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Short communication

Serotonin transporter null male mouse pups have lower ventilation in air and 5% CO_2 at postnatal ages P15 and P25

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ABSTRACT

In Wild Type (WT) and serotonin transporter (5HTT) null mice, we studied oxygen consumption, ventilation and heart rate in air and 5% CO_2 at postnatal (P) days P5, P15, and P25 using either a head-out (younger mice) or whole body plethysmograph (older mice). Body weight and temperature did not differ between the groups. Oxygen consumption differed significantly only in females at P15 when it was reduced in 5HTT nulls ($P<0.01$). Heart rate similarly differed only in female 5HTT nulls at P15 being decreased in both air and CO_2 ($P<0.01$). Ventilation in air and 5% CO_2 was significant reduced via an effect on tidal volume at P15 ($P<0.02$) and P25 ($P<0.05$) but only in males. Ventilation in air and 5% CO_2 was greater in 5HTT null females at P25. We conclude that the gender specific effect (male predominant) on the CO_2 response reported in 5HTT null adult mice (Li and Nattie, 2008, J. Physiol. 586.9, 2321–2329, 2008) appears to have origins in early postnatal life (P15) when ventilation in both air and 5% CO_2 is reduced.

1. Introduction

1.1. Serotonin and chemoreception

Serotonergic neurons of the medulla contribute to central chemoreception, the ventilatory response to changes in brain CO_2/H^+ (Guyenet et al., 2010; Nattie, 2010), as detectors which can then stimulate respiratory neurons (Corcoran et al., 2009) and as modulators which can affect the sensitivity and output of other chemodetector neurons, e.g., the retrotrapezoid nucleus (Dias et al., 2008; Guyenet et al., 2010). One approach to study the role of serotonin in chemoreception is to use a transgenic mouse in which the serotonin transport protein (5HTT) is absent (Li and Nattie, 2008). These mice have increased extracellular serotonin (5HT) but reduced tissue 5HT levels and reduced $5HT_{1A}$ receptor binding (see Li and Nattie, 2008). Adult 5HTT null mice have a significantly reduced response to CO_2 which is present to a greater degree in males than in females (Li and Nattie, 2008). There are *in vitro* data to support the hypothesis that during postnatal development the participation of 5HT neurons in chemoreception occurs later in postnatal life, at P15 or so (Corcoran et al., 2009). Here we study the ventilatory and heart rate responses to CO_2 in male and female 5HTT null and WT control pops during postnatal development to determine if and when the absence of the 5HTT affects central chemoreception.

1.2. Serotonin and the Sudden Infant Death Syndrome

In addition to its role in central chemoreception, the serotonergic system has been implicated in the Sudden Infant Death Syndrome (SIDS). Multiple defects have been described including reduced serotonin transporter (5HTT) binding per neuron, reduced $5HT_{1A}$ receptor binding, increased numbers of immature 5HT neurons, and reduced tissue 5HT and TPH (tryptophan hydroxylase) levels (Duncan et al., 2010). SIDS can be viewed as a serotonin deficiency syndrome.

1.3. Serotonin deficiency and cardiorespiratory control

Four animal models of 5HT deficiency have been studied: (1) *Lmx1b* null mice with total absence of brainstem 5HT neurons (Corcoran et al., 2009); (2) *Pet-1* null mice with substantial but not total loss of brainstem 5HT neurons (Cummings et al., 2010); (3) intracisternal treatment with 5,7 DHT (dihydroxytryptamine), which depletes brainstem 5HT neurons in rat pups by 80% (Cummings et al., 2009); (4) dietary treatment of pregnant rat dams with a tryptophan depleted diet, which induces a ~40–50% depletion of brainstem 5HT in the pups (Penatti et al., 2010). Each of these models is associated with substantial abnormalities in cardiorespiratory control during early postnatal life (see Section 4).

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1.4. 5HTT transporter deficiency

The 5HTT null mouse model exhibits a subset of the abnormalities reported in SIDS cases, namely reduced 5HTT and $5HT_{1A}$ receptor binding, but differs from experimental approaches that wholesomely deplete 5HT neurons in that tissue 5HT levels are normal. Thus the 5HTT null mouse allows examination of the potential physiological role of this particular subset of 5HT system abnormalities in SIDS.

2. Methods

2.1. General

All experimental protocols were within the guidelines of the National Institutes of Health for animal use and care, and were approved by the Dartmouth College Institutional Animal Use and Care Committee. The mouse dams and pups were kept in the Animal Research Facility at Dartmouth in controlled temperature and light conditions with lights on from 7 AM to 7 PM and light off from 7 PM to 7 AM. All experiments were performed between 9 AM and 5 PM. Food and water were available *ad libitum* to the dam. The mouse pups were separated from the dam for the physiology experiments and were immediately brought back to the litter upon their completion. A total of 122 pups from both genders were studied from 8 litters of WT (29 males; 34 females) and 5HTT nulls (30 males and 29 females).

2.2. Genotyping

Genotyping was performed as described in our previous publication (Li and Nattie, 2008).

2.3. Head-out and whole body plethysmography

Physiological studies were conducted using either a head-out (for P5 pups) or whole body plethysmograph (for P15 and P25 pups) to measure ventilation ($\dot{V}_E$), tidal volume (V_T), frequency (f), and oxygen consumption ($\dot{V}_{O_2}$) as previously described (Penatti et al., 2010). The rectal temperature was measured before and after each experiment. The inflow gas for the plethysmograph was humidified and matched to the outflow connected to a vacuum system. The flow rate was ~1 l min^{-1} for the whole body plethysmograph and approximately 100 ml min^{-1} of outflow gas served O_2 and CO_2 analyzers (S3-A from Applied Electrochemistry Inc. and Capstar-100 from CWE Inc., respectively). In the head-out box, the chamber with the pup's head was not sealed and was constantly flushed by either room air or the 5% CO_2 challenge gas at a flow rate of ~0.5 l min^{-1}. A port very close to the pup's head was connected to the O_2 and CO_2 analyzers, which, due to the flow rate of the gas analyzer sampling, obtained expired gas from the pup's nose. We measured chamber pressure by transducer and calibrated the plethysmograph with injections of 0.1 ml for the head-out and 0.2 ml for the whole body plethysmograph. The chamber temperature for both the head-out and whole body plethysmographs was measured by a thermometer continuously and it was kept at ~32 °C for the head-out P5 data and at ~30 °C at P15 and P25 for the head-out experiments using a feed-back system. The measurement of breathing for the whole body plethysmograph during air or 5% CO_2 was recorded for approximately 3 min.

Pups were studied at P5, P15 and P25 during room air and hypercapnia (5% CO_2 challenge) periods. The measurement of heart rate (HR) was performed using a telemetric device (CTA-F40 from Data Systems International) with 2 ECG leads that were placed on the pup's chest skin and kept in place with an elastic bandage. After placing the ECG leads, the pup was placed inside the box and measurements of breathing and HR were recorded when the animal was settled and calm (~15 min of acclimation period). For the head-out box, tidal volume (V_T) was directly proportional to the pressure deflection caused by the pup's thorax movements and it was calculated based on the calibration of the box. For the whole body box, V_T was calculated using plethysmograph temperature at that time and pup's averaged temperature measured before placing it in the box and after removing it from the box, and breathing frequency (f) to estimate ventilation ($\dot{V}_E$) per breath.

Table 1
Oxygen consumption ($\dot{V}_{O_2}$) and body weight (BW) of WT and 5HTT null male and female mouse pups at P5, P15, and P25. Mean ± SEM values are shown. *$P < 0.01$.

		Male	Female
		P5	
$\dot{V}_{O_2}$ (ml/min/g)	WT	0.06 (0.007)	0.072 (0.01)
	Null	0.06 (0.009)	0.053 (0.006)
BW (g)	WT	4.3 (0.2)	4.0 (0.3)
	Null	4.6 (0.2	4.1 (0.3)
		P15	
$\dot{V}_{O_2}$ (ml/min/g)	WT	0.083 (0.011)	0.11 (0.009)*
	Null	0.097 (0.005)	0.064 (0.007)
BW (g)	WT	7.5 (0.3)	7.4 (0.4)
	Null	7.4 (1)	6.9 (0.3)
		P25	
$\dot{V}_{O_2}$ (ml/min/g)	WT	0.102 (0.012)	0.093 (0.009)
	Null	0.099 (0.009)	0.099 (0.009)
BW (g)	WT	11.9 (0.4)	11.8 (0.7)
	Null	12.4 (0.5)	11.2 (0.4 h)

2.4. Statistical analysis

We used a repeated measures (RM) ANOVA with room air and CO_2 as the RM to analyse at each age and in each gender ventilation ($\dot{V}_E$), tidal volume (V_T), respiratory rate (RR), oxygen consumption ($\dot{V}_{O_2}$), body weight (BW) and heart rate (HR) between WT and 5HTT null pups. In some cases we compared values in WT and 5HTT nulls using a t-test.

3. Results

3.1. Body weight and oxygen consumption

Table 1 shows data for body weight (BW) and oxygen consumption during air breathing for 5HTT null and WT mice at P5, P15 and P25. At P5 and P25 there was no significant difference between WT and null male or female pups in respect to BW or oxygen consumption (T-test). At P15 there was no significant difference between WT and null male or female BW or male oxygen consumption. But in females, oxygen consumption was significantly lower in the 5HTT nulls ($P < 0.01$; t-test).

3.2. Ventilation in air and 5% CO_2

Fig. 1 shows ventilation data for males and females at P5, P15 and P25 obtained while breathing air and 5% CO_2. At each age, in males and females, ventilation increases significantly in 5% CO_2 ($P < 0.01$; repeated measures ANOVA) due to significant increases in tidal volume and frequency ($P < 0.01$; repeated measures ANOVA). At P5 there is no difference between groups nor is there an interactive effect in respect to ventilation, tidal volume, or frequency. In males there is a significantly lower ventilation in 5HTT nulls compared to WT at P15 ($P < 0.02$; treatment effect) and P25 ($P < 0.03$; treatment effect) but the interactive term is not significant. This difference in ventilation was entirely due to a lower tidal volume in 5HTT nulls