行政院國家科學委員會專題研究計畫　期中進度報告

在肥胖症中內因性 opioids 作用在 mu, delta, or kappa 接受體對心肺功能和運動的調節(1/2)

計畫類別：個別型計畫
計畫編號：NSC92-2314-B-040-015-
執行期間：92 年 08 月 01 日至 93 年 07 月 31 日
執行單位：中山醫學大學物理治療學系

計畫主持人：李信達

計畫參與人員：吳芳俐

報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 93 年 5 月 17 日

行政院國家科學委員會專題研究計畫成果報告

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Preparation of NSC Project Reports

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成果報告類型(依經費核定清單規定繳交)：精簡報告

摘要

我們的主要目的是在調查在肥胖症中不正常心肺反應導因於不正常 opioids 作用在 mu, delta, or kappa 接受體的機制。24 隻基因瘦鼠和 24 隻基因肥胖鼠被研究約 12 週大。換氣的反應用 barometric 和 sphygmomanometric 技術測試之前注射安慰劑或周圍 opioid 接受體阻斷劑 naloxone methiodide (NM) or 中央 opioid 接受體阻斷劑 naloxone hydrochloride (NHCl), 或顱內微量注射 opioid 接受體阻斷劑如 mu1 接受體阻斷劑 naloxonazine (NAL), mu-接受體阻斷劑 CTOP, delta 接受體阻斷劑 naltrindole (NALT), 和 kappa-接受體 阻斷劑 nor- Binaltorphimine (NB).

在基因瘦鼠 NM 和 NHCl 沒有影響正常的換氣、血壓和心跳。相似的，NM 沒有影響正常的換氣、血壓和心跳在基因肥胖鼠。相反的，NHCl 比較安慰劑有意義增加換氣和心跳在基因肥胖鼠。 NAL 沒有影響正常的換氣、血壓和心跳在基因瘦鼠和肥胖鼠。CTOP 沒有影響正常的換氣、血壓和心跳在基因瘦鼠然而 CTOP 有意義增加換氣和心跳在基因肥胖鼠。在基因瘦鼠和肥胖鼠換氣、血壓和心跳沒有被 NALT 和 NB 影響。在基因肥胖鼠心臟的 TNFα 有意義高過於在基因瘦鼠。

因此在肥胖症中內因性 opioids 對換氣和心跳的調節主要作用在位於中央神經系統的 mu 接受體而非 mu1, delta, kappa delta, or kappa 接受體。

關鍵詞:換氣、血壓、心跳、心臟

Abstract

To determine whether altered central opioidergic (mu, delta, kappa) mechanisms contribute to the altered cardiopulmonary response in obese Zucker rats. 24 lean and 24 obese Zucker rats were studied. Ventilation (VE), tidal volume (VT), and breathing frequency (f), mean blood pressure (MBP), and heart rate (HR) were measured on separate occasions by the barometric and sphygmomanometric method following the randomized blinded administration of naloxone methiodide (NM, peripheral broad opioid antagonist), naloxone hydrochloride (NHCl, peripheral and central broad opioid antagonist), incracranial microinjections of naloxonazine (NAL, selective mu1 receptor antagonists), CTOP (mu-receptor antagonists), naltrindole (NALT, delta receptor antagonist), and nor-Binaltorphimine (NB, kappa-receptor antagonists).

NM and NHCl in lean animals had no effect on ventilation and MBP, and HR. Similarly, NM failed to alter ventilation in obese rats. In contrast, NHCl significantly ($P<0.05$) increased VE and HR in obese rats. NAL did not affect VE, HR, BP in lean and obese rats and CTOP did not affect VE, HR, BP in lean rats whereas CTOP significantly increased VE, HR in obese rats. In lean and obese Zucker rats, VE, HR, and BP were unaffected by the administration of either NALT and NB. Cardiac TNFα in obese rats is higher than age-matched lean Zucker rats

Thus, endogenous opioids modulate ventilation and heart rate in obese, but not lean, Zucker rats by acting specifically on mu (not on mu1, delta, kappa) receptors located within the central nervous system.

Keywords: Opioid,ventilation, blood pressure, heart rate, cardiac

Introduction

Abnormal respiratory control in some forms of obesity may lead to a decrease in ventilatory compensation and, in time, to the development of chronic alveolar hypoventilation (hypoxemia and hypercapnia), a condition commonly referred to as obesity hypoventilation syndrome (OHS) (Morgan, 1978; Orlowski, 1982; Zwillich, 1975). The obese Zucker rat, a model of morbid obesity, presents many of the same respiratory deficits as noted in morbidly obese humans (Dempsey, 1966; Farkas,1994), including abnormal respiratory control mechanisms (Lee, 2001 [a]; Lee, 2000).

Endogenous opioids and opioid receptors are widely distributed throughout the central nervous system (Mansour, 1988). Opioids, inhibitory neuromodulators, have been demonstrated to have an important role in neural mechanisms of respiratory control (Santiago, 1985). It is now generally accepted that at least three distinct opioid receptors, mu (μ), delta (δ), and kappa (κ), are present in the carotid body and in various brain stem regions, including the nucleus of solitary tract, the nucleus ambiguus, and the hypoglossal nucleus. Each of these structures has established links to ventilatory control (Mansour, 1988; Santiago, 1985).

Our previous investigations have revealed important links between opioidergic mechanisms and impaired breathing control in obesity (Lee, 2000). In obese Zucker rats, ventilation during room air and during acute hypoxic exposure (2 min) was shown to be modulated by endogenous opioids.

Tumor necrosis factor (TNF) is a proinflammatory cytokine that can produce widespread deleterious effects when expressed in large amounts. It is produced in the heart by both cardiac myocytes and resident macrophages under conditions of cardiac stress, and is thought to be responsible for many of the untoward manifestations of cardiac disease. TNF play important role in heart disease and is some potential therapeutic modalities (Retter, 2001). However, the effect of TNFα in the heart of obesity is unknown.

The purpose of this study was therefore to investigate whether endogenous opioids modulate the ventilatory response to hypoxia in morbidly obese Zucker rats. The second purpose is to investigate the effect of TNFα in the heart of obese Zucker rats.

We hypothesized that aberrant opioidergic [mu (mu1, mu2); delta (d1, d2); kappa] receptor-mediated mechanisms contribute to the altered ventilatory response in obese Z rats. Second, we hypothesized that TNFα in the heart of obesity is increased. A parallel study design was used, with lean, age-matched Zucker rats serving as controls.

Methods

Animals: The study was be performed on 24 lean (Fa/Fa or Fa/fa) and 24 obese (fa/fa) age-matched (<12 weeks old) male Zucker (Z.) rats. Animals were breed from Zucker rats purchased from Charles River Lab in France. All protocols were approved by the Institutional Animal Care and Use Committee of Chang Shan Medical University, Taichung, Taiwan.

Guide cannula implantation Five days before the experiments, the rats were deeply anesthetized and placed in a stereotaxic frame (purchase requested) for guide cannula implantation to 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

Pulmonary ventilation: Breathing pattern (V_E, V_T, f,) was be recorded by the barometric technique, of which complete details have been previously been provided (Lee, 2000; Lee, 2001[a]; Lee, 2001[b]). A cylindrical plexiglas chamber (purchase requested) with a volume will be used for the measurement of breathing pattern. The signal will be received and amplified by a Preamplifier and displayed on Biophysical Monitoring System. Injection and withdrawal 0.3 ml of air with a 1-ml syringe will be performed at least 12 times during the recording, for the purposes of calibration.

Tail pressure measurement: After one week of acclimation, tail blood pressure was

measured with a sphygmomanometer (29SSP, IITC INC. /Life Science Instruments, USA), which consisted of a main board machine and a pump machine. The rat was placed on the table and the tail was placed in the sensor area to measure blood pressure. When the pointer started to move, it indicated the maximum blood pressure for the rat. If the rat moved during the measurement, the result was not valid. Blood pressure and heart rates was measured five times and the five values were averaged.

Microinjections. For microinjections into the lateral cerebral ventricle, a 33-gauge needle longer than the guide cannula was connected by PE-10 tubing to a 1-µl syringe. After removal of the occluder, the needle for microinjection will be carefully inserted into the guide cannula. The same procedure will be used in the protocols after 72 hrs for blind solutions including vehicle.

Experimental protocol. Animals were tested 30 min following a subcutaneous injection of equal volumes of saline (vehicle: 1 ml/kg), or naloxone methiodide (N_M, 5 mg/kg, peripheral opioid antagonist), or naloxone hydrochloride (N_{HCl}, 5 mg/kg, central and peripheral opioid antagonist) or incracranial microinjections of naloxonazine (selective mu1 receptor antagonists), CTOP (mu-receptor antagonists), naltrindole (delta receptor antagonist),and nor-Binaltorphimine (kappa-receptor antagonists). Although the binding effect of a single injection of naloxone only persists for 2 hr, the ventilatory test was completed within one hour after injection. The solutions of saline, N_M, and N_{HCl} were prepared daily and placed in vials labeled as solutions I, II, or III. The agents were given in a blinded, randomized design with 72 h elapsing between successive tests.

Tissue Extraction. Cardiac tissue extracts were obtained by homogenizing the left ventricle samples in a PBS buffer (0.14 M NaCl, 3 mM KCl, 1.4 mM KH_2PO_4, 14 mM K_2HPO_4) at a concentration of 1 mg tissue/10 µl PBS for 5 min. The homogenates were placed on ice for 10 min and then centrifuged at 12,000 rpm for 30 min. The supernatant was collected and stored at -70°C for further experiments.

Statistical analysis. Body weights of lean and obese rats were averaged over experimental period and differences between the two groups were tested by unpaired Student t-test. The other parameters (VE, VT, f) were analyzed by analysis of variance using the general linear model (GLM) in a one between (lean and obese) and two within (gases exposures over time, drugs) design. All data presented in the text, tables, and figures are means ± SEM.

Results

Obese rats weighed more than age-matched lean animals in all groug. ($p < 0.01$).

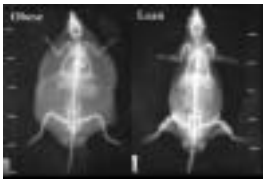

Fig 1. X ray of obese and lean Zucker rats

There was a significant interaction in the overall time-dependent change in $\dot{V}_E$ between lean and obese rats ($P<0.01$). There was a significant interaction in $\dot{V}_E$, MBP, and HR among three factors (phenotypes, time courses, and drugs) ($P<0.01$).

Administration of N_M had no effect at rest on all parameters in lean and obese Zucker rats. In sharp contrast, N_{HCl} infusion altered resting ventilation (V_T, $\dot{V}_E$), and HR in obese Zucker rats (Table 1).

In lean and obese Zucker rats, ventilation ($\dot{V}_E$), tidal volume (V_T), breathing frequency (f), Heart Rate (HR), and Blood Pressure (BP) under room air exposure were unaffected by the administration of either naltrindole (delta receptor antagonist),and nor-Binaltorphimine (kappa-receptor antagonists).

Table 1

	Lean rats		
	Saline	NM	NHCl
f, breaths/min	158 ± 7	154 ± 6	158 ± 9
V_T, ml/kg	7.8 ± 0.5	7.6 ± 0.6	8.0 ± 0.4
$\dot{V}_E$, ml/min/kg	1171 ± 64	1197± 100	1199± 71
HR, beat/min	350± 21	356± 27	361± 24
MBP, mmHg	97 ± 5	95 ± 6	100 ±6
	obese rats		
f, breaths/min	179 ± 5	182 ± 8	188 ± 3
V_T, ml/kg	6.2 ± 0.2	6.2 ± 0.6	7.3 ± 0.4 *
$\dot{V}_E$, ml/min/kg	1108± 44	1106± 60	1367± 78 *
HR, beat/min	378± 17	368± 19	398± 25*
MBP, mmHg	101 ±8	108 ±7	111 ±8

Values are means ± SEM (n=8). f = breathing frequency; V_T = tidal volume; $\dot{V}_E$ = minute ventilation, HR=heart rates, MBP=mean blood pressure. *Significant difference between saline and naloxone methiodide (N_M) or between saline and naloxone hydrochloride (N_{HCl}), P<0.05. ** P<0.01.

Table 2 Effects of mu1 and mu2 antagonist on cardiopulmonary resposes

	Lean rats		
	vehicle	NAL	CTOP
f, breaths/min	148 ± 7	153 ± 6	153 ± 9
V_T, ml/kg	7.8 ± 0.2	7.9 ± 0.3	8.1± 0.4
$\dot{V}_E$, ml/min/kg	1103±85	1019± 144	1260± 188
HR, beat/min	360± 24	360± 24	360± 24
MBP, mmHg	95 ± 5	94 ± 6	102 ±7
	obese rats		
f, breaths/min	180 ± 5	185 ± 7	188± 6.3
V_T, ml/kg	6.9 ± 0.7	7.0 ± 0.8	8.4 ±0.8*
$\dot{V}_E$, ml/min/kg	1066±154	1084± 175	1602±130*
HR, beat/min	358± 17	366± 19	380± 26*
MBP, mmHg	104 ±4	110 ±7	113 ±8

Values are means ± SEM (n=8). (NAL, selective mu1 receptor antagonists), CTOP (mu-receptor antagonists), f = breathing frequency; V_T = tidal volume; $\dot{V}_E$ = minute ventilation, HR=heart rates, MBP=mean blood pressure. *Significant difference between saline and NAL or between saline and CTOP (N_{HCl}), P<0.05. ** P<0.01.

Total body weight (TBW), total heart weight (THW), and left ventricle weight (LVW) in obese rats weighed more than those in age-matched lean Zucker rats (Table 3). Cardiac TNFα in obese rats is higher than age-matched lean Zucker rats (Fig 2).

Table 3

	Obese	Lean
TBW	523±61*	366±28
THW	1.24±0.13*	0.86±0.05
LVW	0.83±0.11*	0.55±0.07
THW / TBW	$(23.8\pm2.2)\times10^{-4}$	$(23.5\pm1.4)\times10^{-4}$
LVW/ TBW	$(16\pm1.45)\times10^{-3}$	$(15\pm1.66)\times10^{-3}$
LVW / THW	0.676±0.059	0.642±0.053

Values are means ± SEM N=7 Total Body weight (TBW), Total heart weight (THW), Left ventricle weight (LVW). *Significant difference between lean and obese, P<0.05.

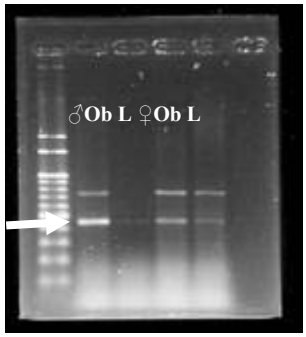

Fig 2 Cardiac TNFα in male obese and lean, and female obese and lean. N=3

Disscussion

Our findings can be summarized as follows: 1) endogenous opioids do not modulate ventilation, Heart rates, Blood Pressure in lean Zucker rats, 2) in obese rats, resting ventilation and heart rates is depressed by increased opioidergic activity via receptors located within the CNS, 3) in obese rats, ventilation (via V_T) is blunted by endogenous opioids acting on mu not on mu1, receptors located within the CNS, suggesting that mu-receptor mediated modulation of ventilation in obesity is possibly via mu2; 4) endogenous opioids acting on delta receptors and kappa-receptors do not modulate ventilation, heart rate, blood Pressure in lean and obese Zucker rats 5) Cardiac TNFα in obese rats is higher than age-matched lean Zucker rats.

Affinity differences between N_M and N_{HCl} for brain μ, δ, and κ opioid receptors

separately have not been identified (Deviche, 1997). The doses selected were based on previous investigations suggesting that 5 mg/kg fully occupies the three major opioid receptors (Santiago, 1985; Gestreau C, 2000). In our experimental design, naloxone methiodide (N_M) and naloxone hydrochloride (N_{HCl}) were injected systemically such that there would be widespread antagonistic actions. After removal of the occluder, the needle for microinjection will be carefully infusing into into the lateral cerebral ventricle. The any modulation here observed are limited in the central nervous system. Further experiments using microinjections into specific brain regions will be required to identify those areas that are directly responsible for the ventilatory responses described below. Endogenous opioids modulate ventilation and heart rate in obese, but not lean, Zucker rats by acting specifically on mu (not on mu1, delta, kappa) receptors located within the central nervous system. Since mu (not in mu1), the finding suggested that mu-receptor mediated modulation of ventilation in obesity is possibly via mu2. Because action of mu2 antagonist is irreversible, our experiment design is not suitable by directly using mu2 antagonist.

Total body weight, total heart weight, and left ventricle weight in obese rats weighed more than those in age-matched lean Zucker rats, suggesting cadiac adaptation occurs in obesity. Cardiac TNFα in obese rats is higher than that in lean Zucker rats. When TNFα is expressed in large amounts, Cardiac TNFα can produce widespread deleterious effects in obese cardium. A increased TNFα in cardium was found in obese Zucker rats, comparing with age-match lean rats, suggesting cardiac myocytes of obesity was under conditions of cardiac stress, and is thought to be responsible for many of the untoward manifestations of cardiac disease.

Significance: Morbidly obese humans often suffer from alveolar hypoventilation and cardiovascular diseases. If elevated endogenous opioids lead to blunted ventilation and heart rates in obese humans, obesity related hypoxemia (decreased PaO_2) can be expected to be exacerbated and in addition, would amplify the likelihood of the development of chronic alveolar hypoventilation. Therefore, altered opioidergic neuromodulation of ventilation in obesity may play an important role in the pathogenesis of obesity hypoventilation syndrome (OHS), a condition characterized by chronic alveolar hypoventilation. Orlowski and colleagues (Orlowski, 1982) presented a case study of a 20-mo-old girl (200% of ideal body weight) admitted to a clinic with respiratory failure caused by OHS. A single dose of naloxone (10 μg/kg) given early in the course of the child's respiratory failure resulted in a dramatic improvement in ventilation that lasted approximately 3 to 4 h. Continuous naloxone infusion led to further improvements in the child's respiratory status.

Cardiac abnormality is often seen in obese patients. However, the development of cardiac pathophysiology in obesity and what mechanism contributes obese cardiac abnormality are still unknown. Except a increased TNFα in cardium in obesity, further studies are required to clarify what mechanism contributes obese cardiac abnormality.

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