呼吸調節機構における摂食・睡眠関連ペプチドの役割に関する研究

課 題 番 号　１４５７０５３３

平成１４年度～平成１６年度　科学研究費補助金（基盤研究C(2)）

研究成果報告書

平成１７年３月

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はじめに

この研究成果報告書は、平成14～16年度の３年間にわたる文部科学省科学研究費補助金　基盤研究C(2)「呼吸調節機構における摂食・睡眠関連ペプチドの役割に関する研究」（研究課題番号１４５７０５３３）の成果をまとめたものである。

本研究は私が平成10年秋～12年夏にかけてアメリカ留学中に経験したZucker肥満ラットとその実験手技をさらに発展させて計画したものである。実験当初は鼠の脳脊髄腔内にどのようにして薬剤を注入するかに関して試行錯誤の毎日であったが、佐々木君（共同研究者）の絶え間ない努力の結果、脳脊髄腔内にカニューレを持続的に留置することに成功した。その結果、全身性の影響を考慮することなく、レプチンやオレキシンの呼吸調節における役割を検討することが可能になった。

大学病院であるがゆえに、高度先進医療を支える信頼された臨床を行い充実した教育・指導を行いつつ、独自の研究を遂行することは容易なことではない。しかし、この３年間で当初の目的を成し遂げることができたのは、ひとえに次ページに挙げた呼吸器グループのスタッフ諸氏のおかげであり、あらためて謝意を表したい。また、この研究の過程で多くの国内・国外の研究者と出会い議論することができたことは、私にとって大きな財産となった。最後に、本研究の財政的支援をして頂いた文部科学省に心からお礼を述べさせて頂きたい。

平成１７年４月

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本研究における研究経費

平成 14 年度	1,400 千円
平成 15 年度	1,000 千円
平成 16 年度	900 千円
計	3,300 千円

過去の研究実績

科学研究費補助金　基盤研究 C－(2)、(平成 14～16 年度)

課題：呼吸調節機構における摂食・睡眠関連ペプチドの役割に関する研究

(平成１４年度１４０万円、１５年度１００万円、１６年度９０万円)

科学研究費補助金　基盤研究 C－(2)、(平成 10～11 年度)

課題：気道上皮内 NO 合成酵素(NOS)の O_2 センサーとしての作用に関する研究

(平成１０年度１６０万円、１１年度１２０万円)

科学研究費補助金　基盤研究 C－(2)、(平成 8～9 年度)

課題：酸素性肺血管収縮と換気・血流再分配における内因性ＮＯの役割

(平成８年度９０万円、９年度１２０万円)

科学研究費補助金　奨励研究Ａ　　(平成７年度)

課題：低酸素性肺血管収縮における一酸化窒素とその関連代謝物系の作用

(平成７年度１００万円)

研究成果(邦文)

平成１４年度

【目的】レプチンは体組織の脂肪細胞から分泌される循環ホルモンであり、肥満肺胞低換気や睡眠時無呼吸症候群などの呼吸障害の病態に深く関わっている可能性が指摘されている。しかし、レプチンに呼吸刺激作用があるか否かについては明らかではない。そこで、安静呼吸、代謝、呼吸の化学感受性に与えるレプチンの影響について検討した。

【方法】16 週齢の Zucker ラットの肥満群（平均体重 765ｇ）と痩せ群（平均体重 420ｇ）８匹ずつを用いた。自発呼吸下に麻酔深度を維持するために腹腔内にペントバルビタールを持続投与した。また、定位脳固定装置を用いて側脳室内にポリエチレン製カテーテルを留置し、レプチンや人工脳脊髄液(CSF)注入用のマイクロシリンダーを接続した。ラットを容積 3.5ℓのアクリル製チャンバーに挿入し、ﾎﾞﾃﾞｲﾌﾟﾚﾁｽﾓｸﾞﾗﾌ法で換気諸量を、吸入気と排出気の O_2 および CO_2 濃度差から酸素消費量と炭酸ガス産生量を求めた。側脳室内に留置したカテーテルより CSF を 10μℓ注入し，呼吸が安定していることを確認し，9%CO_2 ガスを２分間吸入しコントロールの高炭酸ガス換気応答を観察した。その後呼吸が再度安定したところで，レプチン 10μｇを投与し，　90 分間安静換気を観察した後に、高炭酸ガス換気応答を施行した。

【結果】正常痩せラットにおいてはレプチン投与後に分時換気量は時間経過とともに徐々に増加し、60 分以降で有意に増加した。一回換気量は 30 分以降で、呼吸数は 90 分で有意に増加した。一方，肥満ラットにおいてはレプチン投与においても分時換気量，一回換気量，呼吸数に有意な変化を認めなかった。また、正常群においてはレプチン投与後に徐々に VO_2 、VCO_2 が上昇した。換気酸素当量は時間経過ともに上昇傾向を認めるものの有意な変化を認めなかった。肥満群においてはレプチン投与後も VO_2 、VCO_2、換気酸素当量ともに変化を認めなかった。高炭酸ガス換気応答はレプチン投与により両群で有意な変化は認めなかった。

【考案】正常の状態ではレプチンは安静換気刺激作用を有すると考えられる。しかし、レプチンによる安静換気の増大は、直接の呼吸刺激作用というよりも、むしろ交感神経や代謝の亢進に基づく二次的な作用である可能性が高い。一方、レプチン受容体に異常がある場合、外因性のレプチンは呼吸や代謝に影響を与えないことが示唆された。

平成１５年度

【目的】オレキシンは摂食行動や覚醒・睡眠を制御する神経ペプチドであり，睡眠呼吸障害との関連について近年注目されている。しかし、オレキシンに呼吸刺激作用があるか否かについては明らかではない。そこで、安静呼吸、代謝、呼吸の化学感受性に与えるオレキシンの影響について遺伝的肥満を示すZuckerラットを用いて検討した。

【方法】16週齢のZuckerラットの肥満群（平均体重765ｇ）と正常発育群（平均体重420ｇ）１０匹ずつを用いた。自発呼吸下に麻酔深度を維持するために腹腔内にウレタンを投与した。また、定位脳固定装置を用いて側脳室内にポリエチレン製カテーテルを留置し、オレキシンや人工脳脊髄液(CSF)注入用のマイクロシリンダーを接続した。ラットを容積3.5ℓのアクリル製チャンバーに挿入し、ﾎﾞﾃﾞｲﾌﾟﾚﾁｽﾓｸﾞﾗﾌ法で換気諸量を、吸入気と排出気のO_2およびCO_2濃度差から酸素消費量と炭酸ガス産生量を求めた。側脳室内に留置したカテーテルよりCSFを10μℓ注入し，呼吸が安定していることを確認し，9%CO_2ガスを２分間吸入しコントロールの高炭酸ガス換気応答（HCVR）を観察した。その後呼吸が再度安定したところで，オレキシンA10μｇを投与し，90分間安静換気を観察した後に、高炭酸ガス換気応答を施行した。

【結果】正常発育群では，オレキシン投与後で，体温，呼吸数，分時換気量，換気・酸素当量がコントロールに比べ有意に増大したが，VO_2，VCO_2は大きく変化しなかった。HCVRはオレキシン投与後で増大傾向を認めた。一方、肥満群でも分時換気量，換気・酸素当量がコントロールに比べ有意に増大し、VO_2，VCO_2は大きく変化しなかった。

【考察】オレキシンは安静時の呼吸を刺激する作用を有し，呼吸の中枢化学感受性にも影響を与える可能性が示唆された。また、この作用はレプチン受容体を介することなく発揮されることが示唆された。

平成１６年度

【目的】脂肪細胞から分泌されるレプチンは食欲を抑制する。一方、視床下部で産生されるオレキシンは食欲を亢進させる。睡眠・覚醒と深い関わりを持つこれら２つのペプチドは共に心・循環系や交感神経系の調節に重要な役割を担っていることが最近明らかになったが、呼吸調節系や呼吸の化学感受性に与える影響については不明である。本研究ではこれら２つのペプチドの安静換気や高炭酸ガス換気応答に与える影響について検討した。

【方法】Zucker ラットの肥満群（平均体重 770 g）と正常発育群（平均体重 450 g）用い、あらかじめ側脳室内に留置したポリエチレン・カテーテルを介して、レプチン、オレキシンまたは人工脳脊髄液(CSF)を注入する。ﾎﾞﾃﾞｨﾌﾟﾚﾁｽﾓｸﾞﾗﾌ法で換気諸量を、吸入気と排出気のO_2濃度差から酸素消費量を求めた。高炭酸ガス換気応答（HCVR）は 9%CO_2ガスを２分間吸入して測定した。

【結果】正常発育群において、レプチンは安静分時換気量と酸素消費量を増加させたが、HCVR には影響を与えなかった。一方、オレキシンの投与は安静分時換気量，酸素消費量を増加させ、かつ、HCVR を増大させた。肥満群においてもレプチン・オレキシンは同様の反応を呈したが、正常発育群に比べるとその反応性は小さかった。

【考察】レプチンは代謝を活性化することにより換気を増大させるが、オレキシンは直接的に呼吸を刺激する作用を有し，呼吸の中枢化学感受性にも影響を与える可能性が示唆された。また、これらの作用はレプチン受容体を介することなく発揮されることが明らかとなった。食欲・肥満と深い関連を持つこれらペプチドの呼吸との密接な関係が明らかとなり、肥満・肺胞低換気症候群や睡眠時無呼吸症候群の病態のさらなる解明が期待される。

研究成果(英文)

EFFECTS OF LEPTIN AND OREXIN ON VENTILATION AND METABOLISM IN ZUCKER RATS

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Supported by grants from: 14570533 Grant-in-Aid for Scientific Research (C), Japan Society for the Promotion of Science

Short running head: Leptin and orexin on ventilation

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Subject Category:

Word count for the body of the manuscript:

ABSTRACT

Leptin, synthesized in adipose tissue, suppresses food intake and increases sympathetic activity. On the other hand, orexin, produced mainly in lateral hypothalamus, stimulates food intake and may maintain arousal state. Although besides appetites both leptin and orexin are associated with the regulation of cardiovascular and autonomic function, roles of these peptides on control of breathing and metabolism remain unclear. Thus, we investigated the central action of leptin and orexin on resting ventilation, hypercapnic ventilatory response (HCVR) and metabolism in Zucker rats. The effects of leptin and orexin were tested in obese Zucker rat as a model of genetic obesity and its lean counterpart as a model of normal growth. A micro-polyethylene catheter was inserted into the lateral cerebroventricle several days before experimental date. Animals were anesthetized with intraperitoneal injection of urethane allowing to breath spontaneously. Ventilation ($\dot{V}_E$) was measured by the barometric technique of plethysmography and $\dot{V}O_2$ was calculated from inflow-outflow O_2 difference. After resting ventilation for 60 min, 9%CO_2 gas was challenged for 2 min (HCVR). Then after, either 10μg of orexin-A or 10μg of leptin was injected i.c.v. followed by the same protocol as above described was performed. The administration of leptin significantly increased V_T

and $\dot{V}_E$ in lean rats, but not in obese rats. $\dot{V}_E/\dot{V}O_2$ and HCVR were unaffected by leptin in both groups. In lean rats, the administration of orexin significantly increased $\dot{V}_E$, $\dot{V}O_2$, resulting significant elevation in $\dot{V}_E/\dot{V}O_2$. Furthermore, orexin significantly augmented HCVR. We, therefore, conclude that leptin increases metabolic rate leading to a rise in ventilation under condition with a normal leptin receptor. On the other hand, orexin may stimulate resting ventilation and modulate central chemosensitivity directly via respiratory center.

Number of words:

Key words: leptin, orexin, control of breathing, ventilation, metabolism

INTRODUCTION

Obesity often leads to serious health problems, including hypertension, hyperlipidemia, diabetes, and respiratory disturbances during sleep. It is now recognized that obesity and hyperphagia (overeating) is associated with elevated leptin[1], synthesized in adipose tissue, which suppresses food intake by acting on the satiety center in the ventromedial hypothalamus (VMH) [2]. In contrast, orexin, produced mainly in the lateral hypothalamus (LH), stimulates food intake with maintaining arousal response [3]. Although the actions of leptin and orexin on the appetites are controversial, receptors of these peptides are found together in feeding-regulating neurons in the haypothalamic arcuate nucleus [4]. Furthermore, these peptides have been demonstrated to increase arterial blood pressure, heart rate, and sympathetic nerve activity[5]. In addition to the effects on cardiovascular and autonomic function, leptin replacement in obese mutant mice, which lack circulating leptin, increased resting minute ventilation, suggesting that leptin can prevent respiratory depression in obesity[6]. On the other hand, orexin may contribute to the pathophysiology in patients with obstructive sleep apnea syndrome[7][8]. However, precise roles of these peptides on control of breathing remain unclear.

The obese Zucker (Z) rat, a genetic model of obesity with a receptor mutation in leptin access pathway into the brain, presents many of the same abnormalities as observed in morbidly obese humans, including diabetes, metabolic impairments, and a blunted respiratory drive. The underlying mechanisms responsible for the depressed ventilatory drive in the obese Z rat have been unknown.

The purpose of the present study was to determine the role of leptin and orexin in control of breathing and metabolism in obese Z rats. We hypothesized that the blunted respiratory drive previously observed in obese Z rats are secondary to altered leptin and orexin levels in the brain. To test our hypothesis, we investigated central actions of leptin and orexin on resting ventilation, metabolism, and ventilatory response to hypercapnia in obese Z rats. A parallel study design was used with age-matched lean Z rats serving as non-obese controls.

METHODS

Chemicals

Orexin A (human) was from Peptide Institute, INC. Leptin (murine) was from Sigma-Aldrich.

Animals

The studies were performed on all male Z rats (lean phenotype [Fa/?] and obese phenotype [fa/fa] n =41, 478.0 ± 25.2g and n=11, 775.0 ± 19.5g, respectively). Ambient temperature was maintained at 24 °C with a 12-hour light/dark cycle. All animals were provided with standard laboratory chow and water ad libitum. Before 3 to 7 days of experiment, all animals were anesthetized with i.p. pentobarbital (50mg kg ·1bw) and a 0.8mm of outer diameter polyethylene cannula was positioned stereotaxically above a lateral cerebral ventricle at the following co-ordinates: 1.7 mm lateral to the midline, 0.4 mm posterior to the bregma, 3.0 mm from the cranial theca. The cannula was secured to the skull by super glue. All experimental protocols were approved by the institutional animal care and use committee of the Asahikawa Medical College.

Intracerebroventricular injection. Artificial cerebrospinal fluid (ACSF) had the following composition (in mM): 125.1 NaCl 2.6 KCl, 0.9 $MgCl_2$, 1.3 CaCl2, 21.0 $NaHCO_3$, 2.5 Na_2HPO_4, and 120 mg/ml BSA. Leptin and orexin-A were dissolved in 10 µl ACSF and injected into the lateralcerebroventricle over 1 minute. Control experiments were performed with intracerebroventricular injection of 10 µl ACSF in 4 rats.

Techniques and measurements

Ventilation was measured by using the barometric technique of prethysmography. A cylindrical Plexiglas chamber with a volume of 3.7 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 chamber had an inlet tube that was connected to pressurized air tanks. Inlet flow was regulated at 1.5 L/min by a flowmeter (Kofloc Co.Tokyo,Japan). The concentrations of inflowing or outflowing O_2 and CO_2 were monitored by an O_2-CO_2 analyzer (IH26, San-Ei CO.Tokyo. Japan). 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 (TR602T, NIHON KODEN CO.JP). The signal was received, amplified (Mac Lab, Ad instruments Co.) and the data was analyzed by the software (Chart ver.3.0, Ad instruments Co.). The Colonic temperature was measured by Teflon shielded sensor inserted about 50mm beyond the anus and connected to thermo recorder TR51A (T&D corporation, Tokyo, Japan). The temperature and the relative humidity were monitored with digital thermo-hygrometer RD6540 (A&D corporation, Tokyo, Japan).

Respiratory frequency per minute (f) was calculated directly from the $\dot{V}_E$ -induced

pressure swings. Tidal volume (V_T) was obtained as a function of the pressure change inside the chamber. Pulmonary $\dot{V}_E$ was calculated ($\dot{V}_E = V_T \times f$) and expressed in either absolute value (ml/min) or corrected value for body weight ($ml \cdot kg^{-1} \cdot min^{-1}$). O_2 consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) were calculated from the inflow-outflow O_2 and CO_2 differences multiplied by the gas flow, neglecting the small error introduced by a respiratory quotient less than unity. All $\dot{V}O_2$ and $\dot{V}CO_2$ values (presented at STPD) are expressed either in raw data (ml/min) or per unit of effective body mass because lean and obese rats of the same size have different body compositions. Effective body mass for lean and obese Z rats was calculated as $1.00 \times M^{0.75}$and $0.86 \times M^{0.75}$, respectively, where M is the body weight (kg) of the animal [9].

Experimental protocol

Study 1.

To clarify physiological roles of leptin and orexin on resting ventilation and metabolism, lean Z rats were tested at first. To investigate effects of leptin and orexin under condition of morbid obesity, obese Z rats were tested secondly. After an intraperitoneal injection of urethane (1.2 g/kg), the rat was put into the barometric chamber. It allowed to breath room air spontaneously. Baseline T(c), f,

V_T, $\dot{V}_E$ and metabolic parameters were measured at 10 min intervals for 30 min. Then, ACSF (10μl), leptin (10μg dissolved in 10μl ACSF), or orexin (10μg dissolved in 10μl ACSF) was injected into lateral ventricle through micro cannula followed by monitoring each parameter at 10 min intervals for 90min. In obese Z rat, the administration of ACSF was omitted.

Study 2.

To investigate the role of leptin and orexin on respiratory chemosensitivity in normal control and morbid obesity, we tested hypercapnic ventilatory response (HCVR) in both lean and obese Z rats. After anesthetizing with intreperitoneal injection of urethane, the rat was put inside the barometric chamber. The rat breathed room air for 30 min followed by exposing hypercapnic gas (9%CO_2, 40%O_2) for 2 min (control HCVR). After enough recovery duration, leptin or orexin was injected into lateral ventricle. The second protocol as the same as above described performed 30 min after injection of each agent (leptin or orexin HCVR).

Statistical Analysis.

In *study 1*, analysis of variance (ANOVA) with repeated measure was used. In *study 2*, differences in HCVR between lean and obese Z rats were analyzed by one-way ANOVA. A P value of <0.05 was considered statistically significant. All

data presented in the text, tables, and figures are expressed as means ± SE.

RESULTS

Effect of leptin in lean rats

As shown in Figure 1 and Table 1, the administration of leptin gradually increased f and V_T significantly (f: from baseline value of 92.7 ± 6.6 /min to 90 min value of 110.2 ± 7.0 /min, $p<0.01$; V_T: from baseline value of 4.1 ± 0.3 to 90 min value of 4.8 ± 0.3 ml, $p<0.05$). Consequently, $\dot{V}_E$ was significantly increased from baseline value of 366.0 ± 19.8 to 90 min value of 518.7 ± 39.1 ml/kg/min ($p<0.01$). Although leptin showed a slight increase in body temperature T(c) and $\dot{V}O_2$, the differences were not significant (Figure 2 and Table 1). The value of $\dot{V}_E/\dot{V}O_2$ 90 min after the administration of leptin was significantly increased from the baseline value (34.5 ± 2.5 vs. 26.8 ± 1.3, $p<0.05$).

Effect of orexin in lean rats

As compared to the effect of leptin, orexin abruptly increased f and V_T at 30 min followed by showing a gradual decrease (Figure 1 and Table 1). As a result, $\dot{V}_E$ was significantly increased to peak value of 603.6 ± 28.9 ml/kg/min 30 min after the administration of orexin from the baseline value of 373.6 ± 31.2 ml/kg/min ($p<0.01$).

As shown in Figure 2, in contrast to the effect of leptin, orexin significantly increased $\dot{V}O_2$ at 30 min (baseline value of 14.0 ± 0.6 ml/min to peak value of 17.5 ± 1.3 ml/min at 30 min, $p<0.01$). On the other hand, T(c) was gradually increased for 90 min by orexin. $\dot{V}E/\dot{V}O_2$ was significantly increased to peak value of 35.8 ± 2.3 at 30 min from baseline value of 27.0 ± 2.4 ($p<0.01$) and the increase remained for 90 min.

Effects of leptin and orexin in obese rats

In obese Z rats, leptin had no effect on resting f, V_T, $\dot{V}E$, and other metabolic parameters (Figure 3 and 4). On the other hand, orexin abruptly increased f significantly at 20 min and this rise remained until 90 min (Figure 3). V_T showed a gradual elevation but the difference was not significant. $\dot{V}E$ was increased significantly at 40 min by the administration of orexin and this rise lasted until 90 min. In contrast to the result of leptin, orexin significantly increased T(c) as compared to baseline value ($p<0.01$) as shown in Figure 4. Furthermore, $\dot{V}O_2$ was significantly increased at 20 min and remained until 40 min ($p<0.01$) followed by gradual decreasing to the baseline level. $\dot{V}E/\dot{V}O_2$ was not altered by the administration of orexin (Figure 4).

Effect of leptin on HCVR in lean and obese rats

In lean rats, $\dot{V}_E$ during room air breathing was increased by CO_2 exposure, but leptin did not affect this $\dot{V}_E$ response to hypercapnia. In obese rats, leptin also did not influence the $\dot{V}_E$ response to hypercapnia (left panel of Figure 5 and Table 3).

Effect of orexin on HCVR in lean and obese rats

In lean rats, orexin significantly augmented the $\dot{V}_E$ response to hypercapnia ($p<0.05$) as shown in right pannle of Figure 5 and Table 4. However, in obese rats, orexin did not alter the $\dot{V}_E$ response to hypercapnia (right pannle of Figure 5 and Table 4).

DISCUSSION

The important results of the present study are that: 1) The administration of leptin significantly increased $\dot{V}_E$ and $\dot{V}O_2$ in lean rats, but not in obese rats. 2) $\dot{V}_E$ /$\dot{V}O_2$ and HCVR were unaffected by leptin in both groups. 3) In lean rats, the administration of orexin significantly increased $\dot{V}_E$ and $\dot{V}O_2$. And $\dot{V}_E$ /$\dot{V}O_2$ significantly increased in lean rats. 4) orexin significantly augmented HCVR.

The present results indicate that leptin increases metabolic rate leading to a rise in ventilation under condition with a normal leptin receptor. On the other hand, orexin may stimulate resting ventilation and modulate central chemosensitivity directly via respiratory center.

Leptin is first reported as a peptide to control appetite and energy consumption[1)10)11)]. Altered energy homeostasis leads to change in O_2 consumption and that follows modulate ventilation. Recently, leptin is thought to have tight relationship to ventilation. O'donnel et.al reported that leptin replacement improved minute ventilation and ventilatory response to hypercapnia in C57BL/6J-*Lep*^{ob} mice[6)]. Funahashi et al. reported that leptin receptor and orexin receptor are co-existence in the ARC[4)]. So Leptin and orexin may affect with each other. Cai et.al reported that, hypotharamic prepro-orexin mRNA concentration in

Zucker fatty rats were lower than those in lean controls[12]. Lower metabolic rate observed in Zucker fa/fa rats may related with not only leptin receptor disturbance but also orexin containing neuron system.

At present, this manuscript is not completed.

REFERENCES

1) Zhang Y, Proenca R, Maffei M, et al. Positional cloning of the mouse obese gene and its human homologue. *Nature* 372: 425-432, 1994.

2) Jacob RJ, Dziura J, Medwick MB, et al. The effect of leptin is enhanced by microinjection into the ventromedial hypothalamus. *Diabetes* 46: 150-152, 1997.

3) Sakurai T, Amemiya A, Ishii M, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 92: 1 page following 696, 1998.

4) Funahashi H, Yamada S, Kageyama H, et al. Co-existence of leptin- and orexin-receptors in feeding-regulating neurons in the hypothalamic arcuate nucleus-a triple labeling study. *Peptides* 24: 687-694, 2003.

5) Chen CT, Hwang LL, Chang JK and Dun NJ. Pressor effects of orexins injected intracisternally and to rostral ventrolateral medulla of anesthetized rats. *Am J Physiol Regul Integr Comp Physiol* 278: R692-697, 2000.

6) O'Donnell C P, Schaub CD, Haines AS, et al. Leptin prevents respiratory depression in obesity. *Am J Respir Crit Care Med* 159: 1477-1484, 1999.

7) Kanbayashi T, Inoue Y, Kawanishi K, et al. CSF hypocretin measures in patients with obstructive sleep apnea. *J Sleep Res* 12: 339-341, 2003.

8) Nishijima T, Sakurai S, Arihara Z and Takahashi K. Plasma orexin-A-like immunoreactivity in patients with sleep apnea hypopnea syndrome. *Peptides* 24: 407-411, 2003.

9) Refinetti R. Computation of the effective body mass for metabolic studies of lean and obese rats. *Metabolism* 38: 763-766, 1989.

10) Montague CT, Farooqi IS, Whitehead JP, et al. Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* 387: 903-908, 1997.

[11] Pelleymounter MA, Cullen MJ, Baker MB, et al. Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 269: 540-543, 1995.

[12] Cai XJ, Lister CA, Buckingham RE, et al. Down-regulation of orexin gene expression by severe obesity in the rats: studies in Zucker fatty and zucker diabetic fatty rats and effects of rosiglitazone. *Brain Res Mol Brain Res* 77: 131-137, 2000.

Table 1. Effects of leptin and orexin on resting ventilation in lean rats

	ACSF(n=4)			
Time, min	0	30	60	90
f, breaths/min	90.3±3.6	91.1±5.0	95.5±5.3	91.6±3.7
V_T, ml/kg$^{0.75}$	4.0±0.2	4.0±0.2	4.1±0.2	4.1±0.2
V_E, ml/kg$^{0.75}$/min	359.9±18.3	365.8±21.4	390.2±33.3	375.8±23.1
V_E/VO_2	26.6±2.2	26.7±1.2	28.1±0.8	26.8±1.4
VO_2, ml/kg$^{0.75}$/min	13.7±0.8	13.7±0.8	13.8±0.9	14.1±0.8
VCO_2, ml/kg$^{0.75}$/min	8.8±0.7	9.3±1.1	8.6±0.8	9.1±1.4
T, ℃	36.4±0.1	36.3±0.1	36.2±0.3	36.2±0.3
	Leptin(n=10)			
Time, min	0	30	60	90
f, breaths/min	92.7±6.6	103.4±7.3※※	107.0±6.8※	110.2±7.0※※
V_T, ml/kg$^{0.75}$	4.1±0.3	4.4±0.3	4.7±0.3※	4.8±0.3※
V_E, ml/kg$^{0.75}$/min	366.0±19.8	452.5±38.3※	492.2±34.1※	518.7±39.1※
V_E/VO_2	26.8±1.3	30.6±1.7	33.3±2.4	34.5±2.5※
VO_2, ml/kg$^{0.75}$/min	13.8±0.8	14.7±0.8	14.9±0.6	15.2±0.9
VCO_2, ml/kg$^{0.75}$/min	9.2±0.6	9.4±0.7	9.8±0.8	10.3±0.5
T, ℃	36.4±0.4	36.5±0.5	36.7±0.5	36.9±0.5
	Orexin(n=10)			
Time, min	0	30	60	90
f, breaths/min	92.7±5.6	108.4±7.5※※	127.7±5.6※※	117.1±6.5※※
V_T, ml/kg$^{0.75}$	4.0±0.2	4.8±0.2※※	4.6±0.3※	4.4±0.2
V_E, ml/kg$^{0.75}$/min	373.6±31.2	603.6±28.9※※	580.7±39.4※※	513.4±34.6※※
V_E/VO_2	27.0±2.4	35.8±2.3※※	35.5±2.8※※	34.4±3.3※※
VO_2, ml/kg$^{0.75}$/min	14.0±0.6	17.5±1.3※※	16.9±1.1※※	15.5±1.0※
VCO_2, ml/kg$^{0.75}$/min	8.9±0.4	12.2±0.9※※	11.8±0.6※※	10.8±0.4※※
T, ℃	36.3±0.3	36.5±0.3	37.0±1.0※※	37.1±0.3※※

Values are mean ±SE

※ $p<0.05$ significantly different from corresponding control value
※※ $p<0.01$ significantly different from corresponding control value

Table 2. Effects of leptin and orexin on resting ventilation in obese rats

	Leptin(n=6)			
Time, min	0	30	60	90
f, breaths/min	121.4±6.9	117.7±5.5	118.7±5.9	118.7±6.9
V_T, ml/kg$^{0.75}$	4.6±0.3	4.7±0.3	4.9±0.4	5.3±0.5
V_E, ml/kg$^{0.75}$/min	597.1±49.5	618.8±56.8	633.9±56.6	657.4±75.8
V_E/VO_2	51.8±5.2	52.1±4.6	51.9±3.3	56.1±5.9
VO_2, ml/kg$^{0.75}$/min	11.8±1.0	12.1±1.0	12.5±1.4	12.2±1.5
VCO_2, ml/kg$^{0.75}$/min	6.9±0.5	6.9±0.6	7.3±0.9	7.6±1.1
T, ℃	35.1±0.9	34.9±0.8	35.0±0.8	35.0±0.8
	Orexin(n=6)			
Time, min	0	30	60	90
f, breaths/min	123.6±5.3	141.5±9.0	144.3±9.2 ※	144.4±8.6 ※
V_T, ml/kg$^{0.75}$	4.4±0.5	4.1±0.4	4.5±0.5	4.6±0.5
V_E, ml/kg$^{0.75}$/min	609.1±50.2	705.2±51.1	727.3±39.8※	712.7±32.8 ※
V_E/VO_2	52.7±6.2	55.2±4.0	58.1±4.1	58.9±5.7
VO_2, ml/kg$^{0.75}$/min	11.9±0.9	13.0±1.0 ※	12.7±0.8	12.5±1.0
VCO_2, ml/kg$^{0.75}$/min	7.1±0.9	7.9±1.0※	7.9±1.0	8.2±1.1
T, ℃	37.5±0.3	38.1±0.3 ※	38.6±0.4※※	39.0±0.5 ※

※$p<0.05$ significantly different from corresponding control value
※※$p<0.01$ significantly different from corresponding control value

Table 3. Effects of leptin on ventilatory response to hypercapnia *

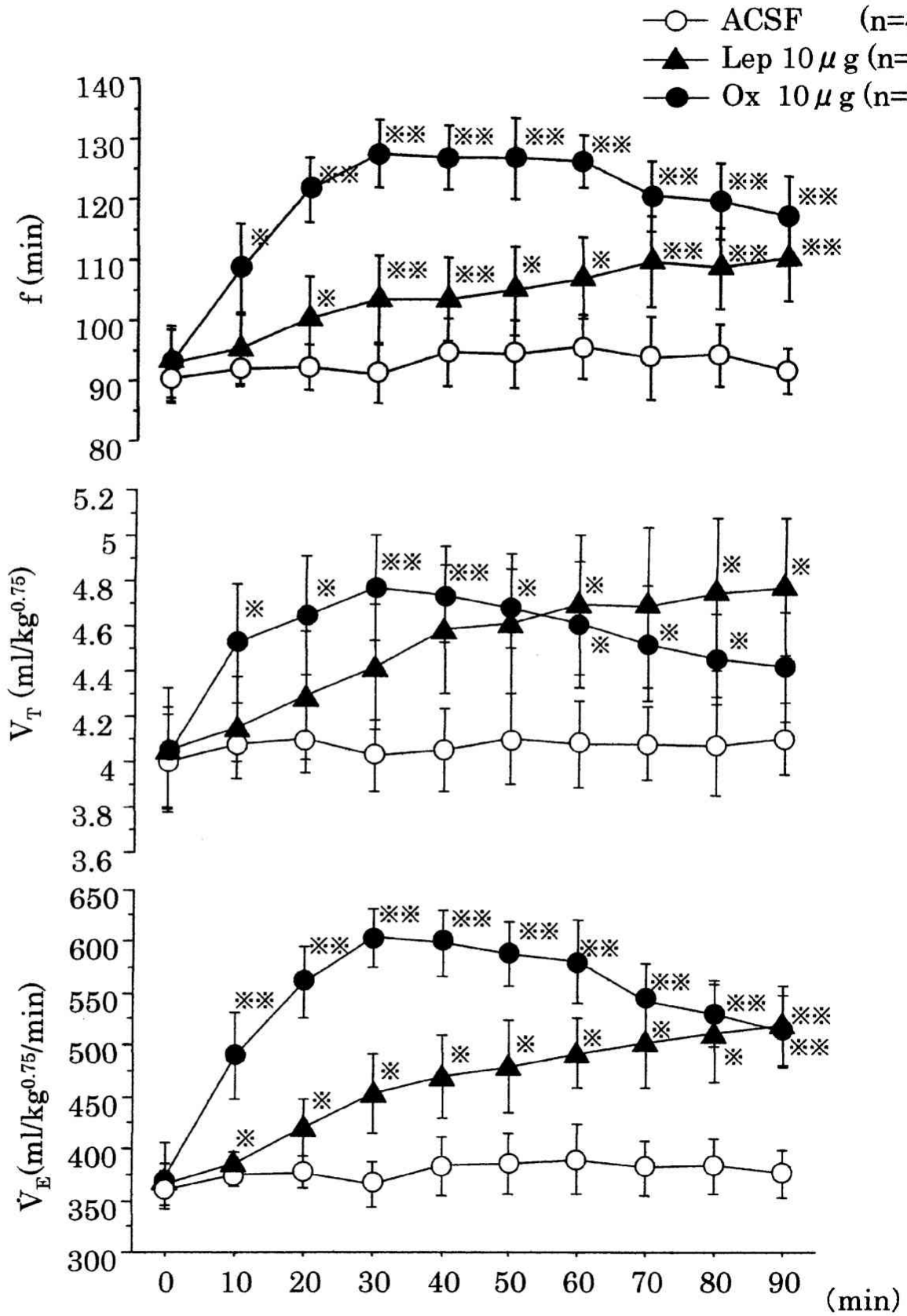
	Lean (n=10)		Obese (n=6)	
	Control	Leptin	Control	Leptin
HCVR($\Delta\dot{V}_E$) ,ml/kg/min	126.0±56.7	170.9±53.8	199.5±33.8	106.7±46.8

Table 4. Effects of orexin on ventilatory response to hypercapnia *

	Lean (n=7)		Obese (n=5)	
	Control	Orexin	Control	Orexin
HCVR($\Delta\dot{V}_E$) ,ml/kg/min	137.8±83.0	325.4±85.7※	127.5±12.2	135.0±26.5

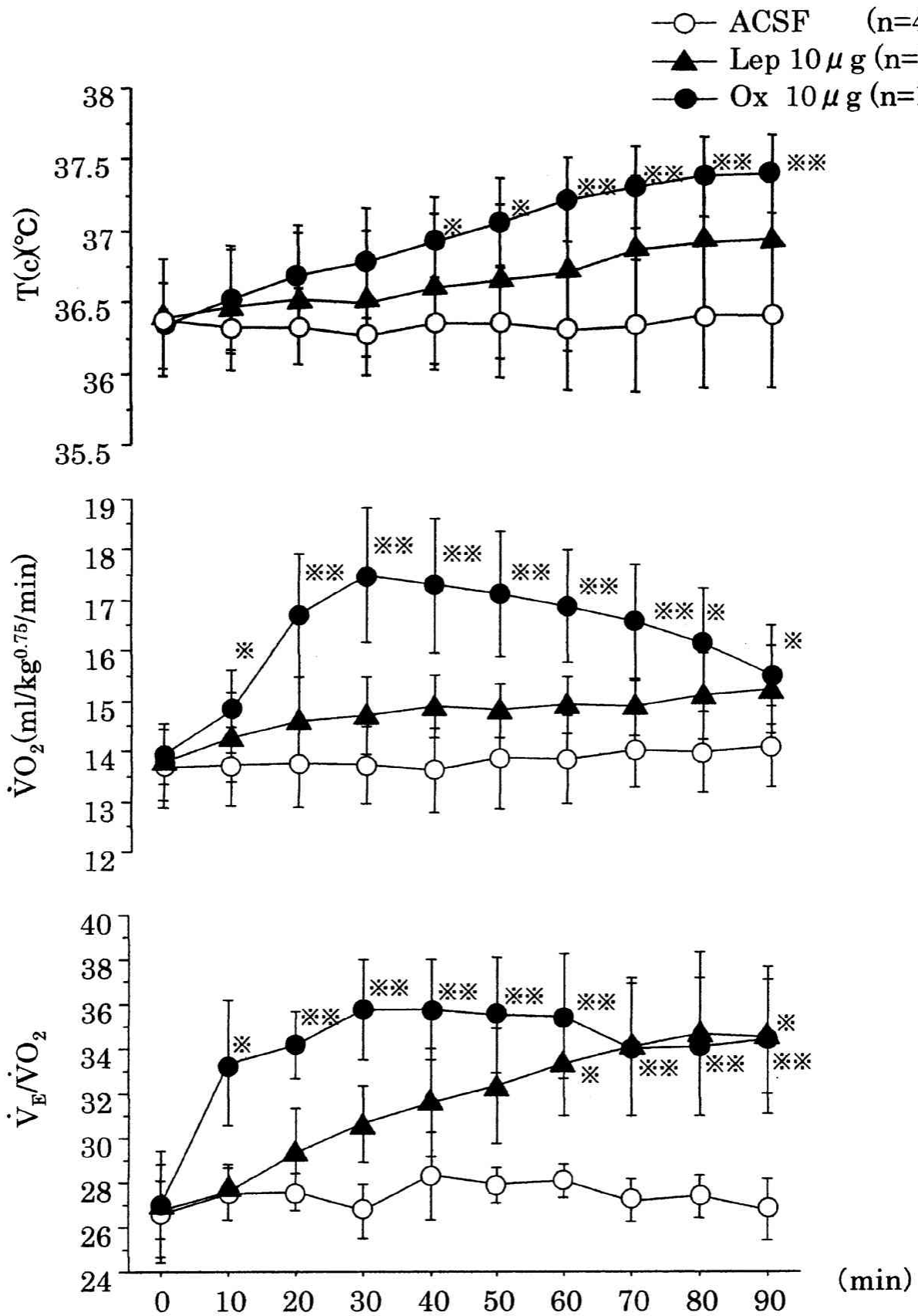
Definition of abbreviation :HCVR=hypercapnic ventilatory response
$\Delta\dot{V}_E$ = increase in $\dot{V}_E$ from room air to hypercapnia.
* values are means ± SD for the number of rats in each group.
※$p < 0.05$ significantly different from control value.

Figure 1. Effects of leptin and orexin on resting ventilation in lean rats



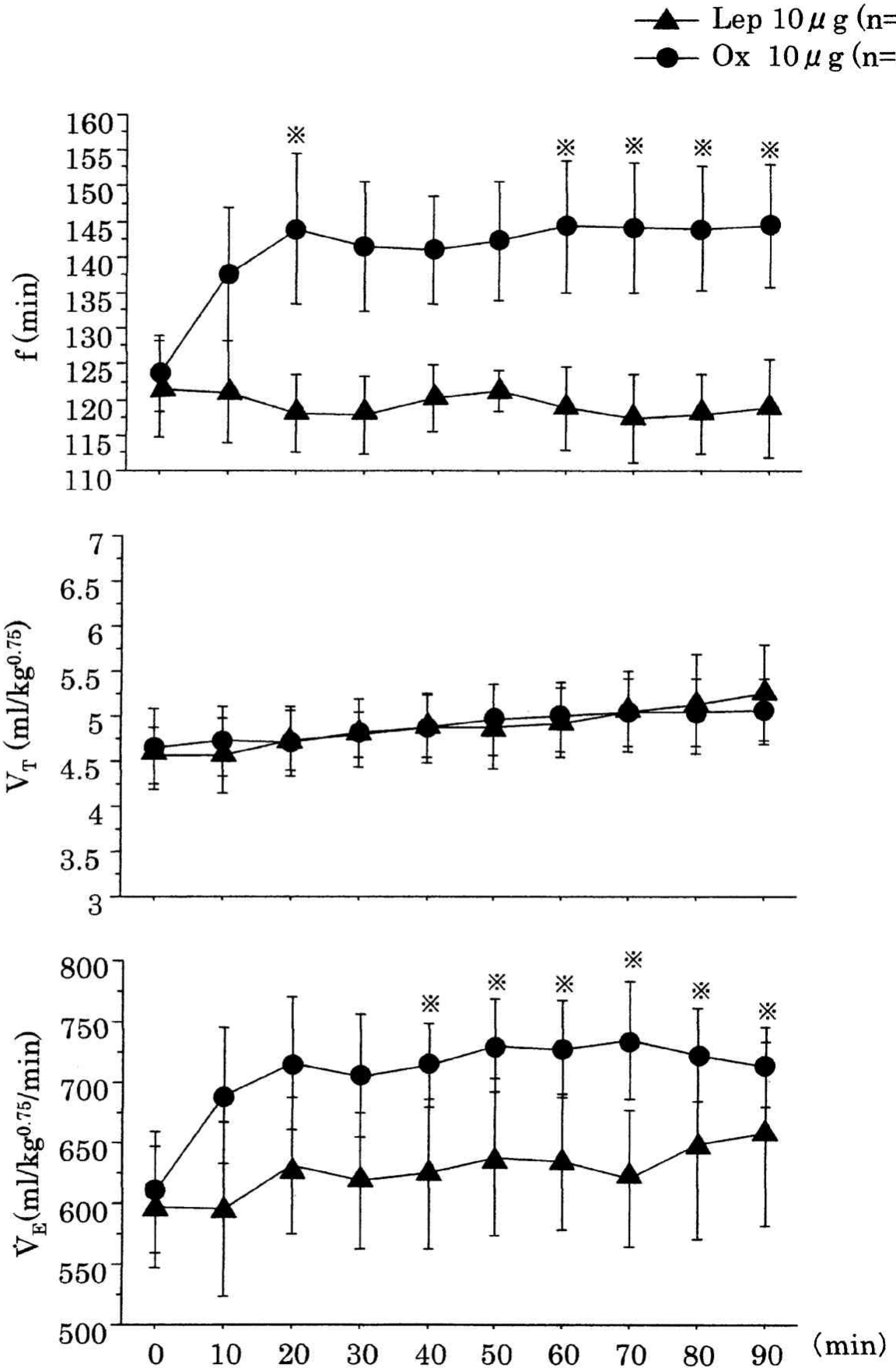
※p<0.05 significantly different from corresponding control value
※※p<0.01 significantly different from corresponding control value

Figure 2. Effects of leptin and orexin on resting metabolism in lean rats



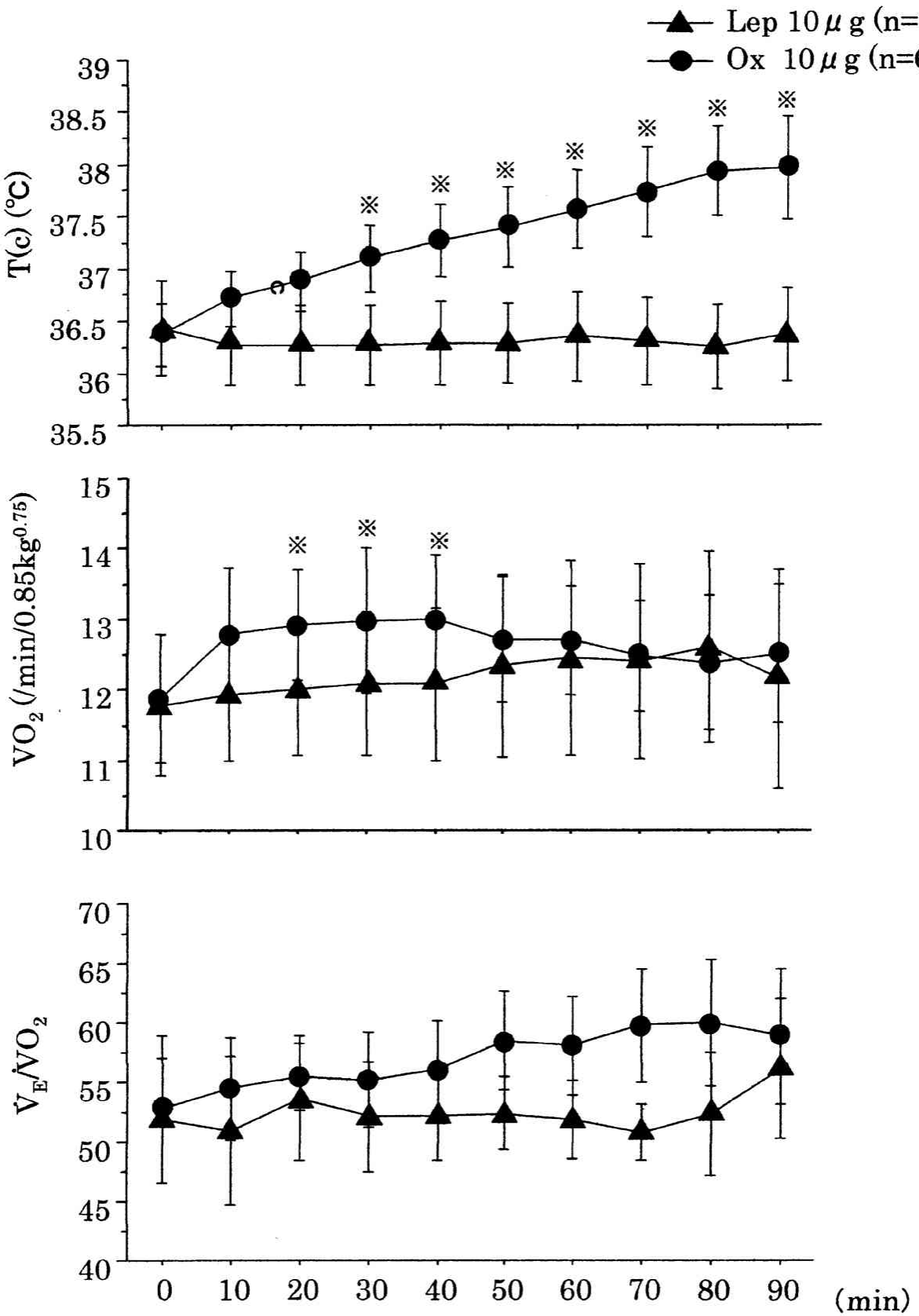
※p<0.05 significantly different from corresponding control value
※※p<0.01 significantly different from corresponding control value

Figure 3. Effects of leptin and orexin on resting ventilation in obese rats



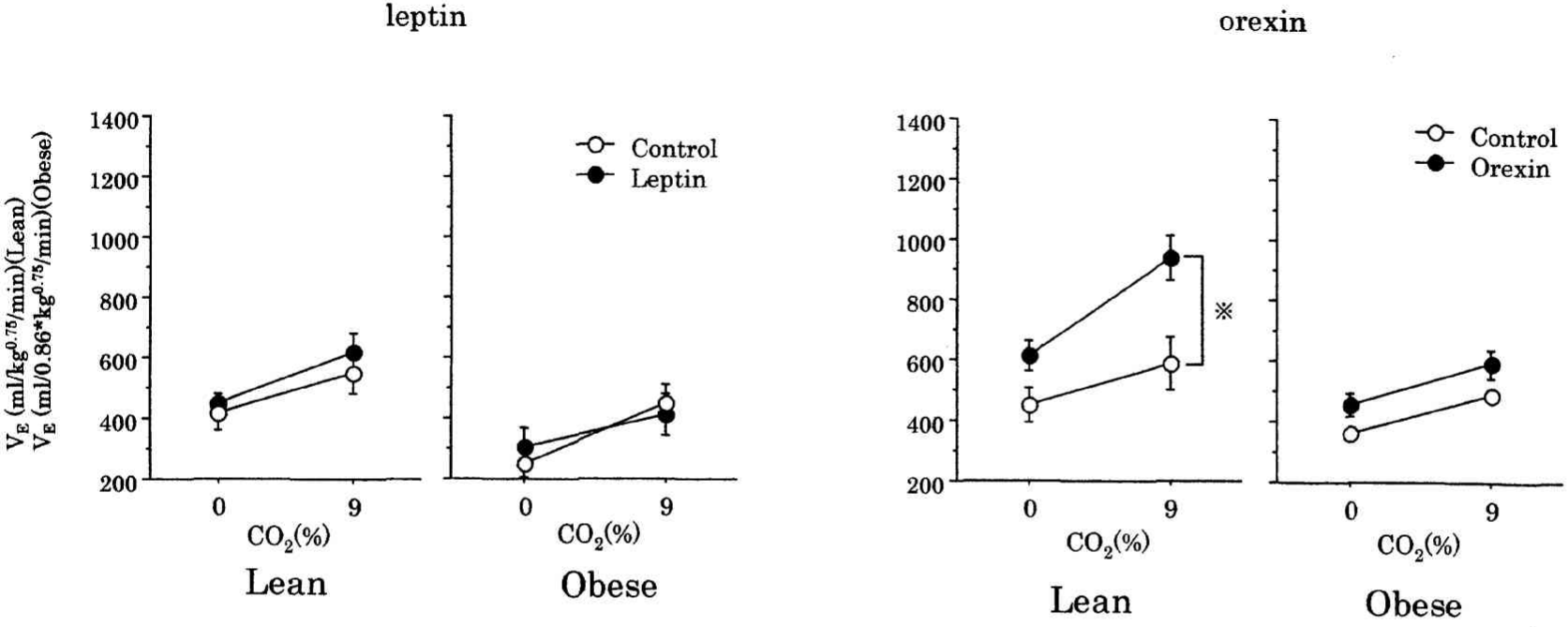
※p<0.05 significantly different from corresponding control value

Figure 4. Effects of leptin and orexin on resting metabolism in obese rats



※$p<0.05$ significantly different from corresponding control value

Figure 5. Effects of leptin and orexin on hypercapnic ventilatory response



$\dot{V}_E$ was not increased by cerebroventricular administration of Leptin in nether lean nor obese Zucker rats during hypercapnia.
$\dot{V}_E$ was significantly increased by cerebroventricular administration of Orexin in Lean Zucker rats during hypercapnia.
$\dot{V}_E$ did not increased by Orexin injection in Obese Zucker rats.
※$p<0.05$ significantly different from Control value

Ⅰ. 研究発表

1. 中野　均、肥満による上気道閉塞におけるセロトニン作動神経の役割、第 42 回日本呼吸器学会総会、東京、2002. 4
2. 佐々木伸彦、中野　均、レプチンの呼吸調節に与える影響－Zucker ラットを用いて－、第 43 回　日本呼吸器学会総会、福岡、2003. 3
3. 佐々木伸彦、中野　均、オレキシンの呼吸と代謝に与える影響について－Zucker 肥満ラットを用いて－第 44 回　日本呼吸器学会総会、東京、2004. 3
4. Nobuhiko Sasaki, Hitoshi Nakano, Effect of leptin on control of breathing in Zucker rats. 2003　ALA/ATS International Conference. Seattle, 2003. 5
5. Nobuhiko Sasaki, Hitoshi Nakano, Effects of leptin and orexin on ventilation and metabolism in zucker rats.　2004　ALA/ATS International Conference. Orlando, 2004. 5

Ⅱ. 原著論文

1. Hitoshi Nakano, Shin-Da Lee, and Graspar A. Farkas Dopaminergic modulation of ventilation in obese Zucker rats. J Appl Physiol 92: 25-32, 2002
2. S. Osanai, T. Takahashi, H. Nakano, K. Kikuchi. Doxapram inhibits carotid sinus baroreceptors in rabbits.　Auto Autaco Pharmacol.　25: 79-84, 2005
3. Toru Takahashi, S. Osanai, H. Nakano, Y. Ohsaki, K. Kikuchi, Doxapram stimulates the caroid body via a different mechanism than hypoxic chemotransduction. Respir Physiol Neurobiol 147:1-9, 2005