure signal conditioner, passed through an analog-to-digital converter and digitized in a microcomputer

equipped with data acquisition software (Acquire 6600 data acquisition system; Gould Instrument Systems, Inc., Valley View, OH). The results were analyzed with the data-analysis software Windaq (v1.32 data acquisition system, DI-720, DATAQ Instruments). Calibration for volume was obtained during each experiment by injecting the animal chamber with a known amount of air (1 ml). Two respiratory variables were measured, respiratory frequency (f_R) and tidal volume (V_T). V_E was the product of f_R and V_T.

V_T was calculated by the formula of Malan (1973):

$$V_T = V_K \frac{P_T}{P_K} \frac{T_A}{T_R} \frac{P_B - P_C}{(P_B - P_C) - (T_A/T_c)(P_B - P_R)}$$

where P_T is the pressure deflection associated with each V_T, P_K the pressure deflection associated with injection of the calibration volume (V_K), T_c the body core temperature, T_A the air temperature in the animal chamber, P_B the barometric pressure, P_R the vapor pressure of water at T_c, P_C the vapor pressure of water vapor in the animal chamber and T_R the room temperature. V_E and V_T are presented at ambient barometric pressure, at body core temperature, and saturated with water vapor at this temperature (BTPS). T_c was monitored by biotelemetry (model: ER-3000; Mini-Mitter Co., Inc., Sunriver, OR) and the air temperature in the animal chamber with the use of a thermoprobe (model 8502-10; Cole Parmer, Chicago, IL). According to Malan (1973), T_R may be slightly lower than T_A, due to the heat production of the animal in the chamber. P_C (vapor pressure of water vapor in the animal chamber) was calculated indirectly by using an appropriate table (Dejours, 1981).

2.3. Determination of plasma lactate levels, pH, blood gases and HCO_3^-

Arterial blood samples were obtained from the implanted arterial catheter of the normoxic and hypoxic rats treated with saline or DCA. For lactate dosages, 150 μl of arterial blood samples was collected into heparinized tubes, centrifuged at $10{,}000 \times g$ for 10 min at 4 °C. The samples were stored at −70 °C until the time for assay. Lactate concentration was then measured using a colorimetric method based on the cleavage of lactate to pyruvate by the enzyme lactate oxidase (Lactate–PAP, Randox Laboratories, UK). Another 150 μl of arterial blood was sampled for immediate analysis of arterial pH (pHa), arterial carbon dioxide partial pressure ($PaCO_2$), arterial oxygen partial pressure (PaO_2) and plasma bicarbonate (HCO_3^-) with the use of a blood gas analyzer (model ABL 5; Radiometer, Copenhagen). Measurements were performed during normoxia and hypoxia.

2.4. Determination of oxygen consumption

Oxygen consumption (VO_2) was measured with a closed-flow system using an oxygen analyzer (type OA 272, Saylor Servomex). Each time VO_2 was measured, the flow was interrupted, the chamber was sealed and six samples of chamber air (30 ml) were taken at 2 min intervals and passed through O_2 analyzer. Therefore, the chamber remained sealed for 10 min, the time needed to collect sufficient data to obtain accurate slopes. Assuming a respiratory exchange ratio (CO_2 production/VO_2) of 0.8, which is a general rule for air breathers (Dejours, 1981), and considering the measured VO_2, at the end of the 10-min period the CO_2 level inside the chamber will be $\sim 0.6\%$. A curve of $\%O_2$ evolution was constructed, and its slope gave the value of VO_2. This metabolic variable is reported at STPD.

2.5. Experimental protocol

2.5.1. Effect of DCA on V_E, plasma lactate levels, pHa, blood gases, plasma HCO_3^- and VO_2 during normoxia

After the animals habituated to the experimental condition (~ 1 h), basal ventilation was determined as the mean of three measurements made at 15 min intervals, after which the rats received an i.p. injection of vehicle (pyrogen-free saline) or DCA (100 mg/kg; Sigma-Aldrich, St. Louis, MO). The volume of each injection was 1 ml/kg. V_E was measured at 15, 30, 45, 60, 90, 120 and 150 min post-injection. Blood samples were collected at 90 min after the injection for plasma

lactate levels, pHa, blood gases and plasma HCO_3^- measurements. Immediately after that, VO_2 was measured.

2.5.2. Effect of DCA on V_E, plasma lactate levels, pHa, blood gases, plasma HCO_3^- and VO_2 during hypoxia

The identical protocol was followed during hypoxia, except that animals were subjected to a hypoxic atmosphere (7% inspired oxygen) for 2 h, starting 30 min after the injection.

2.6. Data analysis

Values are reported as mean ± S.E.M., a repeated measures multivariate analysis of variance (MANOVA), factors being treatment (DCA or saline), time and stimulus (hypoxia or normoxia). In case of significant interactions, one-way ANOVA was performed at each time. Duncan test was used for multiple comparisons. Statistical analysis was performed using a software program (SPSS for Windows 6.0). Values of $P < 0.05$ were considered significant.

3. Results

During the experiments the mean chamber temperature was 24.2 ± 0.3 °C and the basal levels did not differ between saline- and DCA-treated groups.

Fig. 1 shows that injection of DCA or saline did not affect V_E of normoxic rats.

Fig. 2 shows the effect of hypoxia on DCA and saline groups. Hypoxia caused an increase in V_E that was due to an elevation in V_T and f_R in saline group. Intraperitoneal DCA attenuated hyperventilation induced by hypoxia, with V_T being the only ventilatory parameter affected.

Table 1 shows the plasma lactate levels, pHa, $PaCO_2$, PaO_2, plasma HCO_3^- and VO_2 of rats injected with DCA or saline and subjected to normoxia or hypoxia. Under normoxic conditions, DCA caused a decrease only in plasma lactate levels. Hypoxia significantly increased plasma lactate levels ($P < 0.001$), a response which was attenuated by DCA ($P < 0.001$). Exposure to a 7% oxygen atmosphere in saline group caused significant reductions in $PaCO_2$ ($P < 0.05$), PaO_2 ($P < 0.05$) and VO_2 ($P < 0.05$) and an increased pHa ($P < 0.05$). Injection of DCA attenuated $PaCO_2$ reduction and the alkalosis observed during hypoxia when compared with saline group ($P < 0.05$). None of the experimental conditions had any significant effect on plasma HCO_3^-. Hypoxia also caused a similar drop in VO_2 in both groups ($P < 0.05$).

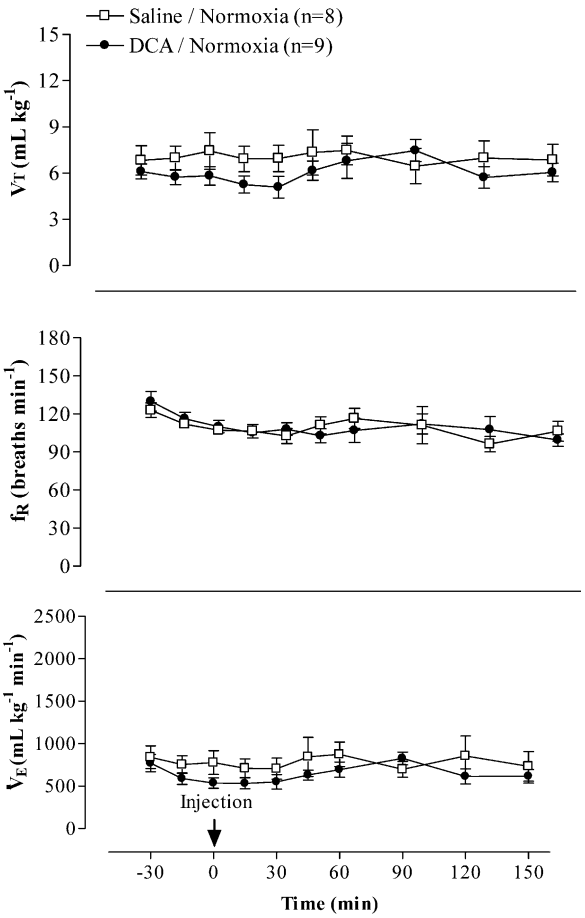

Fig. 1. Effect of i.p. injection of dichloroacetate (DCA, 100 mg/kg) or saline on tidal volume (V_T), respiratory frequency (f_R) and pulmonary ventilation (V_E) of normoxic Wistar rats. The arrow indicates the time of injection. Values are expressed as mean ± S.E.

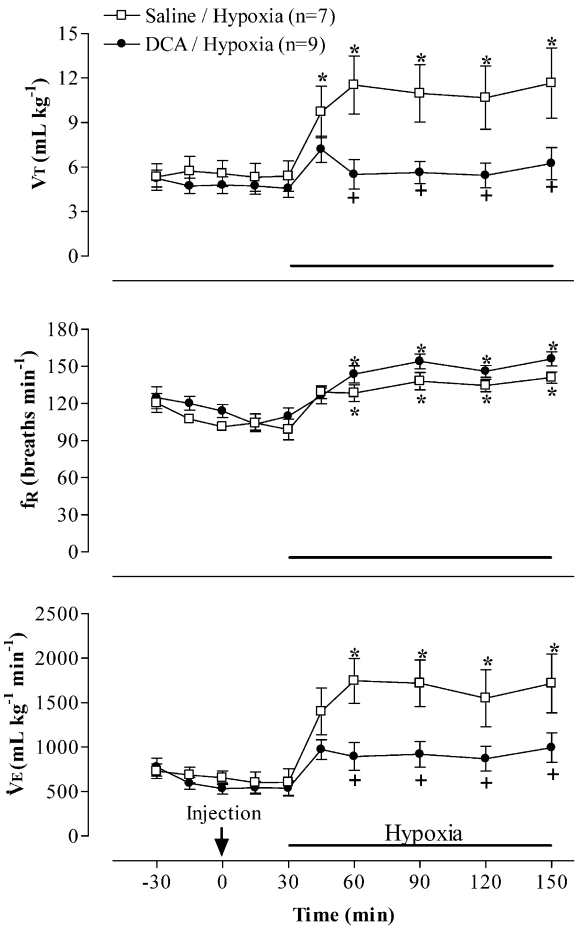

Fig. 2. Effect of i.p. injection of dichloroacetate (DCA, 100 mg/kg) or saline on tidal volume (V_T), respiratory frequency (f_R) and pulmonary ventilation (V_E) of Wistar rats subjected to hypoxia (7% inspired oxygen). The arrow indicates the time of injection and the horizontal bars the period of hypoxia exposure. Values are expressed as mean ± S.E. (*) Depicts $P < 0.01$ in relation to basal values (time: −30 min). (+) Indicates significant ($P < 0.05$) differences between DCA- and saline-treated groups.

4. Discussion

The present study provides evidence that lactate participates in the control of V_E in rats, exerting an important role in the genesis and maintenance of the ventilatory response to hypoxia. This conclusion is based on the finding that pretreatment of rats with DCA, at a dose (100 mg/kg) that significantly reduced lactate production during hypoxia, also blunted hypoxia-induced hyperventilation. We then suggest that the failure of a smaller dose of DCA (50 mg/kg) to markedly affect the ventilatory response to hypoxia in previous studies (Georgopoulos et al., 1990; Aaron et al., 1996) is likely to be due to an insufficient inhibition of lactate production, although interspecies differences cannot be ruled out.

It is currently accepted that the hyperventilation induced by hypoxia results from activation of peripheral chemoreceptors located in the aortic and carotid bodies, where electrically excitable cells capable of sensing arterial O_2 tension exist (López-Barneo et al., 2001). Interestingly, glomus cells in the carotid body have been shown to produce and release a series of neurotransmitters in response to hypoxia, among which dopamine seems to be most proximal to activate afferent nerve fibers that carry chemoreceptive information to the brain (Monti-Bloch et al., 1993). These afferent fibers then make their first synapse in the nucleus of the tractus solitarius, where excitatory amino acids seem to play an important role in the control of breathing (cf. Burton and Kazemi, 2000). An intricate neural network, involving several neurotransmitters and neuromodulators, e.g. amino acids (Ang et al., 1992), peptides (Dwinell et al., 2001) and nitric oxide (Haxhiu et al., 1995), is involved in hypoxia-induced hyperventilation.

Lactate has been recognized as a classical companion of hypoxic stress in vertebrates, being the major anaerobic end product (Pörtner et al., 1991). Oxygen limitation to the mitochondria evokes pyruvate accumulation and the consequent formation of lactate by the action of the enzyme lactate dehydrogenase (Brooks et al., 1999). The study of the role of endogenously produced lactate in the physiological adjustments to hypoxia is now possible with the use of DCA. After i.p. administration, DCA is rapidly distributed throughout the body, being found in skeletal and cardiac muscles, liver, kidneys, adipose tissue, platelets, fibroblasts and brain (Peeling et al., 1996). By enhancing the activity of pyruvate dehydrogenase, DCA reduces pyruvate accumulation and, consequently, lactate production (McAllister et al., 1973). Accordingly, in the present study, i.p. administration of DCA at

Table 1
Plasma lactate levels, arterial pH (pHa), arterial oxygen partial pressure (PaO_2), arterial carbon dioxide partial pressure ($PaCO_2$), plasma bicarbonate (HCO_3^-) and oxygen consumption (VO_2) of rats injected i.p. with dichloroacetate (DCA; 100 mg/kg) or saline, subjected to normoxia or hypoxia (7% inspired O_2)

	Saline		DCA	
	21% O_2 ($n=8$)	7% O_2 ($n=7$)	21% O_2 ($n=9$)	7% O_2 ($n=9$)
Plasma lactate (mg dl^{-1})	11.6±1.3	60.6±4.0*	6.6±0.6**	35.7±3.8*, **
pHa	7.44±0.01	7.55±0.02*	7.42±0.01	7.48±0.01*, **
$PaCO_2$ (mmHg)	36.98±1.77	22.50±2.13*	36.58±2.55	30.85±2.74**
PaO_2 (mmHg)	83.21±2.32	31.35±1.13*	85.95±2.08	30.16±1.42*
HCO_3^- (mM)	24.31±1.53	20.78±2.95	23.15±2.56	21.96±2.17
VO_2 (ml kg^{-1} min^{-1})	20.77±0.91	8.52±1.09*	20.39±0.95	9.02±1.19*

Values are expressed as mean±S.E.M.
* Significantly different from normoxia value.
** Significantly different from saline group.

the dose 100 mg/kg reduced, but not abolished, the hypoxia-induced plasma lactate accumulation (Table 1). This is in line with the fact that lactate dehydrogenase is fully active, even though the availability of pyruvate is decreased by DCA. The tendency of increased ventilation during hypoxia exposure of rats treated with DCA (Fig. 2) may result from the residual lactate buildup.

It is unlikely that DCA has a direct inhibitory effect on V_E independent of lactate, since DCA did not change V_E in normoxic animals (Fig. 1). Another possibility is that the pathway affected by DCA may not be active or minimally active under normoxia. However, no previous data exist to support this hypothesis.

During hypoxia many factors affect V_E, most probably, synergistically (for review, see Richter et al., 1999; Burton and Kazemi, 2000). The present study focuses on the effect of lactate alone and confirms that it may indeed be one of the factors involved in increased V_E. However, the mechanisms involved are not fully understood. We assessed if the lactate effect was dependent on blood gases. During normoxia, PaO_2, $PaCO_2$ and pH were not altered by DCA treatment (Table 1). Hypoxia induced a decrease in PaO_2, which was not affected by DCA, whereas this drug significantly reduced hypoxia-induced alkalosis (Table 1) most likely after the decreased ventilatory response (Fig. 2). Accordingly, Hardarson et al. (1998) reported that lactate infusion stimulates ventilation despite normal pHa and $PaCO_2$. Moreover, it has been recently reported that after systemic administration of platelet-activating factor receptor antagonist, rats show a markedly reduced ventilatory response to hypoxia, despite there is no significant change in hypoxia-induced alterations of blood gases between control and experimental groups (Simakajornboon et al., 1998). Alternatively, hypoxia-induced lactate accumulation in the cerebrospinal fluid (Javaheri et al., 1994) might have decreased the difference between the concentrations of strong cations and strong anions (strong ion difference, SID) and this would lead to an increased ventilation (for review, see Boyle and Baldwin, 2002).

Another possibility is that lactate could act on metabolism to increase V_E during hypoxia. However, no significant difference was observed in VO_2 between saline and DCA groups (Table 1), suggesting no effect of lactate on VO_2. In agreement, a recent study reported that DCA does not affect anapyrexia (a regulated decrease in T_c) induced by hypoxia, which is known to be accompanied by a drop in metabolism (Bicego et al., 2002).

Since the mechanism by which lactate is acting to promote the excitatory role on V_E remains unknown, some speculation is natural. Lactate might enhance ventilation by directly interacting with the carotid body chemoreceptors, a hypothesis that is supported by the observation that the increased V_E produced by lactic acid injection to

anesthetized rats is substantially attenuated by denervation of the carotid body chemoreceptors (Lee et al., 1996). Accordingly, lactic acid has been reported to depolarize chemoreceptor glomus cells (Monti-Bloch et al., 1993) and to cause increases in the oscillation of chemoreceptor discharge (McLoughlin et al., 1995). Another possibility is that lactate increases V_E by acting on the central nervous system. Actually, several studies (Abemayor et al., 1984; Cremer et al., 1976; Kaplan et al., 1987; Kuroda et al., 1984) have indicated that both lactate and DCA cross the blood–brain barrier. Since a recent study has demonstrated that lactate release depends on the uptake of the excitatory neurotransmitter L-glutamate (Demestre et al., 1997), it is possible that lactate influences neuronal activity by affecting glutamatergic synapses, which are known to play a major role in the control of breathing (Bonhan, 1995).

Considering that distinct brainstem neuronal populations account for the respiratory adjustments in f_R and V_T (Berger, 1994), the present observation that DCA inhibits hypoxia-induced hyperventilation by affecting V_T, but not f_R, further indicates that lactate is acting in some specific pathway of the respiratory control. Corroborating this finding, intravenous infusion of the lactate anion has been shown to increase V_T of rats without affecting f_R (Hardarson et al., 1998).

In conclusion, the present study supports the hypothesis that lactate plays a role in the genesis and maintenance of hypoxia-induced hyperventilation, modulating the adjustments in V_T.

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References

Aaron, E.A., Foster, H.V., Lowry, T.F., Korducki, M.J., Ohtake, P.J., 1996. Effect of dichloroacetate on $PaCO_2$ responses to hypoxia in awake goats. J. Appl. Physiol. 80, 176–181.

Abemayor, E., Kovachich, G.B., Haugard, N., 1984. Effects of dichloroacetate on brain pyruvate dehydrogenase. J. Neurochem. 42, 38–42.

Ang, R.C., Hoop, B., Kazemi, H., 1992. Role of glutamate as the central neurotransmitter in the hypoxic ventilatory response. J. Appl. Physiol. 72, 1480–1487.

Barros, R.C.H., Branco, L.G.S., 2000. Role of central adenosine in the respiratory and thermoregulatory responses to hypoxia. Neuroreport 1, 193–197.

Bartlett, D., Jr., Tenney, S.M., 1970. Control of breathing in experimental anemia. Respir. Physiol. 10, 384–395.

Berger, A.J., 1994. Control of breathing. In: Murray, J.F., Nadel, J.A. (Eds.), Textbook of Respiratory Medicine. Saunders, Philadelphia, PA, pp. 199–218.

Bicego, K.C., Gargaglioni, L.H., Steiner, A.A., Branco, L.G.S., 2002. Is lactate a mediator of hypoxia induced anapyrexia? Pflüglers Arch. 444, 810–815.

Bonhan, A.C., 1995. Neurotransmitters in the CNS control of breathing. Respir. Physiol. 101, 219–230.

Boyle, M., Baldwin, I., 2002. Introduction to an alternate view of acid/base balance: the strong ion difference or Stewart approach. Aust. Crit. Care 15 (1), 14–20.

Brooks, G.A., Dubouchaud, H., Brown, M., Sicurello, J.P., Butz, C.E., 1999. Role of mitochondrial lactate dehydrogenase and lactate oxidation in the intracellular lactate shuttle. Proc. Natl. Acad. Sci. USA 96 (3), 1129–1134.

Burton, M.D., Kazemi, H., 2000. Neurotransmitters in central respiratory control. Respir. Physiol. 122 (2-3), 111–121.

Cremer, J.E., Brawn, L.D., Oldendorf, W.H., 1976. Changes during development in transport process of the blood–brain barrier. Biochem. Biophys. Acta 448, 633–637.

Dejours, P., 1981. Principle of Comparative Respiratory Physiology, 2nd ed.. Elsevier/North-Holland, New York.

Demestre, M., Boutelle, M., Fillenz, M., 1997. Stimulated release of lactate in freely moving rats is dependent on the uptake of glutamate. J. Physiol. London 499, 825–832.

Dwinell, M.R., Kazemi, H., Lam, J.T., Powell, F.L., 2001. Central amino acid neurotransmitters, ventilatory output and metabolism during acute hypoxia in anesthetized rats. Adv. Exp. Med. Biol. 499, 291–296.

Fabris, G., Anselmo-Franci, J.A., Branco, L.G.S., 1999. Role of nitric oxide in hypoxia-induced hyperventilation and hypothermia: participation of locus coeruleus. Braz. J. Med. Biol. Res. 32, 1389–1398.

Georgopoulos, D., Berezanski, D., Anthonisen, N.R., 1990. Effect of dichloroacetate on ventilatory response to sustained hypoxia in normal adults. Respir. Physiol. 82, 115–122.

Gladden, L.B., 2001. Lactic acid: new roles in a new millennium. Proc. Natl. Acad. Sci. USA 98, 395–397.

Hardarson, T., Skarphedinsson, J.O., Sveinsson, T., 1998. Importance of lactate anion in control of breathing. J. Appl. Physiol. 84, 411–416.

Haxhiu, M.A., Chang, C.H., Dreshaj, I.A., Erokwu, B.N.R., Prabhakar, N.R., Cherniack, N.S., 1995. Nitric oxide and ventilatory response to hypoxia. Respir. Physiol. 101, 257–266.

Heymans, C., Bouckaert, J.J., Boutrebande, L., 1930. Sinus carotidien et reflexes respiratoires. II. Influences respiratoires reflexes de l' acidose, de l' alcalose, de l' anhydride carbonique, de l' ion hydrogene et de l' anoxémie. Sinus carotidiens et changes respiratoires dans lês poumons et au dela dês poumons. Arch. Int. Pharmacodyn. Ther. 39, 400–408.

Javaheri, S., Corbett, W., Wagner, K., Adams, J.M., 1994. Quantitative cerebrospinal fluid acid–base balance in acute respiratory alkalosis. Am. J. Respir. Crit. Care Med. 150, 78–82.

Kaplan, J., Dimlich, R., Biros, M., 1987. Dichloroacetate treatment of ischemic cerebral lactic acidosis in the fed rat. Ann. Emerg. Med. 16, 298–304.

Kuroda, Y., Toshima, K., Watanabe, T., Kobashi, H., Ito, M., Takeda, E., Miyao, M., 1984. Effects of dichloroacetate on pyruvate metabolism in rat brain in vivo. Pediatr. Res. 18, 936–938.

Lee, L.Y., Morton, R.F., Lundberg, J.M., 1996. Pulmonary chemoreflexes elicited by intravenous injection of lactic acid in anesthetized rats. J. Appl. Physiol. 81, 2347–2348.

Linton, R.A., Band, D.M., McLoughlin, P., 1995. Adaptation of carotid chemoreceptors to step increases in $PaCO_2$ in anesthetized cats. J. Neurophysiol. 74, 2707–2712.

Long, W.Q., Anthonisen, N.R., 1995. Dopaminergic influence on the ventilatory response to sustained hypoxia in the cat. J. Appl. Physiol. 78, 1250–1255.

López-Barneo, J., Pardal, R., Ortega-Sáenz, P., 2001. Cellular mechanisms of oxygen sensing. Annu. Rev. Physiol. 63, 259–287.

Malan, A., 1973. Ventilation measured by body plethysmography in hibernating mammals and poikilotherms. Respir. Physiol. 17, 32–44.

McAllister, A., Alisson, S.P., Randle, P.J., 1973. Effects of dichloroacetate on the metabolism of glucose, pyruvate, acetate, 3-hydroxybutyrate and palmitate in rat diaphragm and heart muscle in vitro and on extraction of glucose, lactate, pyruvate and free fatty acids by dog heart in vivo. Biochem. J. 134, 1067–1081.

McLoughlin, P., Linton, R.A., Band, D.M., 1995. Effects of potassium and lactic acid on chemoreceptor discharge in anesthetized cats. Respir. Physiol. 99, 303–312.

Monti-Bloch, L., Abudara, V., Eyzaguirre, C., 1993. Electrical communication between glomus cells of the rat carotid body. Brain Res. 622, 119–131.

Peeling, J., Sutherland, G., Brown, R.A., Curry, S., 1996. Protective effect of dichloroacetate in a rat model of forebrain ischemia. Neurosci. Lett. 208, 21–24.

Pörtner, H.O., MacLatchy, L.M., Toews, D.P., 1991. Acid–base regulation in the toad Bufo marinus during environmental hypoxia. Respir. Physiol. 85, 217–230.

Richter, D.W., Schimidt-Garcon, P., Pierrefiche, O., Bischoff, A.M., Lalley, P.M., 1999. Neurotransmitters and neuromodulators controlling the hypoxic respiratory response in anesthetized cats. J. Physiol. London 514, 567–578.

Simakajornboon, N., Graff, G.R., Torres, J.E., Gozal, D., 1998. Modulation of hypoxic ventilatory response by systemic platelet-activating factor receptor antagonist in the rat. Respir. Physiol. 114, 213–225.

Soto-Arape, I., Burton, M.D., Kazemi, H., 1995. Central amino acid neurotransmitters and the hypoxic ventilatory response. Am. J. Respir. Crit. Care Med. 151, 1113–1120.

Taylor, E.W., Jordan, D., Coote, J.H., 1999. Central control of the cardiovascular and respiratory systems and their interactions in vertebrates. Physiol. Rev. 79, 855–916.