中 國 醫 藥 大 學
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碩士學位論文

糖尿病鼠在缺氧和高二氧化碳下 GABA 和 NMDA 受體對呼吸反應的調節

GABAergic and NMDA-mediated modulation of ventilatory response to hypoxia and hypercapnia in diabetes rats

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中 華 民 國 九 十 八 年 七 月

中文摘要

在糖尿病病人身上可以發現到，當處於在一個缺氧的環境下其呼吸反應會相較一般人來的遲鈍，但是其中的機轉並未讓人所明白。在其他的研究指出，糖尿病病人體內的神經調節物質會受到改變或損傷，而在呼吸反應調控方面會因抑制性或興奮性的神經傳導物質影響而有所不同。本研究的目的是在於探討當神經傳導物質- gamma-aminobutyric acid A ($GABA_A$)和 N-methyl-D-aspartic acid (NMDA)的改變而使得糖尿病病人在急性缺氧時的呼吸反應改變。實驗 1, 實驗爲兩組老鼠，一組爲正常另一組使用 streptozotocin(STZ, 65 mg/kg i.p.)誘發成胰島素依賴型糖尿病，接給注射 DMSO 和 bicuculline (0.5 ml/kg s.q.)，利用 BUXCO 系統給予一般空氣（２１％　O_2）３０分鐘和缺氧氣體（１０％　O_2）３０分鐘接著再給一般空氣(21 % O_2) 10 分鐘和高二氧化碳氣體(8% CO_2)20 分鐘並給予記錄。實驗 2,　同樣爲兩組 8 周大的老鼠,一組是正常狀態下而另一組是使用 streptozotocin (STZ, 65 mg/kg i.p.)誘導爲胰島素依賴型糖尿病模式，分別給予注射 saline 和dextromethorphan (DxM, 0.5 ml/kg s.q.)，　同樣是利用 BUXCO 系統給予一般空氣（２１％　O_2）３０分鐘和缺氧氣體（１０％　O_2）３０分鐘接著再給一般空氣(21 % O_2) 10 分鐘和高二氧化碳氣體(8% CO_2)20 分鐘同樣給予記錄。實驗 1, 結果發現糖尿病組在急性缺氧時的呼吸反應明顯較正常組來的遲鈍。在糖尿病組中給予 bicuculline 的介入後其呼吸反應有顯著的恢復，而在正常組的老鼠給予 bicuculline 後沒有任何的改變。此實驗結果顯示當ＧＡＢＡ$_A$接受器被阻斷後糖尿病病人在急性呼吸反應有恢復的現象，顯示其呼吸反應遲鈍是因內因性ＧＡＢＡ的作用特別是對ＧＡＢＡ$_A$接受器。ＧＡＢＡ調節的改變會損害糖尿病病人在面對睡眠呼吸中止症相關的代償反應。實驗 2,結果發現在糖尿病組在急性缺氧時的呼吸反應明顯較正常組來的鈍。在糖尿病組中給予dextromethorphan的介入後其呼吸反應並無顯著差異，在正常組的老鼠予dextromethorphan介入後沒有任何的改變。

ABSTRACT

Diabetes mellitus (DM) patients exhibit blunted ventilatory responses to acute hypoxia whereas the underlying mechanism is unknown. The purpose of the study is to determine whether altered gamma-aminobutyric acid (GABA)ergic mechanisms acting in GABAA receptors contribute to the abnormal ventilatory response to hypoxia in diabetes mellitus. Ventilatory function ventilation (VE), tidal volume (VT), and breathing frequency (f) was assessed using in an unrestricted whole body plethysmograph (Buxco System) in eight non-DM Wistar rats and 8 streptozotocin(STZ)-induced diabetic rats (65 mg/kg i.p., DM). Part 1, Ventilation (VE), tidal volume (VT), and breathing frequency (f) during room air breathing and in response to acute (<10 min) and sustained (10-30 min) hypoxic (10% O_2) and hypercapnia (CO_2) challenges were measured on two separate occasions following the randomized blinded administration of equal volumes of DMSO (vehicle), bicuculline (0.5 mg/kg, $GABA_A$ receptor antagonist). Part 2, Ventilation (VE), tidal volume (VT), and breathing frequency (f) during room air breathing and in response to acute (<10 min) and sustained (10-30 min) hypoxic (10% O_2) and hypercapnia (8% CO_2) challenges were measured on two separate occasions following the randomized blinded administration of equal volumes of saline (vehicle) and dextromethorphan (DxM, 0.5 mg/kg, NMDA receptor antagonist).

Part 1, Ventilatory response to acute (not sustained) hypoxia in DM group was significantly ($P<0.05$) blunted compared to non-DM group. Bicuculline administration in non-DM Wistar rats had no effect on ventilation either during room air breathing, or acute and sustained exposure to hypoxia. In contrast, bicuculline administration in DM group significantly increased ventilatory response to acute hypoxia. However, bicuculline administration in DM Wistar rats had no effect on ventilation either during room air breathing or sustained hypoxia. Blunted ventilatory responses to acute hypoxia in diabetes mellitus appeared to be suppressed by endogenous GABA by acting specifically on $GABA_A$ receptors. Altered GABAergic modulation of acute ventilatory response in diabetes might potentially impact sleep apnea episode (acute hypoxia) related ventilatory compensation. Part 2, Ventilatory response to hypoxia in DM group was significantly blunted compared to non-DM group. Dextromethorphan administration in non-DM Wistar rats had no effect on ventilation either during room air breathing, or acute and sustained exposure to hypoxia. In contrast, dextromethorphan administration in DM Wistar rats had no effect on ventilation either during room air breathing or acute and sustained hypoxia. In hypercapnia, to compare vehicles and drugs it no effect on ventilation for either DM and non DM group.

致謝

時光飛逝兩年的碩士生涯一下子就過去，在這不長不短的時間裡，一路上面對太多事情，有太多的人助我在挫折中一一的面對難關，這些要感謝的人太多了，一時之間不知該如何表明謝意。

首先能讓本篇論文順利的完成，要先感謝我的指導教授李信達老師，感謝他對於我的研究所生涯不斷的給予鼓勵與指導，讓我能有許多不同的觀點面對問題去解決困難，並耐心的教導我做報告及撰寫論文。感謝口試委員駱明潔老師、吳柏蒼老師對我所做的研究及論文提出許多不同的見解並給予指導。

謝謝系所上吳柏蒼老師、陳郁文老師及謝悅齡老師，給予我許多學業上和生活鼓勵與幫助，不論是儀器借用或使用都給我很多的協助，讓我實驗能順利進行。謝謝凡妮、憶帆教我許多實驗的技術，也協助我的實驗能順利進行。也謝謝實驗室的學弟妹泳璁、凱玲、智超與曉玲，有你們的加入使得研究所生活不再枯燥單調，讓生活更活潑。謝謝昆霖，能從大學有緣到現在，協助處理研究所的大小事情，解決很多莫名的雜事。更特別感謝乃瑜，能在我身邊一直給予我許多幫忙及很大的鼓勵，讓我能有走下去動力。

最後要感謝家人，在我研究所的過程中，一直給我莫大的鼓勵與支持；特別感謝我弟弟，能給我許多正面的思考幫助我熬過難關。最後，感謝所有在我研究所給予我幫助的人，謝謝您們讓我能不斷的成長與進步的動力。

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Chapter 1

Background review

1.1 Ventilatory responses to hypoxia

In mature animals the peripheral arterial chemoreceptors in the carotid body are the first step in a closed-loop feedback control system that acts to normalize arterial oxygen and carbon dioxide levels by effecting changes in ventilation. This ventilatory control system is critically important in promoting arousal from sleep during hypoxia, altering ventilatory pattern due to changes in inspired oxygen, and in responding to decreases in arterial oxygen as may occur in numerous circumstances such as apnea and airway obstruction(18). The difference in ventilatory response to hypoxia between neonatal and adult subjects is well documented(54). The hypoxic ventilatory response is susceptible to developmental plasticity, and that a carotid chemoreceptor deficit is the primary cause(33). The ventilatory response to sustained hypoxia in humans and in some animals is characterized by an initial increase in ventilation (early phase), followed by a gradual decline in ventilation (late phase)**.** Evaluation of breathing pattern revealed that during constant hypoxia there was little alteration in respiratory timing and that the changes in inspiratory minute ventilation were related to significant alterations in tidal volume and mean inspiratory flow (VT/Ti) (16). Hyperoxia might elicit this plasticity by inhibiting chemoreceptor activity during early life(3).

1.2 Ventilatory responses to hypercapnia

Elevated CO_2 (hypercapnia) is a major stimulus for increased ventilation. Increases in blood CO_2 are proposed to be monitored by specialized neurons known as central chemoreceptors, which are believed to be localized within several areas of the brainstem including the ventrolateral medulla (VLM), the nucleus of the solitary tract (NTS), the locus coeruleus (LC) and the medullary raphe(11, 12). Recent reports have shown that there are developmental changes in the ventilatory response to hypercapnia in the rat. These are characterized by an initial large response to carbon dioxide immediately after birth followed by a decline with a trough at one week of age, followed by a return in sensitivity(6). In the linear increase in minute ventilation was observed with step-wise increases

of inspired CO_2 from 0 to 10%. Other studies dealing with the ventilatory response to CO_2 tend to make comparisons between a control (normocapnia) and a single hypercapnic stimulus rather than step-wise increases over a range of CO_2 concentrations(50).

1.3 Diabetes mellitus and ventilatory responses to hypoxia

The insulin-dependent diabetes mellitus (IDDM) can lead to an overall depression in ventilatory control mechanisms. Rats with streptozotocin (STZ)-induced IDDM also exhibit decreased baseline ventilation, as well as attenuated hypercapnic and hypoxic ventilatory responses (42). In IDDM, the greater perception of dyspnea is associated with changes in inspiratory effort being out of proportion to changes in ventilation. The greater increase in dynamic elastance and the lower increase in ventilation may, account for the greater perception of breathlessness during hypoxia (47). Type 2 diabetic human subjects exhibit blunted ventilatory responses to acute hypoxia, suggesting that this group of diabetic subjects possesses a chemoreflex ill-equipped to respond homeostatically to hypoxic challenge (55).

1.4 Diabetes mellitus and ventilatory responses to hypercapnia

In the past study seven diabetics had an impaired response to hypercapnia. Loss of integrity of motor descending pathways to the respiratory muscles is a possible cause of a reduced ventilatory response to hypercapnia(56). In other study was conducted to elucidate the changes in ventilatory responses to hypercapnia and hypoxia and the effects of insulin in long-term DM rats. Acute ventilatory responses to progressive hypercapnia and hypoxia increased with age in the normal rats, whereas they were relatively constant at a lower level in the DM group. The significant reduction in the ventilatory response in the DM group appeared 16 w after STZ injection, and it was not recovered by insulin treatment(59). A decreased ventilatory response to hypercapnia may occur as a

result of a reduction in the amount of afferent information arising from the carotid bodies(21).

1.5 Neurotransmitter GABA and Glutamate in diabetes

The other studies, insulin modulates synaptosomal GABA, thus having a neuroprotective role under oxidizing and/or diabetic conditions (15) . The in vivo basal striatal GABA levels of streptozotocin diabetic rats are similar to non-diabetic rats, suggest that diabetes may change GABA homeostasis and modify behavioral responses in an animal model of depression (20). In a other theme the gamma amino butyric acid (GABA) and its related enzymes have been demonstrated in pancreatic beta cells of normal rat. It was shown that the number of GABA-LIR cells is reduced significantly in diabetes. Moreover, GABA is a strong secretagogue of insulin from the pancreas of normal rat (1). The GABA transport might be implicated in the neuroprotective role of insulin (14). The lower susceptibility of synaptosomes isolated from diabetes rats to lipid peroxidation as compared with synaptosomes isolated from non-diabetes Wistar rats. The diabetes rat synaptosomes, that state affected the uptake of the neurotransmitters GABA (13).

Insulin administration to neurons regulates the cell surface localization, or activity, of a variety of neurotransmitter receptors. Insulin modulates glutamate receptors and $GABA_A$(29, 35) . These effects on NMDA receptors can potentially explain some of the known CNS effects of diabetes(4). The modulation of the surface localization of the NMDA subtype of glutamate receptor could explain various central defects indiabetic animals and diabetic patients, including the impairment of learning and memory (independent of altered glucose levels), impaired synaptic plasticity(5, 41, 52, 60), reduced central sensory responses(2), dendritic shortening in the hippocampus (36) and altered hypothalamic function (61). In prior studies find that there can be amarked inter-animal and intra-animal variability in the NMDA receptor response to diabetes; this may also account for the disparity between prior studies and correspond to the variability in sensory impairments(4).

Insulin insufficiency has multiple actions on the CNS. These effects on NMDA receptors can potentially explain some of the known CNS effects of diabetes.(4) Previous studies have indicated an association between diabetes mellitus and impairments in synaptic plasticity in the hippocampus. The results showed that glutamate levels were significantly decreased in diabetes group compared to the control group. The diabetes affects the concentration of glutamate in extracellular space in the DG. That is a possibility for involvement of pre-synaptic component in synaptic plasticity defect in the hippocampus under diabetic conditions(46).

1.6 Neurotransmitter GABA and ventilatory response to hypoxia

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS) and acts at approximately 25-40% of the synapses within the CNS (7). GABA-A receptors have been shown to be involved in the generation and the modulation of respiration (23, 28). During hypoxic challenges, brain GABA levels increase and exert an inhibitory modulation during the sustained hypoxic ventilatory response (37, 57). During hypoxia, the respiratory drive is determined by a balance between the stimulation of peripheral chemoreceptors and the central depression of hypoxia on respiration(49). It has been postulated that the ventilatory response to hypoxia is modulated by a variety of neurotransmitters, including GABA (28, 49). Brain GABA content is elevated during hypoxic (57).

1.7 Neurotransmitter NMDA and ventilatory response to hypoxia

Ventilation in response to hypoxic exposure is closely related to the release of excitatory neurotransmitters, in particular glutamate, acting specifically on N-methyl-D-aspartate (NMDA) receptors(31).The N-methyl-D-aspartate (NMDA) receptor has many functions throughout the central nervous system (CNS) including its role within the centers controlling respiration(53). The

increase in ventilationduring hypoxia is closely related to the release of excitatory neurotransmitters, in particular glutamate, acting on Nmethyl- D-aspartate (NMDA) receptors located in brainstem respiratory motor neurons (32). Chronic hypoxia altered the effect of MK-801 on the acute HVR, primarily because of increased effects on tidal volume. This indicates that changes in NMDA receptor-mediated neurotransmission may be involved in ventilatory acclimatization to hypoxia(45). An attempt has been made to test the hypothesis that, in the caudal part of nucleus tractus solitarii (NTS) where carotid sinus nerve (CSN) afferents project, L-glutamate (Glut) modulates the hypoxic ventilatory response. Pretreatment with MK-801 or kynurenate reduced the hypoxic ventilatory response. This reduction in ventilation was mainly due to the decrease in tidal volume. (38).

1.8 Neurotransmitter and ventilatory response to hypercapnia

Hypercapnia stimulates ventilation in general, but it seems to elicit little or no metabolic response that might modify the demand for ventilation. Although the ventilatory response to CO_2 may lack a metabolic component and is generally stable during the short duration exposure periods that have been studied, the ventilatory response to CO_2 is not stable over the period of development. The developmental changes of the responsiveness of individual chemosensitive neurons to hypercapnia or to a developmental change in the number of chemosensitive neurons (43). The particular patterns of activity present in the central nervous system seem to prune the dendritic arbor and decrease the number of synapses, so that certain synapses are strengthened and others are removed. Glutamate, acting through NMDA receptors, seemed to be particularly important in the process of stabilizing favored synaptic pathways while eliminating others (51). Neurons from two of the three brainstem chemosensitive regions studied, the locus coeruleus and nucleus of solitary tract, seemed to have a fully developed response to hypercapnia at birth that showed no detectable change with development. The development of chemosensitivity of neurons did not appear to play a critical

role in the triphasic developmental pattern of the ventilatory response to hypercapnia seen in intact neonatal rats(50).

1.9 Neurotransmitter and diabetes for ventilatory response

In the present research, the releance between diabetes respiratory reaction and the neurotransmitter is currently unknown. In the hypoxia and hypercapnia situations, it can affect respiratory reaction of most people, but the effects on bodies of diabetes people are also currently unknown.

Chapter 2

Aims of this study

2.1 Aims of this study

Whether the altered ventilatory response to hypoxia and hypercapnic observed in DM Wistar rats is associated with altered $GABA_A$ receptors and glutamatergic NMDA function has not been previously studied, and formed the basis of our study.

Part 1: Since STZ-induced DM rats are known to possess altered brain GABAergic mechanisms, we hypothesized that ventilatory response to hypoxia and hypercapnic in DM Wistar rats would also be modulated by GABAergic mechanism. We hypothesized that the altered ventilatory response to hypoxia in diabetes rats is mediated in part via altered $GABA_A$ receptors function. We used bicuculline, $GABA_A$ receptors antagonist to investigate whether endogenous GABA modulates ventilation at rest and ventilation during hypoxic and hypercapnic exposure in diabetes rats. The agents were given in a blinded-randomized design with 72-hour recovery between successive ventilatory tests. A parallel study design was used, with non-DM Wistar rats serving as controls. The role of GABA in mediating breathing control during hypoxic exposure in diabetes has to our knowledge, not been previously investigated.

Part 2: Since STZ-induced DM rats are known to possess altered brain glutamate NMDAergic mechanisms, we hypothesized that ventilatory response to hypoxia and hypercapnic in diabetes rats would also be modulated by NMDA-mediated mechanism. We hypothesized that the altered ventilatory response to hypoxia in diabetes rats is mediated in part via altered NMDA receptors and glutamatergic NMDA function. We used dextromethorphan (DxM), a noncompetitive NMDA receptor antagonist to investigate whether endogenous glutamate modulates ventilation via NMDA

at rest and ventilation during hypoxic and hypercapnic exposure in diabetes rats. The agents were given in a blinded-randomized design with 72-hour recovery between successive ventilatory tests. A parallel study design was used, with non-DM Wistar rats serving as controls. The role of glutamate NMDA receptors in mediating breathing control during hypoxic exposure in diabetes has to our knowledge, not been previously investigated.

Chapter 3

Material and methods

3.2 Material and methods

3.2.1 Animals

Part 1: The studies were performed on 8 non-DM Wistar rats and 8 induce DM Wistar rats (streptozotocin, 65 mg/kg i.p., Sigma Chemical, Co., Louis, MO, USA) age-matched male Wistar rats. Animals were purchased from BioLASCO Taiwan Co., Ltd at 4 weeks of age. One non-DM and one DM rat were housed per cage. Ambient temperature was maintained at 21°C and an artificial 12-h light-dark cycle was set. The light period began at 7:00 AM. Rats were provided with standard laboratory chow (LabDiet® Dealers, St. Louis, MO) and water ad libitum. All protocols were approved by the Institutional Animal Care and Use Committee of the China Medical University at Taiwan. Animals underwent testing at 8 weeks of age.

Part 2: The studies were performed on 14 non-DM Wistar rats and 14 induce DM Wistar rats (streptozotocin, 65 mg/kg i.p., Sigma Chemical, Co., Louis, MO, USA) age-matched male Wistar rats. Animals were purchased from BioLASCO Taiwan Co., Ltd at 4 weeks of age. One non-DM and one DM rat were housed per cage. Ambient temperature was maintained at 21°C and an artificial 12-h light-dark cycle was set. The light period began at 7:00 AM. Rats were provided with standard laboratory chow (LabDiet® Dealers, St. Louis, MO) and water ad libitum. All protocols were approved by the Institutional Animal Care and Use Committee of the China Medical University at Taiwan. Animals underwent testing at 6 weeks of age.

3.2.3 Blood glucose

Part 1: We use the streptozotocin (STZ)-induced diabetic rats. STZ (65 mg/kg) was dissolved in sodium citrate (50 mM) buffered saline. All injections were given intraperitoneally (i.p.). Rats were randomly divided into two groups. Rats were denoted as having diabetes if blood glucose level measured ≥220 mg/dl by 2 days post-STZ injection. Accu-ChekAdvantage technology uses two components: electronic meter and disposable biosensor. The inserts a biosensor into the meter and applies a small drop of blood from rats tail.

Part 2: We use the streptozotocin (STZ)-induced diabetic rats. STZ (65 mg/kg) was dissolved in sodium citrate (50 mM) buffered saline. All injections were given intraperitoneally (i.p.). Rats were randomly divided into two groups. Rats were denoted as having diabetes if blood glucose level measured ≥220 mg/dl by 2 days post-STZ injection. Accu-ChekAdvantage technology uses two components: electronic meter and disposable biosensor. The inserts a biosensor into the meter and applies a small drop of blood from rats tail.

3.2.3 Pulmonary ventilation

Part 1: Breathing pattern was recorded by the whole body plethysmography. Subjects can move freely in the chambers for minimum stress and study under the most natural conditions. The unrestrained whole body plethysmography application involves measuring a "box flow" which is the net (or sum) of nasal and thoracic flows. Actual flows are calculated in the software, taking into account temperature, humidity, and pressure. (Buxco research systems©, Wilmington, North Carolina USA). To measure minute volume (V_E), tidal volume (V_T), breathing frequency (f), inspiratory time (T_i), expiratory time (T_e), peak inspiratory flow (PIF), peak expiratory flow (PEF). The rat was placed into the chamber and exposed to room air (21% O_2, balance N_2) for 30 minutes, hypoxia (10 % O_2, balance N_2) for 30 minutes, room air for 15 minutes and hypercapnic (8% CO_2). Ventilatory patterns were recorded at the end of 30 min room air, at 30 min during the hypoxic exposure, and at

the end of the room air 10min. At hypercapnic situation were recorded at the end of 15 min.

Part 2: Breathing pattern was recorded by the whole body plethysmography. Subjects can move freely in the chambers for minimum stress and study under the most natural conditions. The unrestrained whole body plethysmography application involves measuring a "box flow" which is the net (or sum) of nasal and thoracic flows. Actual flows are calculated in the software, taking into account temperature, humidity, and pressure. (Buxco research systems©, Wilmington, North Carolina USA). To measure minute volume (V_E), tidal volume (V_T), breathing frequency (f) , inspiratory time (T_i), expiratory time (T_e), peak inspiratory flow (P IF), peak expiratory flow (PEF). The rat was placed into the chamber and exposed to room air (21% O_2, balance N_2) for 30 minutes, hypoxia (10 % O_2, balance N_2) for 30 minutes, room air for 15 minutes and hypercapnic (8% CO_2). Ventilatory patterns were recorded at the end of 30 min room air, at 30 min during the hypoxic exposure, and at the end of the room air 15 min. At hypercapnic situation were recorded at the end of 15 min.

3.2.4 Experimental protocol

Part 1: Animals were tested 30 min following a sub-cutaneous (S.Q.) injection of equal volumes (0.5ml/kg) of DMSO (vehicle) or bicuculline (0.5mg/kg) (Sigma Chemical, Co., Louis, MO, USA). Bicuculline effects are noted within 10 minutes of injection and lasts for more than 2 hours in rodents (58). The current studies were carried out 5 min after injection. The agents were given in a blinded randomized design, with 72 hours elapsing between successive tests. The solutions were prepared daily and placed in vials labeled as solutions A or B. The agents were given in a blinded design and randomized order. To reduce the stress level during the study, 3 days before the first ventilation all animals were habituated to an experimental protocol for 60 minutes. To minimize any potential differences related to circadian rhythms, each rat was injected and tested at the same approximate time of day.

Part 2: Animals were tested 30 min following a sub-cutaneous (S.Q.) injection of equal

volumes (0.5ml/kg) of saline (vehicle) or dextromethorphan (0.5mg/kg) (Sigma Chemical, Co., Louis, MO, USA). Effects are noted within 10 minutes of injection and lasts for more than 2 hours in rodents (37, 58). The current studies were carried out 5 min after injection. The agents were given in a blinded randomized design, with 72 hours elapsing between successive tests. The solutions were prepared daily and placed in vials labeled as solutions A or B. The agents were given in a blinded design and randomized order. To reduce the stress level during the study, 3 days before the first ventilation all animals were habituated to an experimental protocol for 60 minutes. To minimize any potential differences related to circadian rhythms, each rat was injected and tested at the same approximate time of day.

3.2.5 Statistical analysis

Part 1: Due to interactions among the three factors, the effects of bicuculline on minute volume (V_E), tidal volume (V_T), breathing frequency (f), mean inspiratory flow (V_T / T_i), inspiratory time (T_i), expiratory time (T_e), peak inspiratory flow (P IF), peak expiratory flow (PEF), were subsequently tested as a single group repeated measure with contrast transformation during room air, during hypoxic exposure in non-DM and DM rats, separately. Minute volume (V_E) was also calculated ($V_E = V_T \times f$) and neural respiratory drive was assessed as the mean inspiratory flow (V_T / T_i). The contrast transformation is a useful approach when one level of the repeated measures is a control (i.e. DMSO) against which the others (i.e. bicuculline) are compared. In all cases, use the paired t-test measures and has a difference at $P<0.05$ was considered statistically significant. All data presented in the text and tables represent means ± SD, and for figures represent means ± SDE

Part 2: Due to interactions among the three factors, the effects of dextromethorphan on minute volume (V_E), tidal volume (V_T), breathing frequency (f), mean inspiratory flow (V_T / T_i), inspiratory time (T_i), expiratory time (T_e), peak inspiratory flow (PIF), peak expiratory flow (PEF), were subsequently tested as a single group repeated measure with contrast transformation during room

air, during hypoxic exposure in non-DM and DM rats, separately. Minute volume (V_E) was also calculated ($V_E = V_T \times f$) and neural respiratory drive was assessed as the mean inspiratory flow (V_T / T_i). The contrast transformation is a useful approach when one level of the repeated measures is a control (i.e. saline) against which the others (i.e. dextromethorphan) are compared. In all cases, use the paired t-test measures and has a difference at $P<0.05$ was considered statistically significant. All data presented in the text and tables represent means ± SD, and for figures represent means ± SDE.

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Chapter 4

Results

4.3 RESULTS

4.3.1 DM vs non-DM

During room air breathing, V_E in DM rats was similar to that in non-DM rats (Table 1, Fig 1). During hypoxic exposure in the acute phase (after 4 min), V_E response and V_T / T_i, in DM rats has significantly decreased than non-DM rats ($P<0.05$) (Table 2, Fig 1). During hypoxic exposed late phase, V_E in DM rats was not significantly different from that in non- DM rats (Table 3, Fig 1). The breathing frequency (f) and tidal volume (V_T) were similar between DM and non-DM rats (Table 3, Fig 1). During hypercapnia exposure in the acute phase (after 4 min) and late phase (10 min), breathing frequence (f) and V_E, in DM rats has significantly decreased than non-DM rats ($P<0.05$) (Table 5, Table 6, Fig 3, Fig7).

4.4.2 GABAergic Modulation

During the acute and late phase of the ventilatory response to hypoxia, non-DM rats injected with either control values or bicuculline had no change in any of the various parameters (f, V_T , V_E, T_i, T_e, V_T / T_i, PIF, PEF and EF50) (Table 2, Table 3, Fig 2). In contrast, during room air breathing, DM rats injected with either control values or bicuculline had no change in any of the various parameters (f, V_T , V_E, T_i, T_e, V_T / T_i, PIF, PEF and EF50) (Table 1, Fig 2). In contrast, DM rats exhibited an increased ventilation (V_E), frequence (f) and mean inspiratory flow (V_T / T_i) following bicuculline administration in acute hypoxic exposure(Table 2, Fig 2). During the late phase of the ventilatory response to hypoxia, DM rats injected with either control values or bicuculline had no change in any of the various parameters (f, V_T , V_E, T_i, T_e, V_T / T_i, PIF, PEF and EF50) (Table 3, Fig 2). GABAergic modulation in hypercapnia, DM and non-DM rats injected with either control values or bicuculline had no change in any of the various parameters (f, V_T , V_E, T_i, T_e, V_T / T_i, PIF, PEF and EF50) (Table 4, Table 5, Table 6, Fig 4).

4.4.3 NMDA Modulation

During the acute and late phase of the ventilatory response to hypoxia, non-DM rats injected with either control values or dextromethorphan had no change in any of the various parameters (f, V_T and V_E) (Table 8, Table 9, Fig 6). In contrast, during room air breathing, DM rats injected with either control values or dextromethorphan had no change in any of the various parameters (f, V_T , V_E, T_i, T_e, V_T / T_i, PIF, PEF and EF50) (Table 8, Table 9, Fig 8). In contrast, during acute hypoxic breathing, DM rats injected with either control values or bicuculline had no change in any of the various parameters (f, V_T , V_E, T_i, T_e, V_T / T_i, PIF, PEF and EF50). During the late phase of the ventilatory response to hypoxia, DM rats injected with either control values or dextromethorphan had no change in any of the various parameters (f, V_T , V_E, T_i, T_e, V_T / T_i, PIF, PEF and EF50) (Table8, Table9, Fig 6).

NMDA modulation in hypercapnia DM and non-DM R rats injected with either control values or bicuculline had no change in any of the various parameters (f, V_T , V_E, T_i, T_e, V_T / T_i, PIF, PEF and EF50) (Fig 6, Table 2).

Chapter 5

Discussion

5.5 DISCUSSION

Our major findings:

Our major findings can be summarized as follows:

Part 1: 1) Antagonism of $GABA_A$ receptors does not change ventilation at rest or during ventilatory challenges in non-DM Wistar rats; 2) Breathing at rest in DM Wistar rats is modulated by endogenous GABA acting on $GABA_A$ receptors; 3) Ventilation during hypoxic, exposure is modulated by endogenous GABA acting on $GABA_A$ receptors in DM Wistar rats.

Part 2, 1) Dextromethorphan administration does not alter resting ventilation in Non-DM or DM rats; 2) During the early phase of hypoxic exposure, ventilation appears to does not modulated by NMDA receptors in Non-DM or DM rats; 3) Ventilation during late phase hypoxic, it is not exposure for modulated by NMDA receptor antagonism in DM Wistar rats.

GABA modulation of Ventilation in DM

GABA is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS) and acts at approximately 25-40% of the synapses within the CNS (7). GABA can exert its effect via either ionotropic ($GABA_A$ and $GABA_C$) receptors to produce fast synaptic inhibition, or metabotropic ($GABA_B$) receptors to produce slow, prolonged inhibitory signals(8) . GABA may be involved as a neurotransmitter in the generation, the transmission, and the modulation of respiratory related neural activities (22-24, 28, 30). In the present study, bicuculline, a selective antagonist of $GABA_A$ receptors, was chosen because previous studies have shown that GABA inhibits respiratory activity mainly via $GABA_A$ receptors(22). In GABAergic neurons, $GABA_A$ receptors facilitate Cl^- flux into neurons, resulting in hyperpolization, whereas antagonism of $GABA_A$ receptors by bicuculline will decrease Cl^- flux, resulting in depolarization and increased excitation (8, 28). Thus, any effect noted in the present study is restricted to a modulatory role exerted by endogenous GABA acting specifically on $GABA_A$ receptors. $GABA_A$ receptors are located throughout the neural axis and modulate

numerous systems. In the present study, bicuculline was injected systemically, which consequently produced a widespread antagonistic action. Thus, any effect noted herein cannot be localized to any specific system or brain region. The goal of the present study, however, was to determine whether GABAergic mechanisms regulate ventilationv at acute hypoxia in DM Wistar rats. Clearly, additional experiments using a reductionist approach will be required in order to specifically identify those brain areas that are directly responsible.

In non-DM rats, bicuculline administration did not alter resting ventilation, ventilation during hypoxic exposure. Indeed, in normal human subjects, increasing brain GABA concentration by vigabatrin administration, an agent which prevents the breakdown of GABA, had no effects on resting ventilation or on chemical ventilatory drive (17). Thus, consistent with the human literature, GABA does not exert a significant effect on the control of respiration in normal rats.

In contrast, bicuculline administration elevated resting ventilation, ventilation during hypoxic exposure in age-matched DM Wistar rats. Following 8 weeks of chronic artificial respiratory loading in rats, brain GABA levels are increased and responsible for depressing ventilation (44). Thus, the increased chest wall loading or the airway narrowing that is present in diabetes (15) may represent a possible stimulus responsible for the altered GABAergic mechanisms. In the present study, bicuculline administration significantly increased resting ventilation in DM rats, which was attributed to an increase in tidal volume and not breathing frequency. The selective effect on tidal volume is consistent with previous reports indicating that direct exogenous central administration of GABA or $GABA_A$ receptor agonist produces a dose-dependent depression in respiratory amplitude with only minor effects noted on respiratory frequency (26, 30). During hypoxia, the respiratory drive is determined by a balance between the stimulation of peripheral chemoreceptors and the central depression of hypoxia on respiration (49). It has been postulated that the late phase of the ventilatory response to hypoxia is modulated by a variety of neurotransmitters, including GABA (28, 49). Brain GABA content is elevated during hypoxic(57) and hypercapnic exposures (25, 30). The rise in

ventilation following treatment with bicuculline during hypoxia is consistent with previous studies in either anesthetized cats (37), in sedated newborn piglets (26), or in anesthetized rats (49).

NMDA modulation of Ventilation in DM

Glutamate, an excitatory neurotransmitter, has an important role in the central mechanisms of respiratory control(28).The NMDA receptor family has been extensively studied due to their pivotal roles in regulating synaptic plasticity, learning, psychosis and cell death in various neuropathological conditions(10, 34, 39). NMDA receptors are ligand-gated ion channels, or ionotropic receptors, that allow the transmembrane flux of Na+, K+ and Ca++ ions after the binding of glutamate and glycine to their respective binding sites on the NMDA receptor complex. NMDA receptors exist as heteromeric tetramers and are thought to be most commonly composed of two NR1 subunits and two NR2 subunits(40).

In previous experiments, insulin was shown to significantly increase native NMDA receptor activity in rat hippocampus and recombinant receptors expressed in Xenopus oocytes (9, 35). This increase in activity is due to a rapid insulin-induced increase in the surface expression of NMDA receptors from intracellular pools (48). These observations suggest that in diabetes there may be a reduced cell surface expression of NMDA receptors. Such a reduced expression could underlie some of the adverse effects of diabetes in the CNS. Presently, using rats made diabetic by streptozotocin (STZ) administration, reduction of [^{3}H]-AMPA binding varied in different brain structures, being more pronounced in the striatum, cerebral cortex, and hippocampus and almost absent in the cerebellum. It has reported that there is no effect on brain NMDA receptor levels when measured in horizontal sections of ventral brain by NMDA sensitive L-[^{3}H]glutamate binding site autoradiography. The effect of STZ-induced diabetes appeared to be specific to the AMPA subtype of glutamate receptors, as the same treatment did not modify L-[^{3}H]glutamate binding to NMDA receptors(19).

The primary purpose of the current study, however, was to assess the role of NMDA receptors in modulating ventilation. The non DM and DM rats were used as their own control such that weight differences between both phenotypes cannot account for our finding in NMDA receptor-mediated modulation. Dextromethorphan administration had no effects at rest in both non DM and DM rats.

No finding in NMDA modualtion of ventilatory response to hypoxia and hypercapnia. In under the hypoxia and hypercapnia environment, as if is comes from regarding the ventilatory response influence is the elsewhere function no by the NMDA modualtion means. Possibly is change NMDA receptors which causes of diabetes. But was about the reason not to be still clear.

Significance: When stay on acute hypoxia situation in general person have promptly responses to modulate avoid to dsmage. In the diabetes mellitus, they cannot promptly to modulated for hypoxia. That was to deepen on sleep apnea episode damage for apparatus. In the present study, humans and animals have demonstrated that intermittent hypoxia and reduced sleep duration due to sleep fragmentation, as occur in obstructive sleep apnea, exert adverse effects on glucose metabolism(27). Blunted ventilatory responses to acute hypoxia in diabetes mellitus appeared to be suppressed by endogenous GABA by acting specifically on $GABA_A$ receptors. Altered GABAergic modulation of acute ventilatory response in diabetes might potentially impact sleep apnea episode (acute hypoxia) related ventilatory compensation.

Table and Figure

Table 1. Ventilatory parameters in non-DM and DM rats treated with vehicle or bicuculline on hypoxia resting time.

Hypoxia	Non-DM rats		DM rats	
Body Weight, g	476.39±69.23		387.63±75.89	
BG mg/dl	77±7.19		433.69±90.30	
Resting	Vehicle	Bicucuclline	Vehicle	Bicuculline
f, breaths/min	93.75±6.27	90.25±6.09	75.98±3.84	97.01±4.49
VT, ml	2.71±0.04	2.64±0.22	2.93±0.09	2.93±0.10
VE, ml/min	245.95±11.09	235.31±6.29	216.90±15.95	245.02±14.48
Ti, sec	0.25±0.01	0.23±0.00	0.32±0.00	0.28±0.00
Te, sec	0.46±0.03	0.50±0.03	0.52±0.02	0.49±0.00
PIF, ml/sec	18.13±0.81	18.23±1.07	14.62±0.57	17.88±1.16
PEF, ml/sec	13.43±0.22	12.83±0.42	12.71±1.60	16.81±0.74
EF50, ml/sec	0.73±0.07	0.64±0.02	0.67±0.14	0.76±0.05
VT /Ti	11.05±0.47	11.26±0.83	9.19±0.28	10.44±0.21

Non-DM rats = non- diabetes mellitus rats, DM rats = diabetes mellitus rats, BG = blood glucose, f = Frequency, TV =Tidal Volume, VE=Minute Volume, Ti = Inspiratory Time, Te = Expiratory Time, PIF = Peak Inspiratory Flow, PEF = Peak Expiratory Flow, EF50 = The flow at the point 50% of TV is expired, VT /Ti = mean inspiratory flow. Values are means ± SD (n=8). *P<0.05 DM with Non DM vehicle to compare have a significant difference. ＃P<0.05Non DM vehicle and bicuculline to compare have a significant difference. ＋P<0.05 DM vehicle and bicuculline to compare have a significant difference.

Table 2. Ventilatory parameters in non-DM and DM rats treated with vehicle or bicuculline on hypoxia 4 min.

	Non-DM rats		DM rats	
Hypoxia 4 min	Vehicle	Bicucuclline	Vehicle	Bicuculline
f, breaths/min	132.86±3.57	141.17±0.97	107.84±3.66	140.20±5.56
VT, ml	3.76±0.83	3.53±0.53	3.29±0.52	3.72±0.43^{+}
VE, ml/min	469.84±116.80	470.95±64.04	335.51±74.04*	464.69±73.21^{+}
Ti, sec	0.19±0.00	0.19±0.00	0.26±0.00	0.21±0.00
Te, sec	0.35±0.05	0.31±0.03	0.38±0.05	0.30±0.05
PIF, ml/sec	29.55±4.47	28.67±2.22	19.52±2.34*	28.86±0.94
PEF, ml/sec	27.49±5.91	24.56±3.09	17.38±3.49	24.54±2.75
EF50, ml/sec	1.69±0.43	1.62±0.15	1.14±0.29	1.59±0.27
VT /Ti	19.49±4.35	18.81±2.40	12.70±2.06*	17.90±1.68^{+}

Non-DM rats = non- diabetes mellitus rats, DM rats = diabetes mellitus rats, f = Frequency, TV =Tidal Volume, VE=Minute Volume, Ti = Inspiratory Time, Te = Expiratory Time, PIF = Peak Inspiratory Flow, PEF = Peak Expiratory Flow, EF50 = The flow at the point 50% of TV is expired, VT /Ti = mean inspiratory flow. Values are means ± SD (n=8). *P<0.05 DM with Non DM vehicle to compare have a significant difference. ＃P<0.05Non DM vehicle and bicuculline to compare have a significant difference. ＋P<0.05 DM vehicle and bicuculline to compare have a significant difference.

Table 3. Ventilatory parameters in non-DM and DM rats treated with vehicle or bicuculline on hypoxia 30 min

	Non-DM rats		DM rats	
Hypoxia 30 min	Vehicle	Bicucuclline	Vehicle	Bicuculline
f, breaths/min	140.75±3.12	154.13±3.10	126.59±2.87	116.62±1.65
VT, ml	3.00±0.03	2.71±0.04	2.91±0.04	3.21±0.04
VE, ml/min	402.17±0.68	397.64±3.19	357.78±5.37	363.10±1.91
Ti, sec	0.19±0.00	0.17±0.00	0.21±0.00	0.21±0.00
Te, sec	0.27±0.01	0.25±0.01	0.30±0.01	0.33±0.01
PIF, ml/sec	22.51±0.34	22.00±0.12	19.43±0.06	20.80±0.11
PEF, ml/sec	20.41±0.18	21.36±0.06	16.83±0.14	17.79±0.55
EF50, ml/sec	1.31±0.02	1.26±0.03	1.15±0.04	1.09±0.02
VT /Ti	15.92±0.02	15.89±0.07	13.94±0.00	14.97±0.07

Non-DM rats = non- diabetes mellitus rats, DM rats = diabetes mellitus rats, f = Frequency, TV =Tidal Volume, VE=Minute Volume, Ti = Inspiratory Time, Te = Expiratory Time, PIF = Peak Inspiratory Flow, PEF = Peak Expiratory Flow, EF50 = The flow at the point 50% of TV is expired, VT /Ti = mean inspiratory flow. Values are means ± SD (n=8). *P<0.05 DM with Non DM vehicle to compare have a significant difference. ＃P<0.05Non DM vehicle and bicuculline to compare have a significant difference. ＋P<0.05 DM vehicle and bicuculline to compare have a significant difference.

Table 4. Ventilatory parameters in non-DM and DM rats treated with vehicle or bicuculline on hypercapnic resting time.

Hypercapnic	Non-DM rats		DM rats	
Resting	Vehicle	Bicucuclline	Vehicle	Bicuculline
f, breaths/min	83.36±5.40	89.24±1.45	66.05±2.31	71.28±11.09
VT, ml	2.93±0.20	2.41±0.04	2.84±0.01	3.10±0.02
VE, ml/min	251.00±46.50	205.74±1.92	183.65±5.38	225.07±42.63
Ti, sec	0.28±0.00	0.24±0.00	0.32±0.01	0.33±0.01
Te, sec	0.52±0.04	0.56±0.00	0.62±0.02	0.61±0.06
PIF, ml/sec	16.74±1.75	16.29±0.05	13.58±0.29	15.21±1.44
PEF, ml/sec	14.38±1.59	12.11±0.18	11.52±0.03	13.08±2.10
EF50, ml/sec	0.77±0.15	0.54±0.02	0.51±0.03	0.69±0.24
VT /Ti	10.41±0.77	9.99±0.04	9.99±0.04	9.45±0.42

Non-DM rats = non- diabetes mellitus rats, DM rats = diabetes mellitus rats, f = Frequency, TV =Tidal Volume, VE=Minute Volume, Ti = Inspiratory Time, Te = Expiratory Time, PIF = Peak Inspiratory Flow, PEF = Peak Expiratory Flow, EF50 = The flow at the point 50% of TV is expired, VT /Ti = mean inspiratory flow. Values are means ± SD (n=8). *P<0.05 DM with Non DM vehicle to compare have a significant difference. ＃P<0.05Non DM vehicle and bicuculline to compare have a significant difference. ＋P<0.05 DM vehicle and bicuculline to compare have a significant difference.

Table 5. Ventilatory parameters in non-DM and DM rats treated with vehicle or bicuculline on hypercapnic 15 min.

	Non-DM rats		DM rats	
Hypercapnic 15 min	Vehicle	Bicucuclline	Vehicle	Bicuculline
f, breaths/min	132.74±2.58	146.95±3.00	120.69±0.40	127.42±0.48
VT, ml	5.06±0.05	4.44±0.10	4.63±0.08	4.90±0.05
VE, ml/min	660.84±14.53	637.29±1.16	551.48±8.19	625.97±4.87
Ti, sec	0.22±0.00	0.20±0.00	0.24±0.00	0.23±0.00
Te, sec	0.25±0.01	0.22±0.00	0.27±0.00	0.25±0.00
PIF, ml/sec	30.82±0.76	29.82±0.62	25.69±0.45	28.87±0.36
PEF, ml/sec	42.73±1.10	41.65±0.99	35.78±0.22	39.75±0.06
EF50, ml/sec	2.49±0.13	2.52±0.03	2.25±0.01	2.55±0.03
VT /Ti	22.65±0.59	21.69±0.05	21.69±0.05	20.90±0.09

Non-DM rats = non- diabetes mellitus rats, DM rats = diabetes mellitus rats, f = Frequency, TV =Tidal Volume, VE=Minute Volume, Ti = Inspiratory Time, Te = Expiratory Time, PIF = Peak Inspiratory Flow, PEF = Peak Expiratory Flow, EF50 = The flow at the point 50% of TV is expired, VT /Ti = mean inspiratory flow. Values are means ± SD (n=8). *P<0.05 DM with Non DM vehicle to compare have a significant difference. ＃P<0.05Non DM vechicle and bicuculline to compare have a significant difference. ＋P<0.05 DM vechicle and bicuculline to compare have a significant difference.

Fig 1. DM and Non DM rats for hypoxia

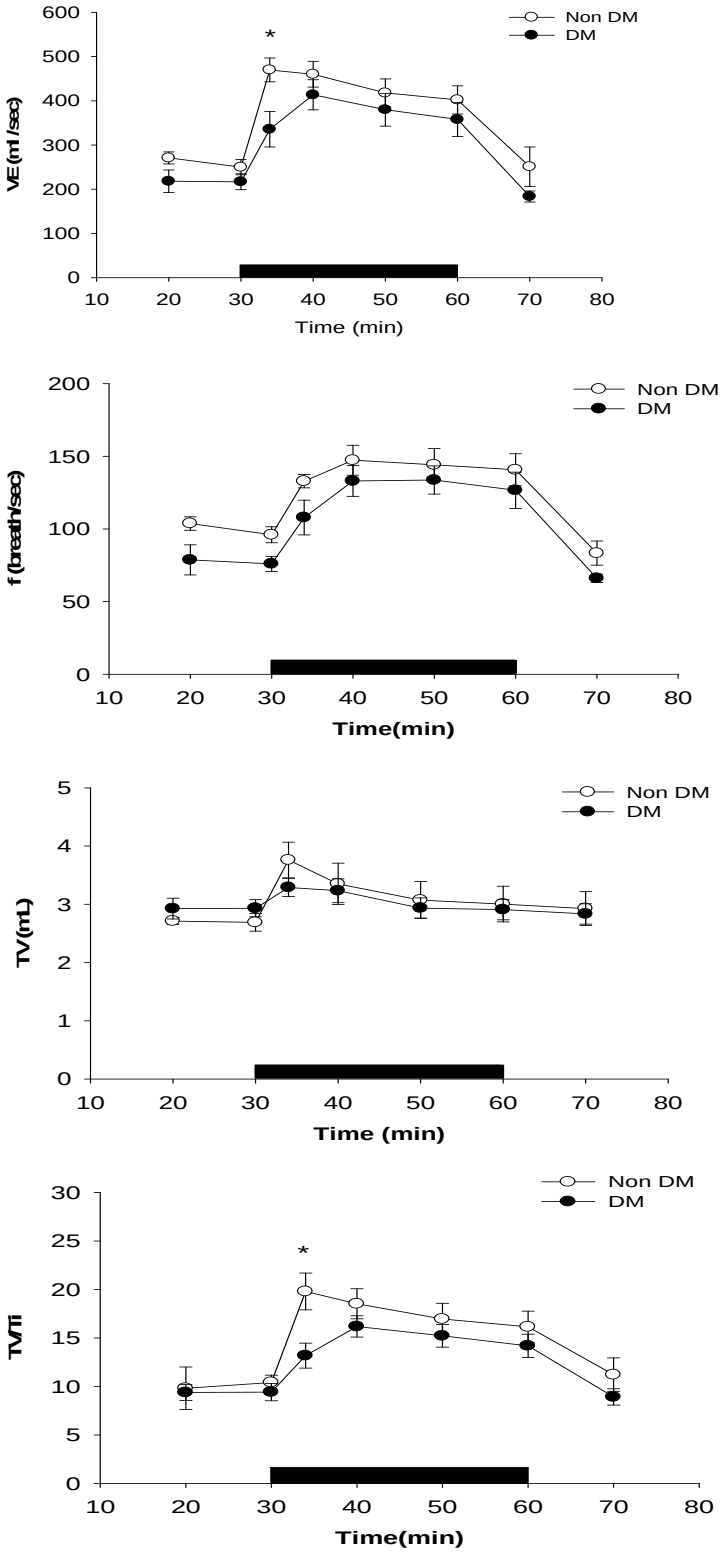

Fig. 1. The ventilation(VE), tidal volume(TV), breathing frequency(f) and TV/Ti of non-DM (vacant circular) and DM (full circular) rats during room air and during 10% O_2 hypoxic exposure(black bar). *p $<$0.05 indicates a significant difference from the value of vehicle at the same time-point. Values represent mean ± SDE.

Fig 2 Bicuculline and DMSO for hypoxia

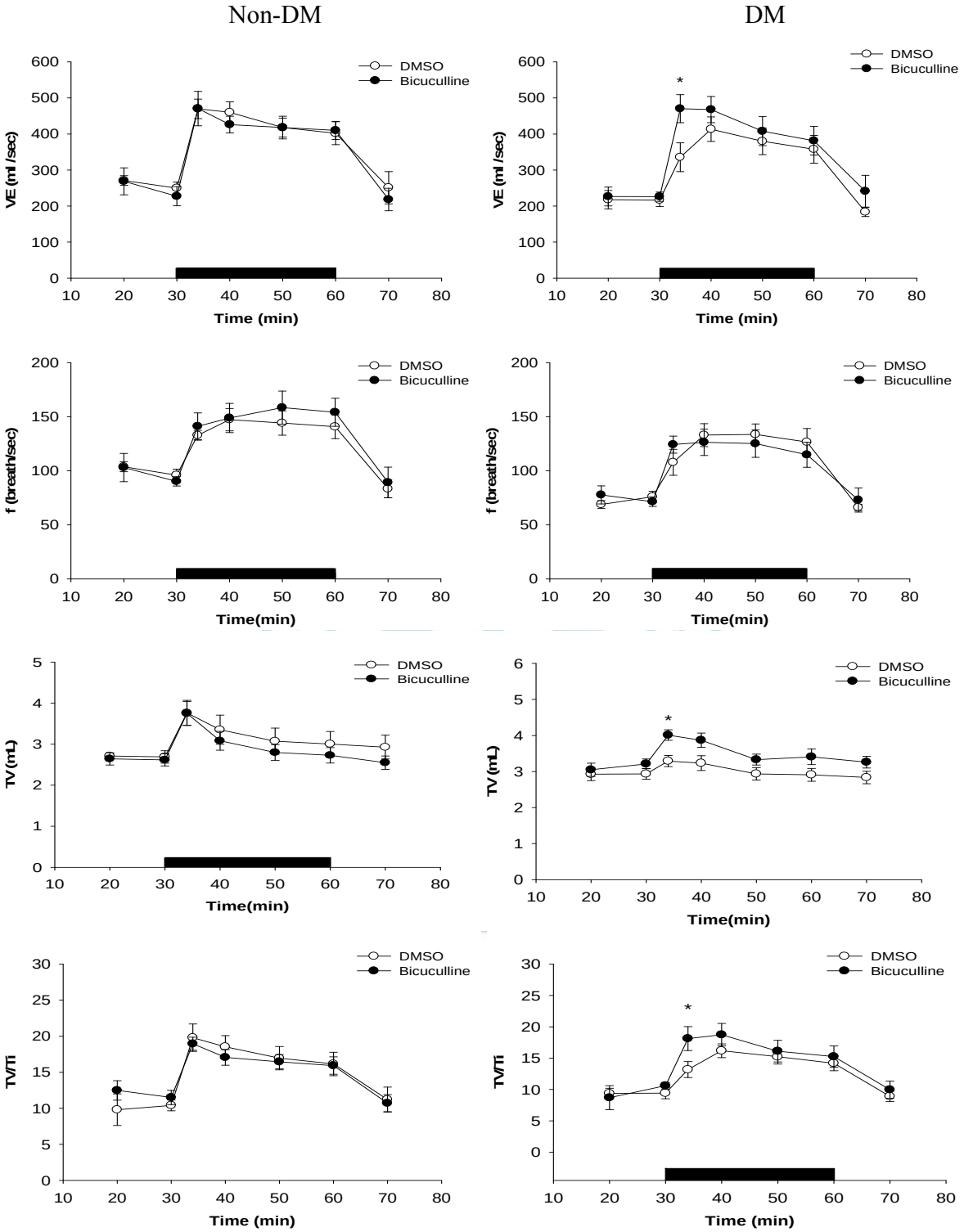

Fig. 2. The effects of vehicle (vacant circular) and bicuculline (full circular)administration on ventilation(VE), tidal volume(TV), breathing frequency(f) and TV/Ti of non-DM and DM rats during room air and during 10% O_2 hypoxic exposure(black bar). *$p < 0.05$ indicates a significant difference from the value of vehicle, bicuculline, at the same time-point. Values represent mean ± SDE.

Fig.3 DM and Non DM rats for hypercapnic

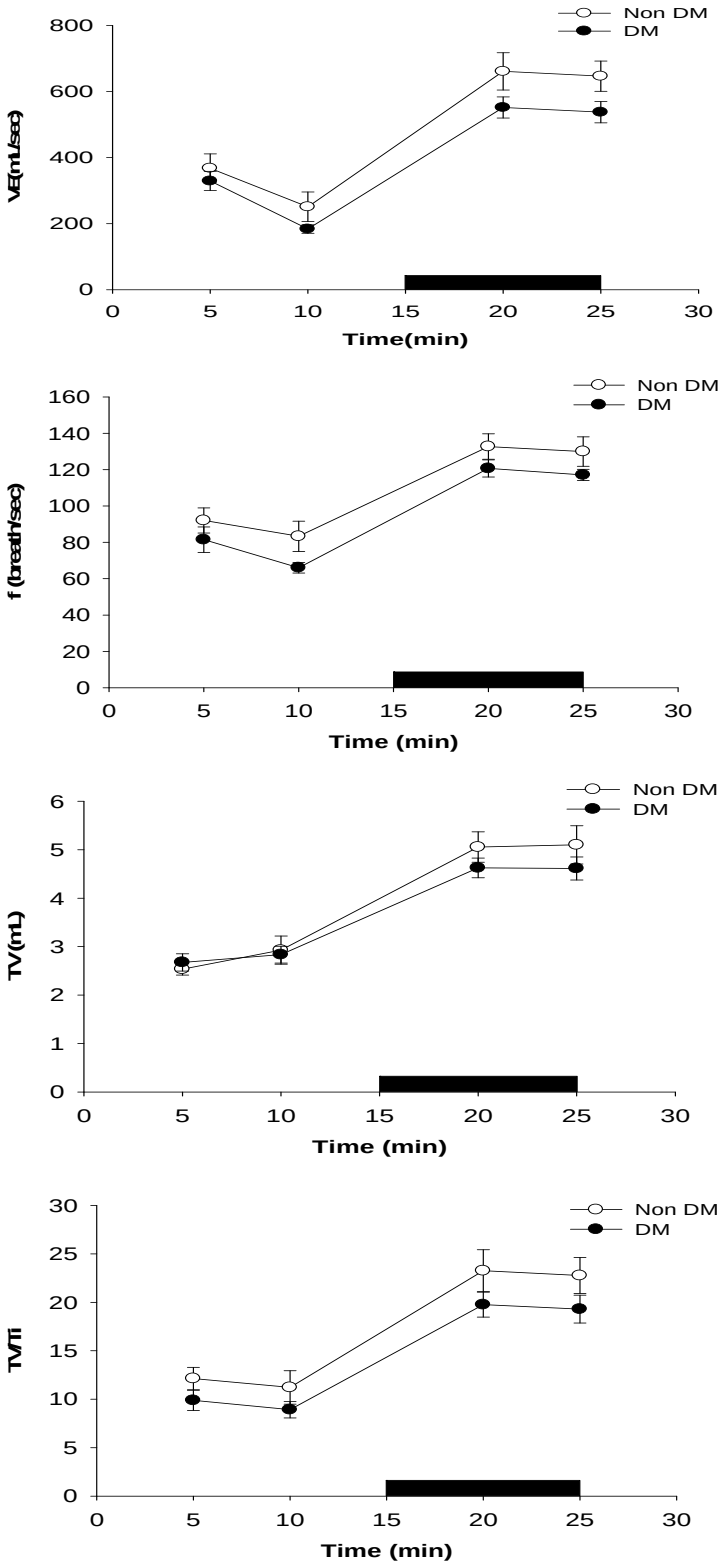

Fig. 3. The ventilation(VE), tidal volume(TV), breathing frequency(f) and TV/Ti of non-DM (vacant circular) and DM (full circular) rats during room air and during 8% CO_2 hypercapnic exposure(black bar). *p $<$0.05 indicates a significant difference from the value of vehicle, at the same time-point. Values represent mean ± SDE.

Fig 4. Bicuculline and DMSO for hypercapnic

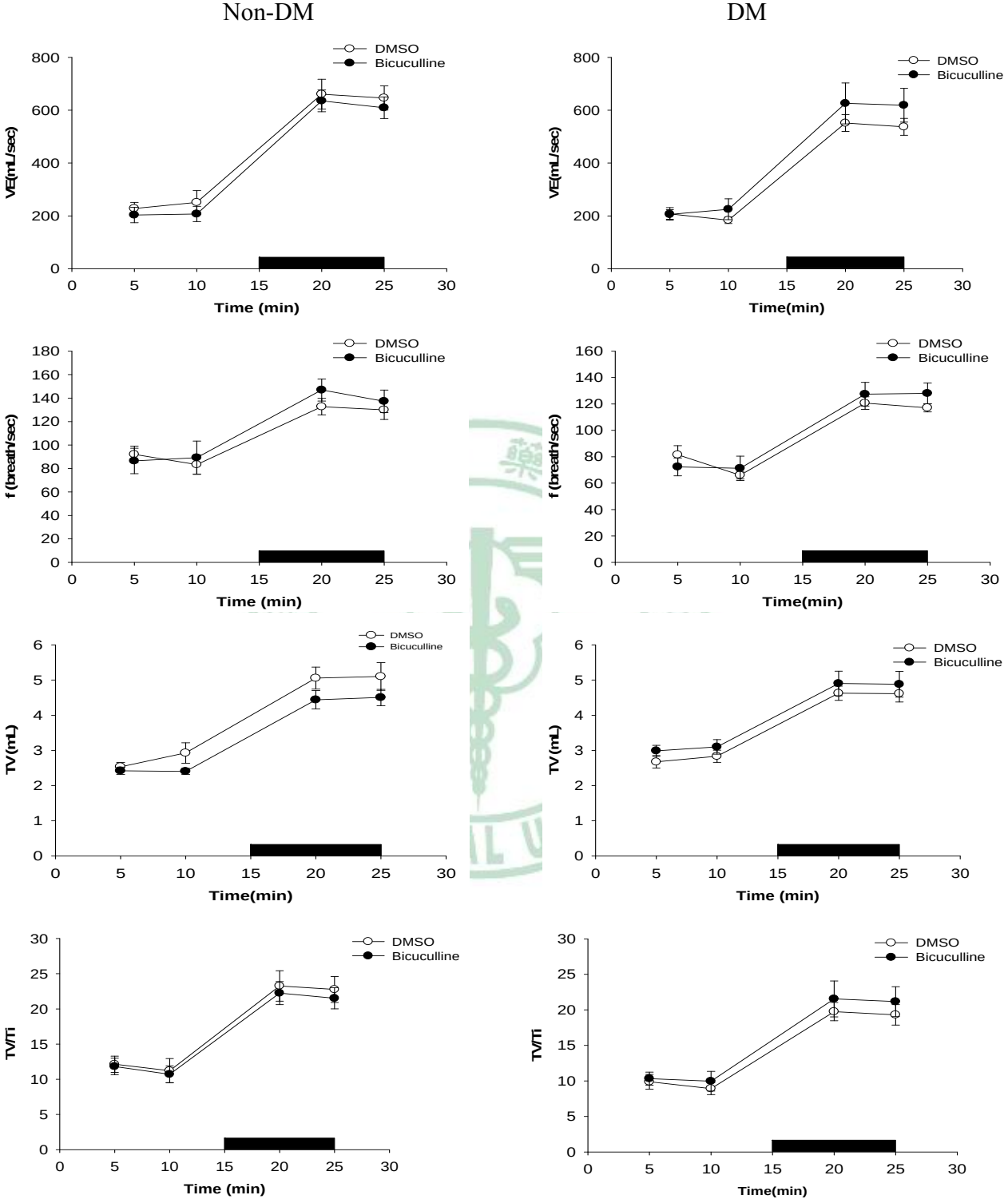

Fig. 4. The effects of vehicle (vacant circular) and bicuculline (full circular)administration on ventilation(VE), tidal volume(TV), breathing frequency(f) and TV/Ti of non-DM and DM rats during room air and during 8% CO_2 hypercapnic exposure(black bar). *$p < 0.05$ indicates a significant difference from the value of vehicle, bicuculline, at the same time-point. Values represent mean ± SDE.

Table 6. Ventilatory parameters in non-DM and DM rats treated with vehicle or dextromethorphan on hypoxia resting time.

	Non-DM rats		DM rats	
Body Weight, g	468.07±42.00		339.92±57.78	
BG mg/dl	97.29±14.82		421.71±106.51	
Resting	Vehicle	Dextromethorphan	Vehicle	Dextromethorphan
f, breaths/min	81.29±1.50	92.09±1.80	82.80±9.37	90.60 ±8.98
VT, ml	2.81±0.04	2.64±0.08	2.63±0.03	2.71 ±0.02
VE, ml/min	224.01±7.11	245.17±10.01	207.90±22.99	230.51 ±17.64
Ti, sec	0.33±0.00	0.28±0.01	0.33±0.01	0.31±0.01
Te, sec	0.49±0.01	0.47±0.01	0.47±0.03	0.43±0.02
PIF, ml/sec	14.75±0.37	17.12±0.86	13.05±0.56	14.06±1.05
PEF, ml/sec	13.02± 0.50	13.27±0.43	11.46±1.46	12.49± 1.65
EF50, ml/sec	0.70±0.03	0.76±0.04	0.67±0.15	0.73±0.13
VT /Ti	8.62± 0.07	9.55±0.52	7.94±0.24	8.81±0.23

Non-DM rats = non- diabetes mellitus rats, DM rats = diabetes mellitus rats, BG = blood glucose, f = Frequency, TV =Tidal Volume, VE=Minute Volume, Ti = Inspiratory Time, Te = Expiratory Time, PIF = Peak Inspiratory Flow, PEF = Peak Expiratory Flow, EF50 = The flow at the point 50% of TV is expired, VT /Ti = mean inspiratory flow. Values are means ± SD (n=14). *P<0.05 DM with Non DM vehicle to compare have a significant difference. ＃P<0.05Non DM Saline and dextromethorphan to compare have a significant difference. ＋P<0.05 DM Saline and dextromethorphan to compare have a significant difference

Table 7. Ventilatory parameters in non-DM and DM rats treated with vehicle or dextromethorphan on hypoxia 4 min.

	Non-DM rats		DM rats	
Hypoxia 4 min	Vehicle	Dextromethorphan	Vehicle	Dextromethorphan
f, breaths/min	118.74±3.34	136.60 ±1.24	114.27±0.30	120.46±5.31
VT, ml	4.43±0.96	4.23 ±1.02	3.84±0.87	3.90±0.71
VE, ml/min	501.02±116.20	562.32± 104.51	409.32±90.71*	441.30±101.93
Ti, sec	0.23 ±0.00	0.20±0.01	0.25±0.00	0.24± 0.02
Te, sec	0.37 ±0.05	0.31±0.02	0.35±0.03	0.32 ±0.04
PIF, ml/sec	31.59± 4.67	34.52±5.01	25.07±3.85*	25.42± 4.87
PEF, ml/sec	26.30 ±7.17	29.65±6.88	22.11±6.20	22.15±5.25
EF50, ml/sec	1.67± 0.51	2.00±0.40	1.51±0.49	1.55±0.35
VT /Ti	19.32±4.43	21.13±4.02	15.47±3.54*	16.48±4.23

Non-DM rats = non- diabetes mellitus rats, DM rats = diabetes mellitus rats, f = Frequency, TV =Tidal Volume, VE=Minute Volume, Ti = Inspiratory Time, Te = Expiratory Time, PIF = Peak Inspiratory Flow, PEF = Peak Expiratory Flow, EF50 = The flow at the point 50% of TV is expired, VT /Ti = mean inspiratory flow. Values are means ± SD (n=14). *P<0.05 DM with Non DM vehicle to compare have a significant difference. ＃P<0.05Non DM Saline and dextromethorphan to compare have a significant difference. ＋P<0.05 DM Saline and dextromethorphan to compare have a significant difference.

Table 8. Ventilatory parameters in non-DM and DM rats treated with vehicle or dextromethorphan on hypoxia 30 min.

	Non-DM rats		DM rats	
Hypoxia 30 min	Vehicle	Dextromethorphan	Vehicle	Dextromethorphan
f, breaths/min	138.71± 3.70	123.20 ±1.18	120.92±2.16	129.17±0.27
VT, ml	3.01±0.08	3.30 ±0.09	2.91±0.01	3.15± 0.05
VE, ml/min	399.82 ±5.21	395.20± 5.57	340.50±4.63	379.70± 4.87
Ti, sec	0.19±0.01	0.20 ±0.00	0.22±0.00	0.21± 0.00
Te, sec	0.27±0.01	0.32 ±0.01	0.30±0.01	0.28 ±0.00
PIF, ml/sec	22.42± 0.11	23.38 ±0.43	18.41±0.12	20.25± 0.24
PEF, ml/sec	18.90±0.15	19.12± 0.21	16.08±0.49	16.94 ±0.31
EF50, ml/sec	1.38 ±0.03	1.14± 0.00	1.16±0.04	1.25 ±0.01
VT /Ti	15.79± 0.05	16.81± 0.45	13.01±0.00	14.78±0.14

Non-DM rats = non- diabetes mellitus rats, DM rats = diabetes mellitus rats, f = Frequency, TV =Tidal Volume, VE=Minute Volume, Ti = Inspiratory Time, Te = Expiratory Time, PIF = Peak Inspiratory Flow, PEF = Peak Expiratory Flow, EF50 = The flow at the point 50% of TV is expired, VT /Ti = mean inspiratory flow. Values are means ± SD (n=14). *P<0.05 DM with Non DM vehicle to compare have a significant difference. ＃P<0.05 Non DM Saline and dextromethorphan to compare have a significant difference. ＋P<0.05 DM Saline and dextromethorphan to compare have a significant difference.

Table 9. Ventilatory parameters in non-DM and DM rats treated with vehicle or dextromethorphan on hypercapnic resting time.

Hypercapnic	Non-DM rats		DM rats	
Resting	Vehicle	Dextromethorphan	Vehicle	Dextromethorphan
f, breaths/min	66.19±2.64	82.94±0.42	68.49± 2.30	76.70± 2.81
VT, ml	2.79± 0.01	2.72 ±0.03	2.60±0.07	2.75±0.02
VE, ml/min	183.53±8.88	226.94±6.17	174.08± 1.33	201.43±6.28
Ti, sec	0.35 ±0.01	0.28±0.00	0.36± 0.01	0.34 ±0.00
Te, sec	0.59±0.02	0.52± 0.01	0.56 ±0.02	0.49±0.03
PIF, ml/sec	12.80± 0.69	16.85± 0.37	11.33 ±0.13	12.46± 0.18
PEF, ml/sec	11.12±0.58	12.93 ±0.54	9.76± 0.06	11.22± 0.19
EF50, ml/sec	0.53±0.06	0.63± 0.03	0.50±0.00	0.61±0.04
VT /Ti	7.96 0.21	9.61 ±0.07	7.26 ±0.05	8.18±0.11

Non-DM rats = non- diabetes mellitus rats, DM rats = diabetes mellitus rats, f = Frequency, TV =Tidal Volume, VE=Minute Volume, Ti = Inspiratory Time, Te = Expiratory Time, PIF = Peak Inspiratory Flow, PEF = Peak Expiratory Flow, EF50 = The flow at the point 50% of TV is expired, VT /Ti = mean inspiratory flow. Values are means ± SD (n=14). *P<0.05 DM with Non DM vehicle to compare have a significant difference. ＃P<0.05 Non DM Saline and dextromethorphan to compare have a significant difference. ＋P<0.05 DM Saline and dextromethorphan to compare have a significant difference.

Table 10. Ventilatory parameters in non-DM and DM rats treated with vehicle or dextromethorphan on hypercapnic 15 min.

	Non-DM rats		DM rats	
Hypercapnic 15 min	Vehicle	Dextromethorphan	Vehicle	Dextromethorphan
f, breaths/min	137.71± 2.31	144.29± 0.83	121.93± 1.41	127.53±4.20
VT, ml	5.51 ±0.12	5.69± 0.02	5.03± 0.03	5.42±0.07
VE, ml/min	744.96±1.09	812.32±7.38	600.16±3.62	678.18±14.02
Ti, sec	0.24±0.00	0.22± 0.01	0.25±0.00	0.24 ±0.01
Te, sec	0.22± 0.00	0.21 ±0.00	0.26± 0.01	0.24±0.01
PIF, ml/sec	32.52± 0.21	36.24± 0.33	26.56± 0.25	29.89±0.36^{+}
PEF, ml/sec	51.80 ±0.28	50.84± 0.01	36.25±0.54	41.46± 0.83^{+}
EF50, ml/sec	3.55± 0.02	3.59± 0.11	2.48± 0.08	2.80±0.14
VT /Ti	23.43±0.03	26.28±0.56	19.92± 0.06	22.80±0.19^{+}

Non-DM rats = non- diabetes mellitus rats, DM rats = diabetes mellitus rats, f = Frequency, TV =Tidal Volume, VE=Minute Volume, Ti = Inspiratory Time, Te = Expiratory Time, PIF = Peak Inspiratory Flow, PEF = Peak Expiratory Flow, EF50 = The flow at the point 50% of TV is expired, VT /Ti = mean inspiratory flow. Values are means ± SD (n=14). *P<0.05 DM with Non DM vehicle to compare have a significant difference. ＃P<0.05 Non DM Saline and dextromethorphan to compare have a significant difference. ＋P<0.05 DM Saline and dextromethorphan to compare have a significant differenc

Fig 5. DM and Non DM rats for hypoxia

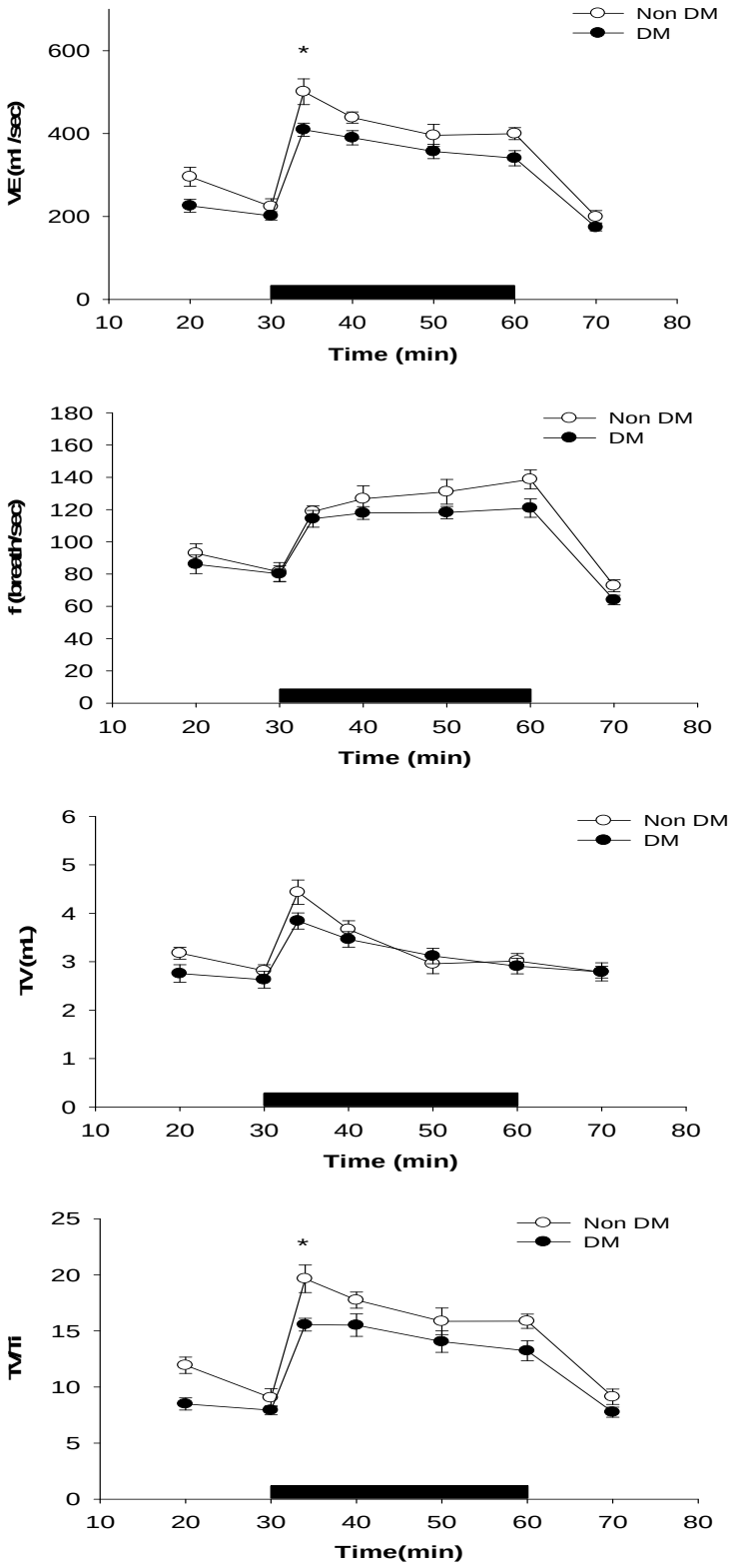

Fig. 5. The ventilation(VE), tidal volume(TV), breathing frequency(f) and TV/Ti of non-DM (vacant circular) and DM (full circular) rats during room air and during 10% O_2 hypoxic exposure (black bar). *p $<$ 0.05 indicates a significant difference from the value of vehicle at the same time-point. Values represent mean ± SDE.

Fig 6. DxM and saline for hypoxia

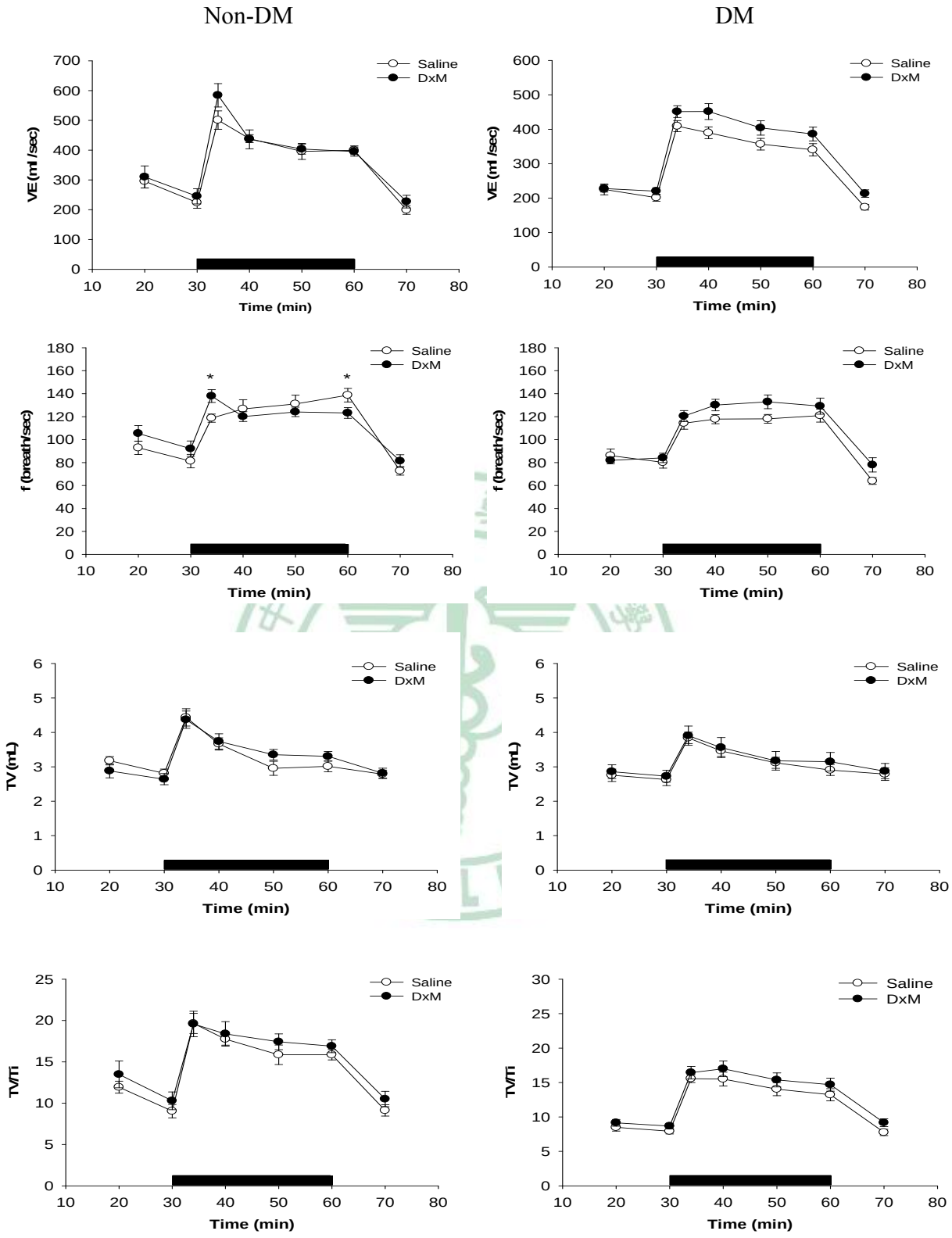

Fig. 6. The effects of vehicle (vacant circular) and dextromethorphan (full circular)administration on ventilation(VE), tidal volume(TV), breathing frequency(f) and TV/Ti of non-DM and DM rats during room air and during 10% O2 hypoxic exposure (black bar). *$p < 0.05$ indicates a significant difference from the value of vehicle at the same time-point. Values represent mean ± SD

Fig. 7 DM and Non DM rats for hypercapnic

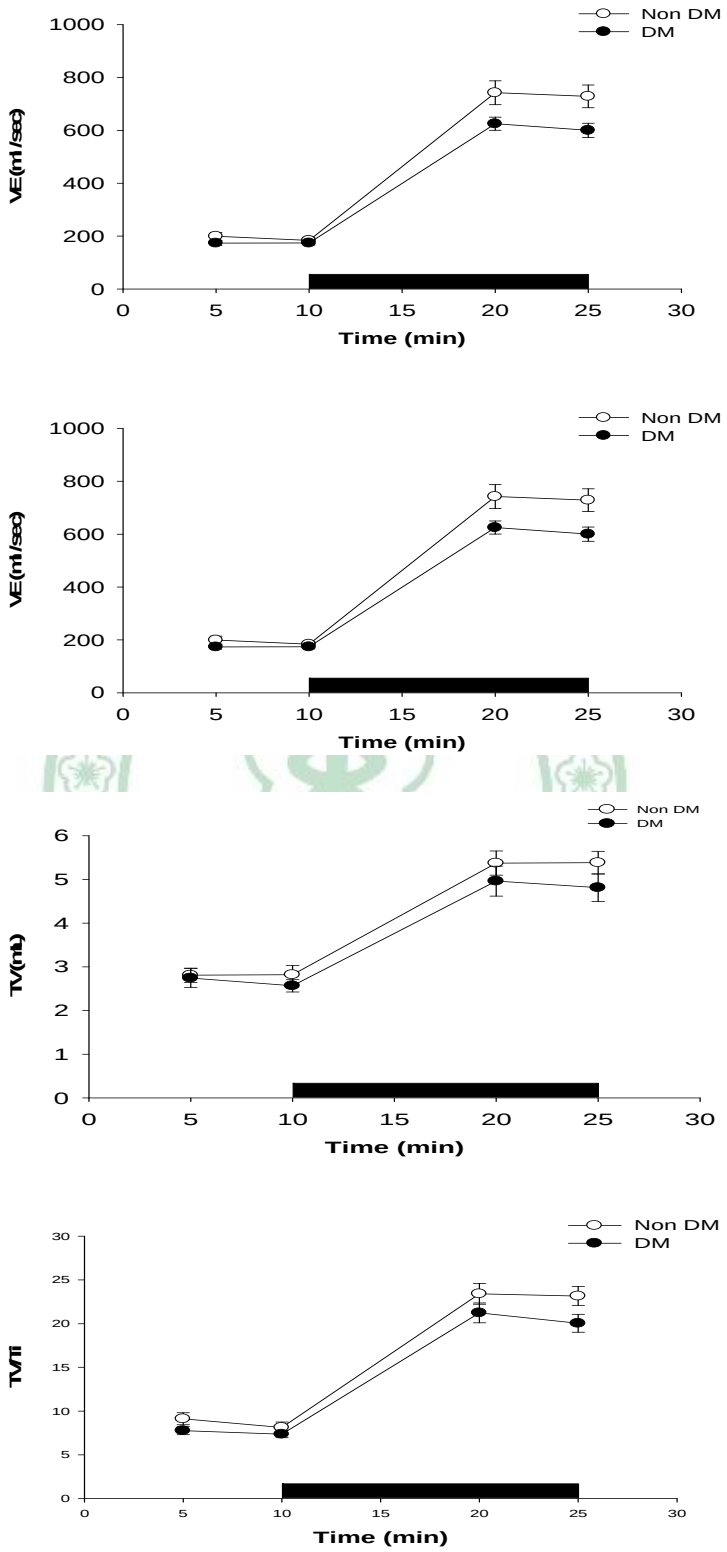

Fig. 7. The ventilation(VE), tidal volume(TV), breathing frequency(f) and TV/Ti of non-DM (vacant circular) and DM (full circular) rats during room air and during 10% O2 hypoxic exposure (black bar). *p ＜0.05 indicates a significant difference from the value of vehicle, saline, at the same time-point. Values represent mean ± SD.

Fig **8**. Dextromethorphan and saline for hypercapnic

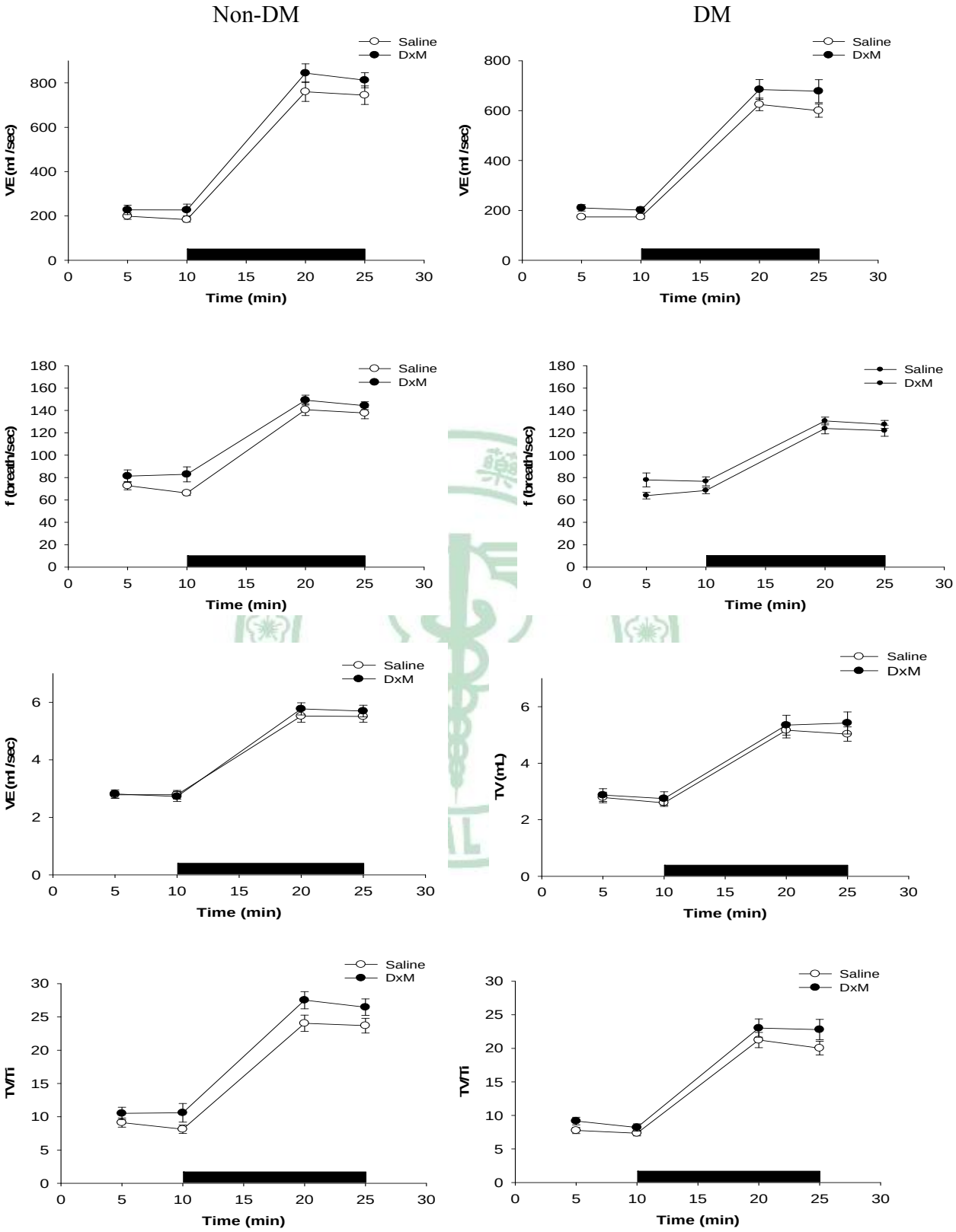

Fig. 8. The effects of vehicle (vacant circular) and dextromethorphan (full circular)administration on ventilation(VE), tidal volume(TV), breathing frequency(f) and TV/Ti of non-DM and DM rats during room air and during 8% CO_2 hypercapnic exposure (black bar). *$p < 0.05$ indicates a significant difference from the value of vehicle, saline, at the same time-point. Values represent mean ± SD.

Chapter 6

Reference

Reference

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