行政院國家科學委員會專題研究計畫　成果報告

在肥胖症中 dopamine 作用在 D1, D 2, D3, or D4 接受體對換氣和運動的調節

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中文摘要

我們的主要目的是在調查在肥胖症中不正常換氣導因於不正常 dopamine 接受體的機制。14 隻基因瘦鼠和 14 隻基因肥胖鼠被研究約 12 週大。換氣的反應用 barometric 技術測試之前注射安慰劑或中央 dopamine D2 接受體阻斷劑 haloperidol (HAL), or 周圍 domperidone D2 (DOM), 或微量注射 dopamine 接受體阻斷劑(D1 antagonist: SCh 23390; D3 antagonist: U-99194A Maleate)。在基因瘦鼠和肥胖鼠 HAL, DOM, SCh 23390 和 U-99194A Maleate 和安慰劑比較沒有影響正常的換氣。DOM 比較安慰劑有意義增加缺氧的換氣反應在基因肥胖鼠但不在基因瘦鼠. HAL 有意義減少缺氧的換氣反應在基因瘦鼠但不在基因肥胖鼠。在基因瘦鼠和肥胖鼠, SCh 23390 和 U-99194A Maleate 沒有影響缺氧的換氣反應。我們的主要發現在周圍缺氧的化學反應在基因肥胖鼠是減少的導因於增加的 dopaminergic D2 的調節在周圍的接受體.中央 D1, D2, D3 對缺氧換氣的調節沒有發現在基因瘦鼠和肥胖鼠。

關鍵詞：dopamine、呼吸、肥胖

ABSTRACT

To investigate the hypothesis that the impaired respiratory drive noted in morbid obesity was attributed to altered dopaminergic mechanisms acting on peripheral and/or central chemoreflex sensitivity, 14 obese and 14 lean Zucker (Z) rats were studied at 11~12 weeks of age. Ventilation (V_E) was measured by the barometric technique during normoxic (21% O_2) and hypoxic (10% O_2) exposures following the administration of vehicle (control), haloperidol (D2 central and peripheral antagonist HAL), or domperidone (D2 peripheral antagonist DOM), and intracranial injecton of vehicle, dopamine D1 antagonist (SCh 23390), and D3 antagonist (U-99194A Maleate). HAL, DOM, SCh 23390 and U-99194A Maleate did not affected V_E during normoxia compared to control in both lean and obese rats. DOM significantly increased V_E during hypoxia compared to control in obese but not lean rats. HAL significantly decreased V_E during hypoxia compared to control in lean but not obese rats. In both lean and obese rats, SCh 23390 and U-99194A Maleate did not affect V_E in response to hypoxia. Our major findings suggest that peripheral chemosensitivity to hypoxia in obese Z rats is reduced as a result of an increased dopaminergic D2 modulation on peripheral chemoreceptors. Central D1, D2, D3 modulation of ventilatory response to hypoxia were not found in lean and obese rats.

Keywords: dopamine, respiration, obesity

INTRODUCTION

The regulation of ventilation relies upon peripheral and central chemical feedback of O_2 levels. Dopamine, a monoamine neurotransmitter, is the most abundant catecholamine in the brain and in carotid body (4). Although dopamine is known to inhibit the afferent neuronal activity from the carotid bodies (4, 11), dopamine stimulates neuronal activity involved in central respiratory regulation (10).

Thus, dopamine appears to exert contrasting effects of ventilatory regulation, a depressive modulation of ventilation on peripheral chemoreflex drive and a stimulatory modulation of ventilation on the central nervous system (CNS) (3, 10, 29).

The obese Zucker (Z) rat, a genetic model of morbid obesity, presents many of the same ventilatory abnormalities as observed in obese humans, including a depressed respiratory drive (8, 14, 15, 19). Obese Z rat display a reduction in brain DA metabolite content compared to lean rats (20). Indeed, the altered dopaminergic function, especially in the hypothalamic dopaminergic system, appears to contribute to the dysfunctional eating patterns noted in obese Z rats and is believed to predispose some individuals to obesity (9). Previously, we have suggested that several neuromodulators, such as endorphins, GABA, nitric oxide and glutamate may partially account for the altered ventilatory response observed in obese Z rats (14, 15, 16, 24,). The role of dopamine acting D1 D2, D3 receptor in mediating breathing control in obesity has, to our knowledge, not been previously investigated.

Since dopamine plays a crucial role in ventilatory response and altered dopamine mechanisms have been noted in obesity, we hypothesized that the impaired respiratory drive noted in obese Z rats could be linked in part to altered dopaminergic mechanisms acting on peripheral and/or central chemoreflex sensitivity. To examine our hypothesis, we measured ventilation during normoxia and hypoxia in obese Z rats following the systemic administration of either vehicle (control), haloperidol (D2 central and peripheral antagonist HAL), or domperidone (D2 peripheral antagonist DOM), and intracranial injecton of dopamine antagonists (D1 antagonist: SCh 23390; D3 antagonist: U-99194A Maleate).

METHODS

Animals. 14 pairs of the lean (Fa/?) and obese (fa/fa) male Z rats were studied at 11~12 week of age. Animals were born by breeders purchased from Charles River Lab in France. One lean and one obese rat were housed per cage. Ambient temperature was maintained at 24 °C, and the animals were kept on an artificial 12-h light-dark cycle. The light period began at 7:00 AM. Rats were provided with standard laboratory chow and water ad libitum. All protocols were approved by the Institutional Animal Care and Use Committee of Chang Shan Medical University, Taichung, Taiwan.

Five days before the experiments, the rats were deeply anesthetized and placed in a stereotaxic frame for guide cannula implantation. A small hole was drilled in the occipital skull. A small cannula (22 G) will be then surgically implanted at lateral cerebral ventricle. The guide cannula will be fixed to the skull with methacrylate and screws on skull closed with an occluder until time of experimentation.

Ventilation was measured using by the barometric technique which have been previously described (14, 15, 16). A cylindrical Plexiglas chamber with a volume of 4 liters was used for the measurements of ventilation. The rat was placed in the chamber within a restrainer, which did not permit backward rotation. The animal chamber had an inlet tube that was connected to pressurized air tanks. Inlet flow was regulated at 2 l/min by a flow meter. An O_2 analyzer and an CO_2 analyzer measured the concentrations of inflowing or outflowing O_2 and CO_2, respectively. To measure ventilation, the chamber was completely sealed after momentarily interrupting the flow through it, and the oscillations in pressure caused by breathing were recorded by a sensitive pressure transducer. The signal was received, amplified and displayed on an oscillographic strip chart recorder.

To reduce the stress level during the experiment, all animals were habituated to an intraperitoneal injection (i.p.) of 0.4 ml of saline, to the insertion of the thermoprobe, and to the restraining device within the chamber for 60 min on two successive days prior to the first experimental study. Each animal was tested at 3-day intervals following an i.p. injection of equal volumes

of vehicle (dimethyl sulfoxide DMSO, 1 ml/kg), haloperidol (HAL, 1 mg/kg, central and peripheral D2 receptor antagonist), or domperidone (DOM, 0.5 mg/kg, peripheral D2 receptor antagonist). Pulmonary parameter (V_E, V_T, f, T_I, T_E, Ttot, V_T/T_I), metabolic rate (VO_2, VCO_2), V_E/VO_2, V_E/VCO_2, and body temperature (Tb) were tested after intracranial microinjecting certain amount of blind solutions, namely either vehicle, dopamine D1 antagonist (SCh 23390), and D3 antagonist (U-99194A Maleate) under normoxia and hypoxia.

After the administration of the agent, the rat was placed into the barometric chamber within the restrainer and exposed to normoxia (21% O_2, balance N_2) for 30 min followed by hyperoxia (100% O_2) for 3 min, normoxia for 10 min, and hypoxia (10% O_2, balance N_2) for 3 min. Thereafter, the rat exposed to hyperoxia (100% O_2) for 5 min. Ventilatory data were collected during the last minute of each gas exposure.

Statistical Analysis. The differences in V_E responses to hyperoxia/hypoxia between lean and obese Z rats were analyzed by factorial analysis of variance (ANOVA) concerning phenotype and gas exposure. The differences between data among the responses following each agent administration within a single group were analyzed by one-way ANOVA. When significance was indicated, a post-hoc t-test with Bonferroni's correction for multiple comparisons was used for point-by-point differences. In all cases, a P value < 0.05 was considered statistically significant. All data presented in the text, Tables and Figures represent means ± SD.

RESULTS

The changes in V_E, f and V_T during hyperoxia, normoxia and hypoxia exposure following vehicle or HAL injection. In lean Z rats during hyperoxia as well as normoxia, the administration of HAL did not change V_E although f decreased and V_T increased. During hypoxic exposure, HAL administration elicited a significant depression in V_E ($P < 0.05$) due to a decrease in f and no changes in V_T in lean rats. In contrast, in obese rats during hypoxia exposure, HAL did not evoke significant depression in V_E due to an increase in V_T.

DOM did not change V_E, f and V_T during hyperoxia and hypoxia in lean rats, compared with control values. In obese rats DOM significantly increased V_T in response to hypoxia compared with vehicle control without changes in f, resulting in a rise in V_E compared with control. Moreover, DOM did not change any parameters during hyperoxia. Incracranial injection of SCh 23390 and U-99194A Maleate did not change V_E, f and V_T during hyperoxia and hypoxia in lean and obese rats.

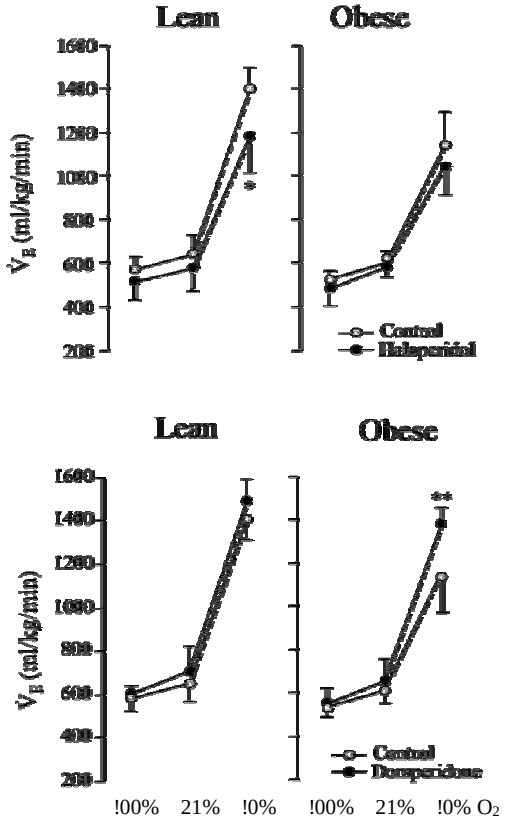

Figure: Changes in V_E hyperoxia, normoxia and hypoxia with vehicle control or domperidone. ** $P < 0.01$; significantly different from corresponding control value.

DISCUSSION

Since HAL (D2 central and peripheral antagonist) crosses the BBB, it produces effects that are a combination of peripheral chemoreceptor stimulation and central dopaminergic neuron inhibition. In the

present study, both lean and obese HAL-treated rats decreased f and increased V_T resulting in an unchanged $\dot{V}_E$ during hyperoxic and normoxic breathing. These results are in agreement with a previous study in rats showing that the intracerebroventricular injection of HAL (D2 central and peripheral antagonist) elicited a depression in f and an increase in V_T, suggesting a tonic influence on dopamine receptors involved in central respiratory regulation (10). DOM (D2 peripheral antagonist), on the other hand, does not cross the BBB thus its effects are limited to peripheral chemoreceptors. Since DOM (D2 peripheral antagonist) administration did not affect f, V_T, and V_E in both lean and obese Z rats during hyperoxic and normoxic breathing, endogenous dopamine acting on D_2 receptors do not modulate ventilation under these conditions. Our findings are consistent with a report in healthy humans reporting that DOM administration produced no significant change in ventilation during normoxic breathing (6).

As stated earlier, HAL crossing BBB antagonizes both central and peripheral D2 receptors, which consists of reversing D2-mediated peripheral ventilatory stimulation and central vetilatory depression. Indeed, it has been demonstrated that HAL (D2 central and peripheral antagonist) greatly attenuates the ventilatory response to hypoxia despite an increase in carotid chemoreceptor activity, suggesting that dopamine acts as an excitatory neurotransmitter in the integrating centers projecting from peripheral chemoreceptor activity (25). In lean Z rats, HAL (D2 central and peripheral antagonist) significantly decreased V_E during hypoxia compared with vehicle control, suggesting that the central inhibitory effect on ventilation by HAL (D2 central and peripheral antagonist) was greater than that of the peripheral stimulation. In contrast, HAL (D2 central and peripheral antagonist) administration had a no (minimal) effect on V_E in obese Zucker rats, which implying that either D2-mediated central ventilatory stimulation is reduced and/or D2-mediated peripheral ventilatory depression is increased during acute hypoxic exposure.

It is known that the peripheral D2 receptor antagonist, DOM, stimulates ventilation and carotid body chemoreceptor afferent neural activity (13, 29). Moreover, in normal awake cats and goats, DOM (D2 peripheral antagonist) increases ventilation in response to hypoxia by removing tonic inhibition from endogenous carotid body D2 receptors (13, 27). We noted that in lean Z rats ventilation in response to acute hypoxia did not significantly increase following treatment with DOM, suggesting no tonic peripheral inhibitory ventilatory modulation by D2 in lean rats. Walsh et al showed that, in humans, half of the population did not augment their hypoxic ventilatory response when pretreated with DOM, suggesting a wide variability in individual hypoxic sensitivities in response to DOM (28). Tatsumi et al also demonstrated in cats that peripheral chemoreceptor responsiveness to hypoxia was highly variable among individual cats as well as was the ventilatory response to DOM (27).

Since incracranial injection of SCh 23390 and U-99194A Maleate did not change V_E, f and V_T during hyperoxia and hypoxia in lean and obese rats. We can speculate Sch 23390 and U-99194 maleate did not affect ventilation during hperoxia, normoxia, and hypoxia. Overdose of SCh 23390 and U-99194A Maleate cause the restless movement and disturb the recording of ventilatory parameter. This is major limitation in the observation of incracranial injection of SCh 23390 and U-99194A.

Our findings from both the HAL and DOM studies suggest that obese Z rats have an enhanced dopaminergic modulation acting on D2 receptor of their peripheral chemoreceptors compared with their lean counter parts. The combined evidence, therefore, suggests that peripheral chemosensitivity to hypoxia in obese Z rats may be blunted as a result of an abnormality originating from dopaminergic mechanisms acting D2 receptors.

In conclusion, the present results suggest that peripheral chemosensitivity to

hypoxia in obese Z rats may be blunted as a result of altered D2 dopaminergic mechanisms.

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