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Unilateral ablation of neurokinin-1 receptor-expressing (NK1R) neurones within the pre-Bötzinger complex (preBötC) in adult rats disrupts breathing during sleep but not during wakefulness

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In adult rats, as the number of ablated preBötC NK1R neurones increases, breathing becomes increasingly disrupted during sleep [1], eventually resulting in an ataxic breathing pattern during wakefulness when cell loss is >80% [2]. Here we determine whether ablation of fewer preBötC NKIR neurones leads to sleep-disordered breathing (SDB). Adult male Sprague Dawley rats (n=8) were anaesthetised (100mg/kg ketamine, 10mg/kg xylazine i.p.) and instrumented to record diaphragmatic, abdominal and neck EMG, ECG, and EEG. Fourteen days post-implantation a second surgery was performed (anaesthesia as before) to stereotaxically inject unilaterally into the preBötC, either the toxin saporin conjugated to substance P (SP-SAP), which selectively ablates NK1R neurones (n=4) or as a control, SP mixed with SAP (unconjugated) that does not ablate NK1R neurones (n=4). Rats were kept on a 12-hour light/dark cycle and monitored within a plethysmograph from day 1 post-injection until they were humanely killed (days 21-50). Post SP-SAP injection, respiratory pattern remained unchanged during wakefulness. During sleep, particularly REM sleep, respiratory pattern became increasingly disordered at ~day 9 post SP-SAP injection, compared to pre-injection control. The disruptions in breathing pattern were characterised by an increase in frequency of apnoeas and hypopnea (~4-6/hour of sleep *vs* <3 control; p<0.05). To test responses to respiratory challenges, rats were exposed to hypercapnia (5% CO_2) and hypoxia (8% O_2) during wakefulness. All SP-SAP injected rats responded by increasing ventilation in a manner similar to pre-injection control until ~day 12 post-injection. Beyond this point SP-SAP injected rats were unable to increase ventilation appropriately (breaths/min: 120±15 *vs* 150±11 in 5% CO_2; 136±12 *vs* 180±8 in 8% O_2, p<0.05 post-injection *vs* pre-injection; mean±S.E.M.). Statistical analysis was performed using ANOVA.

Rats that were monitored up to 50 days post SP-SAP injection continued to have SDB, while breathing during resting wakefulness remained normal. Unlike bilateral SP-SAP injected rats, an ataxic breathing pattern [1,2] did not develop during wakefulness. Histological analysis of the ventrolateral medulla confirmed that only NKIR neurones within the preBötC on one side of the medulla were ablated. There are ~300 preBötC NK1R neurones/side in the adult rat. Ablation of half of these neurones by unilateral SP-SAP injection results in SDB, while breathing during resting wakefulness appears normal, however, when the respiratory system is challenged during wakefulness it does not respond appropriately. We have previously proposed that in the elderly and in individuals who suffer from various neurodegenerative diseases, loss of preBötC NK1R neurones over time may explain why SDB is highly prevalent in these populations.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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Brainstem P_{CO2} modulates phrenic responses resulting from specific carotid body oxygen in an *in situ* dual-perfused rodent preparation

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The chemical control of breathing is mediated through central chemoreceptors, which detect brain tissue P_{CO2}/pH (1), and peripheral chemoreceptors, which detect arterial P_{CO2} in a P_{O2}-dependent manor (2). However, the mathematical nature of the interaction between these chemoreceptors is controversial. Using a novel rat preparation, we demonstrated recently that the magnitude of the phrenic response to a single step of specific carotid body hypoxia (60 Torr P_{O2}) was modulated by the level of brainstem P_{CO2} (25 vs. 50 Torr P_{CO2}; 3). Based on these data, we hypothesized that the interaction between brainstem and carotid body chemoreceptors in the rat is hypo-additive. To test this hypothesis, we evaluated the effects of brainstem P_{CO2} on a broader range of carotid body activation, spanning from 400 to 40 Torr P_{O2}.

We used an in situ, decerebrate, vagotomized, arterially perfused rodent preparation (male Sprague-Dawley albino, decerebrated following halothane inhalation overdose). In this preparation, central and peripheral chemoreceptors are independently perfused with defined medium containing precisely controlled gas mixtures (4) and phrenic nerve activity is used to assess ventilatory responses.

Five minute isocapnic (35 Torr P_{CO2}) carotid body perturbations of 400, 200, 100, 60 and 40 Torr P_{O2} were applied in random order, while the brainstem P_{CO2} was maintained at 25, 35 or 50 Torr.

For the last minute of each 5-min perturbation, the phrenic burst frequency, neural tidal volume and neural minute ventilation were calculated. The effects of brainstem P_{CO2} on specific carotid body responses over the hyperoxic (400 to 100 Torr P_{O2}) and hypoxic (100 to 40 Torr P_{O2}) ranges were compared using a 2-factor ANOVA (factor 1: brainstem P_{CO2}, factor 2: carotid body hyperoxia or hypoxia) and the Student-Neuman-Keuls post-hoc test.

We found that the phrenic responses resulting from carotid body oxygen perturbations were largest in magnitude when the brainstem was held at 25 Torr P_{CO2}. This inverse relationship between oxygen responses and brainstem P_{CO2} was dose-dependent, suggesting that a hypo-additive (i.e. negative) interaction exists between the carotid bodies and the central chemoreceptors within the respiratory network. This is in contrast to the additive model (i.e. no interaction) of the chemical control of breathing that persists in the human literature.

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