# No Effect of Naloxone on Ventilatory Response to Progressive Hypercapnia in IDDM Patients

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**The ventilatory response to hyperoxic progressive hypercapnia was examined by comparing 3 test groups: 7 diabetic patients with AN, 8 diabetic patients without AN, and 8 normal control subjects. In each group, a significant linear correlation was found between $PaCO_2$ and VE. The slopes of the regression curves relating $PaCO_2$ to VE were significantly steeper in the healthy control subjects and diabetic patients without AN than in those with AN ($P < 0.01$). We conclude that the ventilatory response to progressive hypercapnia is reduced in diabetic patients with AN. By analyzing the power spectrum and the amplitude behavior of the diaphragmatic EMG (calculated from the fc and RMS, respectively), we could exclude a disturbance of neural descending pathways and respiratory muscle dysfunction as possible causal mechanisms for the impaired ventilatory response to increasing $CO_2$. By using lung function analysis, causal factors such as alterations in respiratory system mechanics also could be excluded. As diabetes is known to affect the endogenous opioid system, which, in turn, affects the ventilatory response to $CO_2$, naloxone, as a specific opioid antagonist, was administered in all 3 test groups. Naloxone produced a significant increase of ventilatory response to hypercapnia in the healthy control subjects ($P < 0.01$), but produced no effect in either of the diabetic groups. We conclude that the ventilatory response to hypercapnia is impaired in diabetic patients with AN, that lung function alterations and diaphragmatic muscle dysfunction are not responsible for this impairment, and that endogenous opioids produce an effect on the response to $CO_2$ in healthy subjects, but they have no effect on $CO_2$ response in diabetic patients with or without AN. These results suggest that the central control of respiration is pathologically altered in diabetic patients with AN. *Diabetes* 42:282–87, 1993**

Autonomic neuropathy is a complication of diabetes mellitus associated with increased morbidity and mortality (1). Among the most frequent causes of death are renal and cardiovascular diseases (2). However, some deaths, especially among younger diabetic patients with AN, still lack a satisfactory explanation. It has been suggested that an abnormal central control of respiration might account for these deaths (3). This hypothesis was supported by a study that demonstrated that patients with AN had increased sleep apnea (4).

The ventilatory responses to hypercapnia in patients with AN have been the subject of extensive investigations that have produced generally conflicting results (5–9). However, previous investigations failed to determine whether the decreased ventilatory response during $CO_2$-rebreathing tests were caused by impaired sensitivity of the medullary chemoreceptors or to other factors, which, apart from drugs and age, include the mechanical efficiency of the respiratory muscles, the mechanical impedance of the respiratory system, and the integrity of neural descending pathways (10,11). Respiratory muscle function impairment and lung function abnormalities have been described in the scope of diabetes (12–14),

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IDDM, insulin-dependent diabetes mellitus; AN, autonomic neuropathy; $PaCO_2$, arterial $CO_2$ tension; VE, minute ventilation; EMG, electromyogram; fc, centroid frequency; RMS, root mean square; BMI, body mass index; NCV, nerve conduction velocity; $C_{spec}$, specific lung compliance; $PET_{CO2}$, end-tidal fraction of $CO_2$; ECG, electrocardiograph; $fc\%fc_R$, percentage of baseline fc value; $RMS\%RMS_R$, percentage of baseline RMS value; $FEF_{50}$, forced expiratory flow at 50% of forced expiratory vital capacity; $FIF_{50}$, forced inspiratory flow at 50% of forced inspiratory vital capacity; $FEV_1$, forced expiratory volume in 1 s; $FIV_1$, forced inspiratory volume in 1 s; ANOVA, analysis of variance.

and the integrity of neural descending pathways seems to be impaired as well (15). Furthermore, diabetes affects the endogenous opioid system (16), which plays an important role in respiratory control. Opioids are strong depressants of respiration that reduce the chemosensitivity to $CO_2$ (17).

We hypothesized that patients with AN show alterations in the central control of respiration and depressed ventilatory responses that cannot be explained by alterations in peripheral respiratory mechanisms or by the endogenous opioid system. In this study, the integrity of the ventilatory $CO_2$ responses was investigated in diabetic patients with AN and compared with diabetic patients without AN and with healthy control subjects. The influence of peripheral respiratory mechanisms and the endogenous opioid system on the ventilatory response to $CO_2$ was analyzed.

## RESEARCH DESIGN AND METHODS

The study was performed with 7 IDDM patients with AN, 8 IDDM patients without AN, and 8 healthy control subjects. The healthy control subjects were matched with the diabetic patients in age, sex, height, and weight. The 2 patient groups were matched for duration of diabetes. The IDDM patients met the following selection criteria: insulin dependence from the time of diagnosis, age of onset <30 yr, regular attendance at our diabetic department, no hospital admission in the previous 6 mo, BMI <30 kg/m$^2$, and absence of any clinical or radiological cardiorespiratory abnormality. None of the patients had any symptoms of neuromuscular disease or a history of atopy. Their serum urea and creatinine concentrations were within the normal range. Patients and control subjects who had smoked more than five cigarettes a day during the previous 5 yr were excluded.

All patients were examined by an ophthalmologist to detect retinopathy, which was classified according to background and proliferative retinopathy. Diabetic nephropathy was determined by measuring overnight urinary protein excretion. Microalbuminuria was considered present if urinary protein excretion was 20–200 μg/min; proteinuria was diagnosed if the value was >200 μg/min (turbidimetric method; model Turbitimer, Behring, Marburg, Germany). All subjects participating in the study gave their informed consent. The study was approved by the Human Subject Committee of the hospital.

Somatic neuropathy was assessed clinically by evaluating neuropathic symptoms and signs as described recently (18). Motor and sensory nerve conduction studies were performed with conventional techniques in all subjects with a DISA 1500 electromyograph (Disa Electronics, Skovlunde, Denmark). Median nerve (motor and sensory antidromic NCV), peroneal nerve (motor NCV), and sural nerve (sensory orthodromic NCV) were measured, and the results were compared with the reference values listed by Ludin (19).

Three tests were performed to detect AN. *1*) Measurement of orthostatic blood pressure reaction as an indicator for sympathetic damage. Blood pressure was taken after 5 min with the subject in the lying position and immediately after rising. The results were classified according to Ewing et al. (20,21). *2*) Measurement of the beat-to-beat heart rate variation during deep respiration (E/I ratio). *3*) Measurement of the heart-rate variability during a Valsalva maneuver (Valsalva ratio). Examinations 2 and 3 were conducted with a computer-aided system, the Pro-Sci-Card Analyzer (Pro Science, Linden, Germany), after the subjects had remained in the lying position for 5 min. The normal values were determined from 120 healthy subjects, aged 15–67 yr, and the lower limits were defined according to the 2.3 percentile (22). In our patients with AN, all three tests were pathological, indicating severe AN.

Lung function tests consisted of spirometry, plethysmography, and $C_{spec}$. $C_{spec}$ was measured with the subject in the sitting position by an esophageal balloon that was passed into the midesophagus (23). Transpulmonary pressure was measured with a differential pressure transducer (Statham 268B, Cleveland, OH). $C_{spec}$ was expressed as lung compliance/functional residual capacity (24).

The hypercapnic ventilatory responses were recorded according to Read's method (25). With a nose clip in place, the subjects breathed through a mouthpiece connected to a two-way valve (Jaeger, Würzburg, Germany) that separated the inspiratory and expiratory lines. For measuring flow and volume of the inspired air, the inspiratory limb of the valve was attached to a pneumotachograph (model PT18, Jaeger). During the rebreathing tests, the subjects inhaled a gas mixture containing 7% $CO_2$ in $O_2$ from an 8 L anesthetic bag. $PET_{CO2}$ was measured at mouth level by an infrared $CO_2$ absorption analyzer (Jaeger). $PaCO_2$ was computed with $PET_{CO_2}$ according to the formula $PaCO_2 = 5.5 + 0.9\ PET_{CO2} - 0.0021 \times$ tidal volume (26).

The ventilatory responses to progressive hypercapnia were recorded at 30-s intervals. We plotted VE versus $PaCO_2$, and the slopes and intercepts of the linear regression were computed. The coefficient of correlation was >0.96 for all analyses in our test subjects. To further analyze the ventilatory responses to $CO_2$, the mean VEs of all subjects in each group obtained at four different levels of $PaCO_2$ (45, 50, 55, and 60 mmHg) were calculated. The mean VE values were calculated from the actual volume records of each subject at the corresponding $PaCO_2$ measurement and not from the VE versus $PaCO_2$ regression curves.

During the rebreathing tests, the electrical activity of the diaphragm was recorded with silver/silver chloride surface electrodes, positioned at the lower anterolateral rib cage, as described by Gross et al. (27). The signals passed an anti-aliasing filter with a cutoff frequency of 500 Hz and were stored in a computer. The influence of ECG signals on the EMG data was minimized by eliminating the ECG signals from the EMG waveform by a subtraction algorithm (28). From the diaphragmatic EMG, the fc was calculated. As the spectral moment of first order normalized by the spectral moment of order zero, fc provides a stable and sensitive means for describing the spectral shift caused by muscle fatigue (29). In addition, RMS was calculated. RMS represents a statis-

TABLE 1
Physical characteristics of IDDM patients with and without AN and control subjects

| | Patients with AN ($n$ = 7) | Patients without AN ($n$ = 8) | Control subjects ($n$ = 8) |
|---|---|---|---|
| Age (yr) | 39 ± 11 | 38 ± 12 | 41 ± 10 |
| Height (cm) | 174.8 ± 8.4 | 177.3 ± 10.1 | 176.3 ± 8.2 |
| Weight (kg) | 73.5 ± 10.8 | 75.1 ± 12.0 | 76.0 ± 10.8 |
| BMI (kg/m$^2$) | 23.9 ± 3.2 | 24.0 ± 2.7 | 24.5 ± 3.2 |
| $PaO_2$ (mmHg) | 92.5 ± 3.8 | 90.3 ± 4.0 | 91.6 ± 3.4 |
| $PaCO_2$ (mmHg) | 34.6 ± 3.2 | 34.8 ± 3.6 | 35.0 ± 3.1 |
| $FEV_1$ (L/s) | 3.8 ± 0.7 | 4.0 ± 0.8 | 4.3 ± 1.0 |
| $FIV_1$ (L/s) | 4.2 ± 0.4 | 4.5 ± 0.6 | 4.9 ± 0.7 |
| $FEF_{50}$ (L/s) | 5.3 ± 0.5 | 5.6 ± 0.6 | 5.4 ± 0.6 |
| $FIF_{50}$ (L/s) | 5.6 ± 0.3 | 6.0 ± 0.5 | 5.8 ± 0.4 |

tically valid measure for the amplitude behavior of the EMG signal and is further characterized by a very good signal resolution (30). Before each rebreathing run, the fc and RMS values rendered by three inspirations during quiet breathing were averaged and given as 100%. All subsequent fc and RMS values during $CO_2$ loading were expressed as a percentage of these baseline values ($fc\%fc_R$, $RMS\%RMS_R$). The neural activation pattern of the diaphragm was analyzed by computing linear regressions of $RMS\%RMS_R$ versus VE for each rebreathing test.

Immediately before the rebreathing runs, the subjects were given either naloxone (0.1 mg/kg) or volume-matched saline intravenously in a double-blind fashion on 2 successive days. This large dose of naloxone was chosen to block endogenous opiate receptors completely (17,31). The rebreathing tests were started 10 min after injection to guarantee complete penetration of naloxone into the brain (32). All tests were completed within 40 min from injection, which was within the half-life of the drug (32). The tests were performed at least 2 h after the subjects had had their usual lunch, and they were requested to abstain from drinking coffee and tea.

**Statistical methods.** A nonparametric Kolmogoroff-Smirnoff test was used to determine the significance of differences among the 3 groups. A value of $P < 0.05$ was considered significant. All values in the text and tables are means ± SD.

## RESULTS

The general characteristics of the participants are given in Table 1. The group of patients with AN consisted of 3 women and 4 men, and each of the other 2 groups contained 3 women and 5 men. No significant differences in anthropomorphic data were observed among the 2 groups of diabetic patients and the control subjects. Patients and control subjects were not obese and did not show any symptoms of obstructive sleep apnea, like daytime sleepiness or sleep disturbances. Blood gas values were within the normal range in all test subjects (Table 1). Upper airway obstruction was excluded by lung function analysis. The ratio of $FEF_{50}$ to $FIF_{50}$ and the ratio of $FEV_1$ to $FIV_1$ were <1 (Table 1). These data excluded a relevant extrathoracic stenosis (33,34). The clinical features of the diabetic patients are shown in Table 2. In the diabetic patients with AN, duration of diabetes was 17.2 ± 7.5 yr, and $HbA_{1c}$ concentration was 8.4 ± 1.2%. The diabetic patients without AN had a similar duration of disease (15.5 ± 8.1 yr) and $HbA_{1c}$ concentration (8.3 ± 1.1%; $P > 0.05$). Proliferative retinopathy and proteinuria were more common in the group with AN than in the group without AN. Motor and sensory nerve conduction studies bore evidence of somatic neuropathy in all patients with AN and in 4 of 8 patients without AN. Clinical symptoms of somatic neuropathy were found in 6 of 7 patients with AN and in 2 of 8 patients without AN.

Diabetic patients with AN differed from the patients without AN and the healthy subjects with respect to their ventilatory responses to $CO_2$. Table 3 shows the slopes and intercepts of the ventilatory responses to hypercapnia of diabetic patients with and without AN and control subjects. In the patients with AN, the responses were obviously reduced compared with control subjects and patients without AN (2.10 ± 0.50, 2.78 ± 0.55, and 2.55 ± 0.47 $L \cdot min^{-1} \cdot mmHg^{-1}$). Nonparametric ANOVA showed that a longer duration of diabetes and the presence of diabetic retinopathy and nephropathy were not statistically associated with a reduction in ventilatory response to hypercapnia.

Respiratory mechanics were not impaired in diabetic

TABLE 2
Clinical features of IDDM patients with and without AN

| | No. of patients with AN ($n$ = 7) | No. of patients without AN ($n$ = 8) |
|---|---|---|
| Duration of diabetes | | |
| 0–10 yr | 1 | 2 |
| 11–20 yr | 3 | 4 |
| >20 yr | 3 | 2 |
| $HbA_{1c}$ | | |
| <8% | 2 | 3 |
| 8–10% | 5 | 5 |
| Retinopathy | | |
| Background | 2 | 3 |
| Proliferative | 4 | 2 |
| Somatic neuropathy (existing) | 7 | 4 |
| Nephropathy | | |
| Microalbuminuria | 3 | 3 |
| Proteinuria | 3 | 1 |

TABLE 3
Slopes and intercepts for the ventilatory responses to progressive hypercapnia with and without naloxone in IDDM patients with and without AN and control subjects

| | Without naloxone | | With naloxone | |
|---|---|---|---|---|
| | Slope ($L \cdot min^{-1} \cdot mmHg^{-1}$) | Intercept (mmHg) | Slope ($L \cdot min^{-1} \cdot mmHg^{-1}$) | Intercept (mmHg) |
| IDDM without AN | 2.55 ± 0.47 | 40.8 ± 1.3* | 2.65 ± 0.76† | 40.4 ± 1.7 |
| IDDM with AN | 2.10 ± 0.50† | 40.0 ± 1.5* | 2.02 ± 0.60† | 40.0 ± 1.6 |
| Control subjects | 2.78 ± 0.55 | 41.0 ± 1.6* | 3.94 ± 0.73 | 41.8 ± 1.8 |

Intercept indicates extrapolated value for $PaCO_2$ when VE is 0.
†$P < 0.01$, vs. healthy control subjects.

subjects. Diabetic patients with AN had an airway resistance of 0.17 ± 0.07 $kPa \cdot L^{-1} \cdot s^{-1}$ and a $C_{spec}$ of 0.64 ± 0.18 $kPa^{-1}$. Diabetic patients without AN and control subjects had a similar airway resistance (0.18 ± 0.07 and 0.16 ± 0.08 $kPa \cdot L^{-1} \cdot s^{-1}$, respectively) and a similar $C_{spec}$ (0.72 ± 0.21 and 0.68 ± 0.17 $kPa^{-1}$, respectively). Moreover, all other lung function parameters in the diabetic group were also within the age-specific norms, established by Forche (35).

The slopes $\Delta VE/\Delta(RMS\%RMS_R)$ showed no statistically significant difference: 0.42 ± 0.13 L/min for the control subjects, 0.38 ± 0.16 L/min for the diabetic patients without AN, and 0.39 ± 0.15 L/min for the diabetic patients with AN. The coefficient of correlation was >0.90 for all regression analyses. Thus, an abnormality of neural diaphragmatic control can be excluded. No significant decrease of the fc ($fc\%fc_R$) was found in the control subjects and diabetic patients. At the end of the test runs, the mean $fc\%fc_R$ value for the control subjects was 95 ± 5%, for the diabetic patients without AN, 96 ± 4%, and for the diabetic patients with AN, 92 ± 6%. On the basis of these data, we excluded diaphragmatic fatigue as a possible reason for impaired ventilatory response to $CO_2$ in diabetic patients with AN.

The influence of the endogenous opioid system varied among the three test groups. In the control group, the ventilatory response to hypercapnia increased after i.v. injection of naloxone (0.1 mg/kg), with $\Delta VE/\Delta PaCO_2$ increasing from 2.78 ± 0.55 to 3.94 ± 0.73 $L \cdot min^{-1} \cdot mmHg^{-1}$ ($P < 0.01$). Naloxone had no effect on the ventilatory responses to hypercapnia in the diabetic patients with or without AN (Table 3, Fig. 1). Table 4 shows the mean VE of all subjects of each group at $PaCO_2$ levels of 45, 50, 55, and 60 mmHg. Before administration of naloxone, no significant differences among the groups were found at lower levels of $PaCO_2$. However, the average VE of the diabetic patients with AN was significantly lower if the $PaCO_2$ value was >55 mmHg. After the administration of naloxone, the mean VE values even at lower $PaCO_2$ values were statistically significantly lower in the diabetic patients with AN. These data indicate that endogenous opioids do not have an effect on ventilatory response to $CO_2$ in diabetic patients with or without AN, in contrast to healthy control subjects.

## DISCUSSION

According to our findings, diabetic patients with AN have depressed ventilatory responses to rising $CO_2$ that cannot be explained by alterations in the peripheral mechanics of respiration. Furthermore, in contrast to control subjects, no response to naloxone treatment was observed in either diabetic group, which suggests that diabetes mellitus is associated with alterations in the endogenous opioid system.

Research in the field of ventilatory response of diabetic patients to hypercapnia has produced highly contradictory results. Although some authors reported a reduced ventilatory response in diabetic patients with AN (6,8), others did not find any difference between diabetic and healthy subjects. Among the latter are Sobotka et al. (7), who used the same methods as we did. In their study, however, resting VE scattered widely within each group, which may indicate poor reproducibility of their measurements. These factors may explain why, though the mean values were lower in the diabetic group with AN than in the control subjects, the differences in ventilatory response to hypercapnia were not significant. Soler and Eagleton (5) did not find a reduction in ventilatory response to $CO_2$ in the same group of patients, either. Yet, most of their test subjects did not withstand the hypercapnic challenge test; only 4 patients with AN with an end-tidal $CO_2$ >55 mmHg were included in their analysis. It is only at higher $CO_2$ tensions that a significant difference in ventilatory response between diabetic patients with AN and control subjects is seen. This fact also was recognized by Homma et al. (6).

Studies reporting a reduced ventilatory response to $CO_2$ have merely speculated on the mechanisms of this

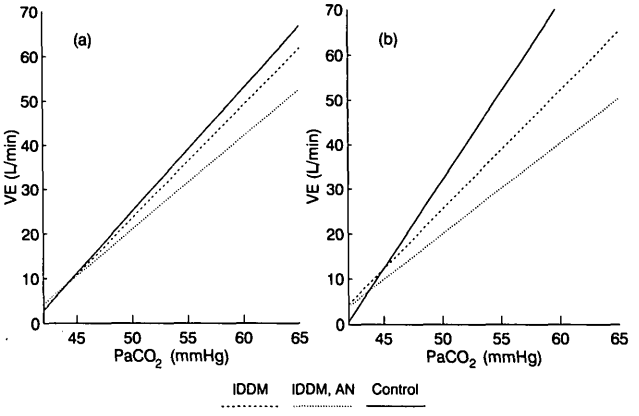


**FIG. 1. $VE/PaCO_2$ curves for healthy control subjects, diabetic patients without AN, and diabetic patients with AN. *A*: without naloxone and *B*: with naloxone.**

TABLE 4
VE (L/min) of IDDM patients with and without AN and control subjects at various $PaCO_2$ values

| | $PaCO_2$ (mmHg) | | | |
|---|---|---|---|---|
| | 45 | 50 | 55 | 60 |
| Without naloxone (L/min) | | | | |
| IDDM without AN | 10.5 ± 2.7 | 23.1 ± 5.1 | 36.2 ± 6.9 | 49.5 ± 5.8 |
| IDDM with AN | 10.1 ± 3.6 | 20.8 ± 4.9 | 31.5 ± 5.8* | 42.0 ± 6.2† |
| Control subjects | 11.0 ± 3.5 | 24.8 ± 5.3 | 38.5 ± 6.0 | 52.2 ± 7.4 |
| With naloxone (L/min) | | | | |
| IDDM without AN | 12.0 ± 4.0 | 24.8 ± 4.8* | 38.6 ± 5.8† | 51.6 ± 6.8† |
| IDDM with AN | 10.1 ± 4.1 | 20.8 ± 5.7‡ | 30.8 ± 5.9‡ | 40.5 ± 7.1‡ |
| Control subjects | 12.1 ± 3.9 | 31.7 ± 6.1 | 51.0 ± 8.3 | 71.8 ± 9.8 |

*$P < 0.05$ vs. healthy control subjects.
†$P < 0.01$ vs. healthy control subjects.
‡$P < 0.001$ vs. healthy control subjects.

phenomenon. Neither the mechanical impedance of the respiratory system nor the respiratory muscle efficiency have been analyzed, although these factors bear a decisive effect on the ventilatory response to hypercapnia. The authors of these studies also tried to exclude an alteration of motor function as a causal mechanism using only general respiratory function parameters. Yet lung function tests are known to be nonspecific, even in patients with severe neuromuscular diseases, and therefore, they cannot be used alone to exclude impaired integrity of neural descending pathways (36). Furthermore, endogenous opioids have not been considered previously as a possible cause of reduction of ventilatory response to hypercapnia.

To recognize the effects of endogenous opioids, we used the opioid antagonist naloxone, which has no agonist activity and is known to cross the blood-brain barrier (32,37). The possibility that diabetes mellitus might have altered our results by changing the pharmacodynamics and pharmacokinetics of naloxone can almost certainly be excluded. According to animal experiments, the potency of naloxone for antagonizing the antinociceptive effects of morphine is not altered by diabetes (38), and the binding affinity of opiate receptors in mouse brain membranes for naloxone is not affected by diabetes (39).

In our study, naloxone increased the ventilatory response to $CO_2$ in the control group, but not in either diabetic group. Therefore, endogenous opioids have a different activity in diabetic than healthy subjects. Streptozocin-induced diabetic and dextrose-induced hyperglycemic animals have been reported to show an elevation of pain threshold (40,41), which might provoke the decline of morphine analgesia in diabetic animals. It was suggested that the elevated threshold of pain is mediated by an endogenous opioid substance (41). However, it has been reported that streptozocin administration reduces the pain threshold (42) and is associated with a decrease of β-endorphin content in the hypothalamus and the neurointermediate lobe of the pituitary (42,43). The latter effect also has been found in alloxan-induced diabetes (44). In our diabetic patients, the endogenous opioids possibly had no effect on the ventilatory response because of a reduced concentration in the brain. This hypothesis would also explain why naloxone had no effect on the $CO_2$ response of either diabetic group. Further research is needed to find out whether the lack of effect of endogenous opioids on the ventilatory response to $CO_2$ is really caused by a reduced brain concentration of endogenous opioids, a decreased opioid receptor binding affinity in the brain (39), or a decreased responsiveness to opioids (45).

We know that carbon dioxide exerts its influence mainly via medullary chemoreceptors (46). During the rebreathing tests, the subjects breathed a hyperoxic gas mixture, which precluded any effect of peripheral chemoreceptors on ventilation by hypoxia (46). Therefore, we have to conclude that the response of the medullary chemoreceptors to hypercapnia is really impaired in diabetic patients with AN.

In summary, the results of our study indicate that the ventilatory response to $CO_2$ is defective in diabetic patients with AN in contrast to patients without AN. Peripheral factors have no impact on the ventilatory response to $CO_2$ in diabetic patients. In both patient groups, however, the endogenous opioids do not reduce the ventilatory response to $CO_2$, in contrast to healthy subjects. These findings add new knowledge to the pathophysiology of the regulation of breathing in IDDM patients.

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