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Influence of differential expression of acetylcholinesterase in brain and muscle on respiration

Eliane Boudinot^a, Véronique Bernard^c, Shelley Camp^b, Palmer Taylor^b, Jean Champagnat^a, Eric Krejci^c, and Arthur S. Foutz^{a,*}

^aNeurobiologie Génétique et Intégrative, Institut de Neurobiologie Alfred Fessard, C.N.R.S., 91190 Gif-sur-Yvette, France

^bDepartment Pharmacology, Skaggs School of Pharmacy & Pharmaceutical Sciences 0657, University of California-San Diego, La Jolla, CA, USA

^cBiologie des Jonctions Neuromusculaires Normales et Pathologiques, Université Paris Descartes, Inserm UMR S686, 45 rue des Saint-Pères, 75006 Paris, France

Abstract

A mouse strain with a deleted acetylcholinesterase (AChE) gene (AChE knockout) shows a decreased inspiration time and increased tidal volume and ventilation. To investigate the respective roles of AChE in brain and muscle, we recorded respiration by means of whole-body plethysmography in knockout mice with tissue selective deletions in AChE expression. A mouse strain with the anchoring domains of AChE deleted (del E5+6 knockout mice) has very low activity in the brain and neuromuscular junction, but increased monomeric AChE in serum. A mouse strain with deletion of the muscle specific region of AChE (del i1RR knockout mice) exhibits no expression in muscle, but unaltered expression in the central nervous system. Neither strain exhibits the pronounced phenotypic traits observed in the complete AChE knockout strain. A third strain lacking the anchor molecule PRiMA, has no functional AChE and butyrylcholinesterase (BChE) in brain and an unaltered respiratory function. BChE inhibition by bambuterol decreases tidal volume and body temperature in del E5+6 and i1RR knockout strains, but not in PRiMA deletion or wild-type controls. We find that: (1) deletion of the full AChE gene is required for a pronounced alteration in respiratory phenotype, (2) BChE is involved in respiratory muscles contraction and temperature control in del E5+6 and i1RR knockout mice, and (3) AChE expression requiring a gene product splice to either exons 5 and 6 or regulated by intron1 influences temperature control.

Keywords

Respiration; Butyrylcholinesterase; Acetylcholine; Gene knockout; Temperature control; Mice

1. Introduction

Acetylcholine (ACh) influences respiratory control in the brainstem (Haji et al., 2000; Kubin and Fenik, 2004), through chemosensory mechanisms of central (Burton and Kazemi, 2000), and peripheral origins (Shirahata et al., 2007). Central cholinergic control may be critical in early life, since its dysfunction has been linked to the pathophysiology of the Sudden Infant Death Syndrome (Kinney et al., 1995). ACh is also the neurotransmitter at the neuromuscular

junction whereby acetylcholinesterase (AChE) rapidly catalyzes its hydrolysis to prevent repetitive and uncoordinated binding to nicotinic receptors. Blockade of AChE by anticholinesterases such as chemical warfare agents produces rapid death by respiratory failure (Holmstedt, 1959; Sidell, 1994). Surprisingly, mice with complete deletion AChE (AChE knockout) survived despite the absence of AChE activity in all tissues (Xie et al., 2000). To ensure survival beyond the first week required liquid enriched food (Ensure®, Duysen et al., 2002) and ambient temperature maintenance near the thermo neutral zone (about 29 °C, Duysen et al., 2002; Sun et al., 2007). The defect of thermoregulation arises from down regulation of nicotinic receptor in the sympathetic ganglia (Sun et al., 2007). Interestingly the AChE knockout strain maintains central cholinergic pathways (Mesulam et al., 2002), and excess ACh resulting from diminished AChE (Hartmann et al., 2007) is compensated for by reduced cholinergic receptor levels (Bernard et al., 2003; Chatonnet et al., 2003; Li et al., 2003). Despite these compensations, AChE knockout mice have characteristic phenotypic traits of excessive cholinergic stimulation, including muscle fasciculations and body tremors, muscle weakness, pinpoint pupils, susceptibility to seizures and excessive salivation during stress (Duysen et al., 2002). AChE knockouts also show a pronounced respiratory phenotype consisting mainly in hyperventilation and increased chemoventilatory response to CO₂ (Boudinot et al., 2004). The component of the system driving respiration (central command, muscle control) through which excess ACh produces these phenotypic traits is not known. To address this question, we compared mutants with selective deficits in AChE expression.

AChE is encoded by a single gene where alternative splicing yields a common catalytic domain (encoded by exons 2, 3 and 4) and 3 alternative 3' splice variations (Li et al., 1993): (1) a rare non-splicing variant that encodes a monomeric AChE_R subunit, (2) a signal or H sequence, encoded by exon 5, that is cleaved and produces a dimer anchored to the plasma membrane AChE_H through a glycopospholipid linkage, and (3) the WAT (tryptophan amphiphilic tetramerization) domain encodes the AChE_T. The WAT domain is essential for interaction of the catalytic subunits with ColQ (Krejci et al., 1997) and PRiMA (Perrier et al., 2002), two AChE associated proteins. ColQ anchors AChE in the basal lamina, whereas PRiMA anchors AChE in the plasma membrane. In PRiMA knockout mice, AChE is reduced to 2–3% of normal levels in brain and the residual enzyme is retained in the endoplasmic reticulum (Alexandre Dobbertin, Anna Hrabovska, Korami Dembele, Shelley Camp, Palmer Taylor, Eric Krejci and Véronique Bernard, unpublished). As in AChE knockout, m2 muscarinic receptors are not expressed in their normal membrane location (Dobbertin et al., unpublished). In the del E5+6 knockout strain, the deleted sequence prevents interaction of AChE with ColQ and PRiMA and transforms AChE in a soluble, shorter monomeric enzyme (Camp et al., 2008). The level of AChE expressed varies with the tissues, being very low in muscle and brain, but higher in serum. In i1RR knockout, the deletion of a short sequence in the intron 1 prevents expression in the skeletal muscle, but keeps expression intact in brain and spinal cord (Camp et al., 2008).

Herein, we compare phenotypes of three knock-out strains, corresponding to the differential accumulation of AChE in brain and muscle, with the reference strain of AChE knockout mice that express no AChE at all. Since a characteristic of AChE knockout mice is that the enzyme butyrylcholinesterase (BChE), plays a vital substituting role in these strains (Xie et al., 2000; Chatonnet et al., 2003; Hartmann et al., 2007), we investigated effects of BChE inhibition in the three mouse strains with impaired AChE function.

2. Methods

2.1. Animals

Animal studies were conducted in accord with European Union and French Government animal protection laws. Mice originating from UCSD (del E5+6 and i1RR knock-out strains) were

bred in our facility in Gif-sur-Yvette, and PRiMA mice were transported from the University of Paris. Gene deletions in these mouse strains are indicated in Fig. 1 (Camp et al., 2008). Measurements were made on mice between ages 6 and 12 weeks.

2.2. In vivo measurement of ventilation

Respiratory activity in adult mice of both sexes was measured using a barometric method (Bartlett and Tenney, 1970). The plethysmograph chamber (700mL), equipped with a temperature sensor, was connected through a small hypodermic needle to a reference chamber of the same volume. Pressure differences between the two chambers were measured with a differential pressure transducer (Validyne, DP-103-10) connected to a sine wave carrier demodulator (Validyne, CD15). The spirogram was stored on a PC computer (CED interface). Calibrations were made during each recording session by injecting 100 μ L of air into the experimental chamber.

During recording sessions, mice were partially restrained by the tail, with a thin temperature probe permanently inserted in the rectum. Within the recording chamber, mice were placed in a small rectangular house opened at both ends that allowed body repositioning, but discouraged movements to chew on the probe. The animal's nose was at the level of the open front end. The chamber was maintained at 27–28 °C and gas input and output closed during 2 min data collection sessions. Between sessions the chamber was flushed with fresh humidified air or gas mixtures.

2.3. Protocols

The mice were habituated to the chamber for 20 min the day before data collection. Ventilation at rest was initially measured in animals breathing room air (control). The chamber was then flushed with one of the following gas mixtures: 3%, 5% and 8% CO₂ (mixed with 20% O₂, balance N₂) for hypercapnia protocols; 10% O₂ balance N₂ for the hypoxia protocol. In each session the animals were challenged with the four gas mixtures (4 min each), at 20 min intervals, with exposures in room air between gas challenges. Single mutant and control wild-type (WT) mice were recorded each afternoon and on distinct days in alternating order, and the results were averaged. In each record, we identified periods of breathing at rest, during quiet waking without limb, body or head movements. These periods of baseline breathing were analyzed as previously described (Boudinot et al., 2004). A computer program (ACQUIS1 software) measured the duration of inspiration (T_I) and expiration (T_E), breathing frequency (f_R), tidal volume (V_T) and ventilation ($\dot{V}_E$).

Separate experiments were conducted to determine the effects of butyrylcholinesterase (BChE) inhibition on ventilation and body temperature. After 15min of baseline data collection, BChE inhibitors bambuterol or tetraisopropyl pyrophosphoramidate (iso-OMPA) were injected s.c. (10 μ L/g), in physiologic saline or 3% DMSO in saline respectively.

2.4. Histology

The mice were deeply anesthetized with sodium chloral hydrate and were perfused transcardially with a mixture of 2% paraformaldehyde and 0.2% glutaraldehyde. Brainstem sections were histochemically stained for AChE and BChE activity by the Koelle–Friedenwald method (thiocholine method) with distinguishing inhibitors (Girard et al., 2007).

2.5. Drugs and data analysis

Bambuterol was a gift from Astra-Zeneca France. Iso-OMPA was purchased from Sigma–Aldrich. Results are expressed as means $\pm$ S.E.M. Respiratory responses to chemosensory

challenges were analyzed using a two-way repeated-measures analysis of variance (ANOVA), and Bonferroni–Dunn post hoc tests. The factors of variation were genotype and treatment.

3. Results

A comparison of the three strains with partial AChE impairment to the respiratory phenotype of AChE knockout mice that are devoid of AChE activity (Boudinot et al., 2004) is presented in Table 1 and Fig. 2.

3.1. PRiMA knockout mice

Despite the virtual lack of surface expression and complete retention of residual AChE within the neuron, PRiMA knockout mice are undistinguishable from their wild-type controls in terms of weight, body temperature and ventilation (Table 1). They respond to hypercapnic (Fig. 3) and hypoxic (Fig. 4) challenges similar to their WT controls, an indication of unaltered chemosensitivity.

3.2. i1RR knockout and del E5+6 knockout mice

3.2.1. Body weight and temperature—i1RR knockout and del E5+6 knockout mice were significantly smaller than their respective WT controls (Table 1). Body temperature in i1RR knockout and del E5+6 knockout mice was not significantly different from their respective WT controls. However, i1RR knockout mice tended to be slightly warmer than their controls (Table 1). To evaluate whether partial restraint during respiration recordings may affect body temperature, rectal temperature was measured in the same animals at different times. Measurements from each unrestrained animal on three different days gave similar values in WT (37.3 ± 0.09 °C) and i1RR knockout mice (37.4 ± 0.13 °C). Thus, i1RR knockout mice might be less adaptable to the stress of restraint than WT animals, resulting in higher temperature.

3.2.2. Ventilation and respiratory pattern—Ventilation and respiratory patterns reveal significant changes in i1RR knockout and in del E5+6 knockout mice (Table 1). These two strains showed higher tidal volume (V_T), ventilation (V_E) and mean inspiratory flow (V_T/T_I) than their respective WT controls. Respiratory timing parameters (T_I , T_E , f_R and T_I/T_{TOT}) were largely unaffected in the two strains with the exception of a significantly shorter T_I in del E5+6 knockout mice.

We examined if the elevated V_T and V_E (normalized by weight) observed in these two strains were an effect of allometry, since normalized V_T was highest in the smallest animals. To take into account scaling factors, normalized V_T and V_T/T_I were plotted against body weight (Fig. 2A). This shows that with comparable weights, i1RR knockout and del E5+6 knockout mice have comparable V_T and V_T/T_I , with the smallest animals showing the largest differences with WT mice. The log–log linear regression curves of tidal volume (not normalized by weight) versus body weight for each knockout strain and their WT controls are very close (Fig. 2C), indicating that the two strains have at best a small phenotypic difference with WT animals.

3.2.3. Ventilation during hypercapnia and hypoxia—The ventilatory response to hypercapnia was unaltered in del E5+6 knockout mice. There were no significant interactions between genotype (WT or mutant) and ventilatory responses (V_E , V_T and f_R) over the range of CO₂ concentrations (Fig. 3). In i1RR knockout mice, however, the V_E response curve to hypercapnia was significantly different than in WT controls ($P < 0.02$). This was due to a blunted response at the highest CO₂ concentration, a possible consequence of muscle fatigue (Fig. 3).

Ventilatory responses to hypoxia were not different in del E5+6 knockout mice compared with their respective controls (Fig. 4), but in AChE i1RR knockout mice the V_T response was significantly blunted ($P < 0.02$).

3.2.4. Comparison with AChE knockout mice—Tidal volume (V_T) and mean inspiratory flow (V_T/T_I), the two parameters previously found to be the most affected in AChE knockout mice, are much lower in i1RR and del E5+6 knockout mice than in AChE knockout mice with comparable weights (Fig. 2B). The relative increases of V_T and V_T/T_I in the two strains over their respective WT controls are only 18–28% of the increases observed in AChE knockout mice (Table 1). For example the increase in V_T/T_I in del E5+6 knockout mice (59% over WT), was a small fraction (24%) of the increase in AChE knockout mice (247% over WT, Table 1 and Fig. 2B). This was due to a smaller increase in V_T and smaller decrease in T_I . Thus, the two knockout mice strains do not present the pronounced phenotypic traits of AChE knockout mice.

3.3. Inhibition of butyrylcholinesterase

Inhibition of BChE by bambuterol (1 mg/kg) did not affect V_E and f_R in del E5+6 knockout mice compared with their WT controls (Fig. 5). However, within 5 min of bambuterol administration, V_T decreased by 25% in mutant mice and remained low 60 min later ($P < 0.01$), whereas the drug had no effect in WT mice. Body temperature progressively decreased in mutant mice and was significantly lower than in WT mice 20–60 min after administration ($P < 0.05$).

In i1RR knockout mice, bambuterol did not affect breathing frequency, but V_E and V_T became significantly lower than in controls 30–90 min after administration. Body temperature also was lower at 20 min and the difference with WT mice widened with time (Fig. 5).

In PRiMA knockout mice bambuterol had no effect on breathing and body temperature (Fig. 6). Because BChE is anchored by PRiMA in the CNS, in PRiMA mutants, we also administered iso-OMPA (6 mg/kg s.c.), which, unlike bambuterol, penetrates the blood–brain barrier. V_E , V_T and f_R did not significantly change from pre-injection control values 20 min ($n = 3$) and 60 min ($n = 2$) after administration (data not shown).

3.4. Localization of AChE in respiratory brainstem areas

In the brainstem of both the PRiMA and del E5+6 knockout strains, AChE activity was restricted to the cell body and not found in the dendrites or axons (Fig. 7). In the mutants no labeling seems apparent in the area of the ventral respiratory group proximal to the nucleus ambiguus.

4. Discussion

The striking outcome of this study is that AChE and BChE in the central nervous system (CNS) appear dispensable in the control of respiration. Compensatory changes that occur in the developmental processes result in an animal strain whose respiratory response to released ACh differs greatly from a mature animal where acute inhibition of AChE is produced chemically (Boudinot et al., 2005). Indeed, the complete absence of AChE and BChE in the CNS (PRiMA knockout) does not alter respiratory function, whereas a strain with a complete absence of AChE in muscle, but normal levels in brain (i1RR knockout), or another strain with dramatic reductions of AChE in brain and muscle (del E5+6 knockout), fail to reproduce the pronounced phenotypic traits of mice completely devoid of AChE expression (Boudinot et al., 2004). We verified that in the ventral brainstem respiratory-related areas of PRiMA and del E5+6 knockout mice, AChE is only detectable inside the neuron, in line with our findings that in the

absence of PRiMA, AChE is retained in the endoplasmic reticulum thus preventing its secretion (Dobbertin et al., unpublished). Del E5+6 and i1RR knockout mice present a variety of behavioral phenotypes, such as impaired locomotion, of diminished intensity compared to AChE knockout mice (Camp et al., 2008), but no locomotor phenotype was detected in PRiMA knockout mice, in line with their unaltered weight and respiration.

4.1. Inspiratory effort and chemosensitivity

Hyperventilation due to a doubling of V_T characterizes AChE null mutants (Boudinot et al., 2004). Tidal volume and mean inspiratory flow were much lower in del E5+6 knockout and i1RR knockout mice of comparable weights, but unaltered in PRiMA knockout mice, showing that neither a complete absence of AChE function in the neuromuscular junction, nor the absence of AChE expression in brain in addition to the lack of expression in muscles can explain the phenotype of AChE null mutants.

The AChE null mutant strain presents an increased chemosensitivity to CO_2 (Boudinot et al., 2004), presumably through up-regulation of some important central chemosensors located near the ventral surface of the medulla (Dev and Loeschcke, 1979; Mulkey et al., 2004), which are cholinceptive and involve predominantly M_3 receptors (Nattie and Li, 1990). The unaffected ventilatory response to CO_2 in del E5+6 and PRiMA knockout mice was therefore unexpected. This might explain why tidal volume, regulated in part by CO_2 chemosensitivity, is marginally or not affected at all in these mutants compared with AChE knockout mice. In i1RR knockout mice, the slightly blunted response to CO_2 at a high concentration (8%), and the blunted V_T response to hypoxia are likely to reflect an inability of these mice to sustain augmented muscle contractions.

4.2. Timing mechanisms

Respiratory frequency was unaffected in all three mutant strains as in AChE knockout mice, in which T_I and T_E are changed in opposite directions with T_I markedly reduced. A smaller but significant reduction of T_I was observed in del E5+6 knockout mice, but not in i1RR knockout mice, indicating that T_I reduction correlates with the absence of functional AChE in the muscle but not in the brain. Hence T_I reduction in AChE knockout mice does not result from the inability of respiratory muscles to maintain nerve-evoked tetanic contractions at high stimulus frequencies (Adler et al., 2004; Girard et al., 2005; Mouisel et al., 2006). Excess ACh is unlikely to decrease T_I by stimulating ACh receptors present on neurons of the Pre-Bötzinger complex rhythm generator (Shao and Feldman, 2000) because the application of cholinergic agonists onto this generator isolated in brainstem slices produces the opposite: it increases the duration of inspiratory bursts. The target might be timing-controlling structures in the pons (Kubin and Fenik, 2004) and/or some hypothetical central cholinergic modulation of the Hering Breuer reflex.

4.3. Role of butyrylcholinesterase

Inhibition of BChE by bambuterol, a drug that does not penetrate the blood–brain barrier (Svensson, 1991), has no effect in WT animals but is lethal to AChE knockout mice (Xie et al., 2000; Chatonnet et al., 2003). Sub-lethal doses decrease V_T (Boudinot et al., 2005), presumably by blocking BChE controlling presynaptically the release of ACh at the neuromuscular junction (Minic et al., 2003; Girard et al., 2007). A dose of 1 mg/kg (20 times the lethal dose in AChE knockout mice) decreased V_T in del E5+6 knockout and i1RR knockout mice without any lethality, and was completely ineffective in PRiMA knockout mice. Thus BChE inhibition affects muscle contraction in mice which lack AChE at the neuromuscular junction, but is lethal only when AChE is completely absent in the animal. The survival of all mice of the three strains studied after BChE inhibition is thus surprising, and might be explained by the presence of AChE in the serum in these animals. Indeed the diffusion of ACh from the

neuromuscular junction was documented in an historical experiment (Dale et al., 1936). This suggests that nerve released ACh can diffuse from the neuromuscular junction to the blood, where AChE and/or BChE may hydrolyze ACh. In this condition, the efficacy of the synaptic transmission should be maintained inside the neuromuscular junction by a gradient of diffusion.

Mature AChE null mutants are normothermic, but these animals show a marked hypothermia by post-natal day 15 (Duysen et al., 2002; Boudinot et al., 2004) due to a reduced nicotinic function in sympathetic ganglia (Sun et al., 2007). Since bambuterol decreased body temperature in *del E5+6* and *i1RR* knockout mice, these animals also present a dysfunction of thermoregulatory mechanisms, conferring a critical role to BChE. A role for BChE in neurotransmission and thermoregulation has indeed been recently demonstrated in BChE knockout strains (Duysen et al., 2007).

5. Conclusions

Taken together, the present results show that the pronounced respiratory phenotype in AChE knockout mice cannot be explained by AChE deficit in the muscle and/or in the CNS. This phenotype might be caused by altered chemosensitivity to CO₂, which is not up-regulated in the three mutants studied here. Both AChE *del E5+6* knockout and AChE *i1RR* knockout mice show altered thermoregulatory mechanisms requiring compensation by BChE.

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Fig. 1.

(A) AChE gene structure showing invariantly spliced exons 1–4 and alternatively spliced exons 5 and 6. ATG denotes translational start signal, TGA and TAA are translational stops. Invariant splicing is shown by solid lines while alternative splicing is indicated by dotted lines. The regulatory region in the first intron is depicted by a oval between exons 1 and 2. (B) Three AChE variants AChE_R, AChE_H and AChE_T RNAs are produced by alternative splicing and generate a diversity of AChE molecular forms. (C) Map of the AChE gene with the wild-type and knockout mouse strains with the regions of deletion shown.

Fig. 2.

Tidal volume (V_T) and mean inspiratory flow (V_T/T_I) plotted against body weight. (A) The phenotypes of individual del E5+6 knockout and i1RR knockout mice are largely overlapping and distinguished from their WT (+/+) controls. Note the wider dispersion of the mutants compared to WT animals. (B) The phenotypes of del E5+6 knockout and i1RR knockout mice are clearly distinguished from AChE knockouts with comparable weights. (C) The log-log linear regressions of tidal volume versus body weight for mutants and WT of the del E5+6 and i1RR knockout strains fit slightly distinct curves, indicating weak phenotypic differences.

Fig. 3. Effects of CO₂ on ventilation in del E5+6, i1RR and PRiMA wild-type (+/+) and nullizygous (-/-) mice.

Fig. 4.
Effects of hypoxia on ventilation in del E5+6, i1RR and PRiMA wild-type (+/+) and nullizygous (-/-) mice.

Fig. 5. Effects of bambuterol (1 mg/kg, s.c.) administered to del E5+6 and i1RR wild-type (+/+) and nullizygous (-/-) mice. The arrows indicate the time of injection. Note in both mutant strains the rapid decrease of V_T and the slow decrease of body temperature. del E5+6 knockout, $n = 6$; all other groups, $n = 5$.

Fig. 6.

Effects of bambuterol (1 mg/kg, s.c.) administered to PRiMA wild-type (+/+) and nullizygous (-/-) mice. The arrows indicate the time of injection. Tidal volume and body temperature are unaffected in the mutants ($n = 5$) relative to their wild-type controls ($n = 5$).

Fig. 7.

Histochemical detection of AChE activity by the Koelle–Friedenwald method in facial nucleus (FN) and nucleus ambiguus (NA) of WT, PRiMA and del E5+6 knockout mice. In WT mice, AChE activity is distributed homogeneously in the two nuclei and in neural processes. In both mutants, AChE labeling is restricted to cell bodies. No labeling is apparent in the ventral respiratory group ventral to the nucleus ambiguus.

Table 1

Ventilation at rest in adult wild-type (+/+) and homozygous (-/-) mice for deletions of intron 1 (i1RR knockout), of exons 5 and 6 (del E5+6 knockout) of the AChE gene, or of the anchor molecule PRiMA.

	i1RR +/+ (n = 6)	i1RR -/- (n=6)	P	% difference
$\dot{V}_E$ (mL/(g min ⁻¹))	2.01 ± 0.1	2.65 ± 0.14	<0.01	+32.5
V_T (μL/g)	10.9 ± 0.3	13.3 ± 0.7	<0.02	+22
f_R (breaths/min)	185 ± 11	204 ± 19	NS	+10
T_i (ms)	105 ± 7	89 ± 5	NS	-15
T_E (ms)	232 ± 18	222 ± 30	NS	-4
T_I/T_{TOT} (%)	31.5 ± 1.10	30.0 ± 2.68	NS	-5
V_T/T_I (μL/(g s ⁻¹))	106 ± 5.9	153 ± 13.2	<0.01	+44
Weight (g)	19.4 ± 0.67	14.5 ± 1.07	<0.01	-25
Temperature (°C)	37.4 ± 0.21	38.1 ± 0.26	NS	+2
	Del E5+6 +/+ (n = 7)	Del E5+6 -/- (n=7)		
$\dot{V}_E$ (mL/(g min ⁻¹))	1.81 ± 0.11	2.57 ± 0.12	<0.001	+42
V_T (μL/g)	10.9 ± 0.42	13.8 ± 0.60	<0.01	+27
f_R (breaths/min)	167 ± 11	188 ± 9	NS	+12
T_i (ms)	109 ± 8	80 ± 1	<0.01	-27
T_E (ms)	266 ± 21	244 ± 15	NS	-8
T_I/T_{TOT} (%)	29.7 ± 1.6	26.8 ± 2	NS	-10
V_T/T_I (μL/(g s ⁻¹))	104 ± 9.5	166 ± 13	<0.01	+59
Weight (g)	23.9 ± 1.0	17.6 ± 1.7	<0.01	-26.5
Temperature (°C)	37.8 ± 0.2	37.8 ± 0.1	NS	0
	PRiMA +/+ (n = 6)	PRiMA -/- (n=8)		
$\dot{V}_E$ (mL/(g min ⁻¹))	1.62 ± 0.16	1.48 ± 0.10	NS	-9
V_T (μL/g)	10.4 ± 0.58	10.4 ± 1.02	NS	0
f_R (breaths/min)	154 ± 8	148 ± 8	NS	-4
T_i (ms)	113 ± 4	118 ± 9.0	NS	+4.5
T_E (ms)	293 ± 20	307 ± 23.0	NS	+5
T_I/T_{TOT} (%)	28.8 ± 1.5	28.2 ± 1.6	NS	-2
V_T/T_I (μL/(g s ⁻¹))	94 ± 7.4	93 ± 13.2	NS	-1
Weight (g)	28.5 ± 2.3	30.1 ± 3.5	NS	+5.5
Temperature (°C)	37.2 ± 0.2	37.1 ± 0.2	NS	0
	AChE +/+ (n = 9)	AChE -/- (n=9)		
$\dot{V}_E$ (mL/(g min ⁻¹))	1.34 ± 0.12	2.28 ± 0.19	<0.001	+70
V_T (μL/g)	9.6 ± 1.1	18.8 ± 1.60	<0.001	+96
f_R (breaths/min)	144 ± 7	130 ± 12	NS	-10
T_i (ms)	130 ± 5	74 ± 4	<0.001	-43

	i1RR +/+ (n = 6)	i1RR -/- (n=6)	P	% difference
T_E (ms)	301 ± 20	448 ± 47	<0.02	+49
T_I/T_{TOT} (%)	30.9 ± 1.4	15.5 ± 1.6	<0.001	-50
V_T/T_I ($\mu\text{L}/(\text{g s}^{-1})$)	76 ± 10	264 ± 31	<0.001	+247
Weight (g)	24.9 ± 0.83	16.3 ± 1.10	<0.001	-34.5
Temperature ($^{\circ}\text{C}$)	36.7 ± 0.4	36.6 ± 0.27	NS	0

Values for mice with deletion of the AChE gene (Boudinot et al., 2004) are presented for reference.