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Alterations of Central Hypercapnic Respiratory Response Induced by Acute Central Administration of Serotonin Re-Uptake Inhibitor, Fluoxetine

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Abstract

Long-term neurochemical changes are responsible for therapeutic actions of fluoxetine. The role of increased central concentration of serotonin by inhibiting its re-uptake *via* fluoxetine on the central hypercapnic ventilatory response is complex and little is known. We aimed to research the effect of acute intracerebroventricular (ICV) injection of fluoxetine on hypercapnic ventilatory response in the absence of peripheral chemoreceptor impulses and the role of 5-HT_2 receptors on responses. Eighteen anesthetized albino rabbits were divided as Fluoxetine and Ketanserin groups. For ICV administration of fluoxetine and ketanserin, a cannula was placed in the left lateral ventricle by the stereotaxic method. Respiratory frequency (f_R), tidal volume (V_T) and ventilation minute volume (V_E) were recorded in both groups. ICV fluoxetine (10.12 mmol/kg) injection during normoxia caused significant increases in V_T and V_E (both $P < 0.01$) in the fluoxetine group. When the animals were switched to hypercapnia $f_{/min}$, V_T and V_E increased significantly. The increases in percentage values in V_T and V_E in Fluoxetine + Hypercapnia phase were higher than those during hypercapnia alone ($P < 0.01$ and $P < 0.05$, respectively). On blocking of 5-HT_2 receptors by ketanserin (0.25 mmol/kg), the ventilatory response to Fluoxetine was abolished and the degree of increases in V_T and V_E in the Ketanserin + Hypercapnia phase were lower than those during hypercapnia alone ($P < 0.01$ and $P < 0.001$, respectively). We concluded that acute central fluoxetine increases normoxic ventilation and also augments the stimulatory effect of hypercapnia on respiratory neuronal network by 5-HT_2 receptors in the absence of peripheral chemoreceptor impulses.

Key Words: acute central fluoxetine, serotonin, hypercapnic ventilatory response, ketanserin

Introduction

Central chemoreceptors that detect increases in CO_2 or H^+ and regulate breathing are located in the brain stem close to the surface of the ventral medulla (11, 28) and in other locations (7, 17, 19, 32) including the caudal nucleus tractus solitarius (nTS), the retrotrapezoid nucleus (RTN), which is a ventral medullary surface area, and the medullary raphe nuclei (MR) (18, 34).

Serotonergic (5-HT) neurons activate and stabilize breathing *via* actions at multiple levels of the

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respiratory network including motorneurons (7, 17). 5-HT is a neuromodulator that has many complex effects on spinal and medullary structures by acting pre- and post-synaptically *via* multiple receptor subtypes (7, 15, 19). In whole animals, the role of 5-HT neurons in response to hypercapnia is complex and is dependent upon several factors like species, anatomical locus in the medullary 5-HT system, age, gender and experimental paradigm (1, 7, 23, 24). More recently, it has been shown that focal acidification of the rostral MR nuclei *via* microdialysis of CO_2 stimulates breathing (10); ventilation was unchanged when the same technique was applied solely to the more caudal raphe obscurus nucleus (11). Furthermore, MR nuclei constitute a major modulatory system in the control of breathing. Stimulation of peripheral chemoreceptors also evokes changes in raphe neuron activity and 5-HT concentration (31). In addition, there are relation between MR and another chemoreceptive site, RTN. Neurons in the RTN respond to brain PCO_2 presumably *via* their intrinsic chemosensitivity and to carotid chemoreceptor activation. However, mechanisms responsible for the carotid body-independent activation of RTN neurons by rising levels of arterial CO_2 are not definitely established (17). In light of this evidence, our experiments were performed in peripherally chemodenervated rabbits to prevent stimulatory effects of afferents raising from the carotid bodies on serotonergic and other chemosensitive neurons during normoxia and hypercapnia.

Fluoxetine is a selective serotonin re-uptake inhibitor that causes an increase in extracellular 5-HT in several subcortical brain regions due to 5-HT transporter blockade at serotonergic cell bodies (4, 8, 20). Because 5-HT is involved in many aspects of the neural control of breathing and it may facilitate the central respiratory chemoreflex, we hypothesized that acute 5-HT re-uptake inhibition with fluoxetine should have significant effects on hypercapnic respiratory response. However, the initial increases in 5-HT are not sufficient for therapeutic effects of fluoxetine. Because, the initial increase in 5-HT by acute administration of fluoxetine decreases the activity of 5-HT neurons through activation of 5-HT_1A autoreceptors. However, with repeated treatment, the inhibition of 5-HT unit activity decreases as the 5-HT_1A autoreceptor desensitizes to the effects of 5-HT (8, 20). Taylor *et al*. (38) observed an increased ventilatory response to hypercapnia after chronic microdialysis treatment with fluoxetine into the medullary raphe, although systemic fluoxetine administration did not alter the ventilatory response to modest hypercapnia. They suggested that chronic 5-HT transporter blockade throughout the brain may not have a cummulative effect on ventilation during CO_2 stress. In contrast, Henderson *et al*. (20) conclude that chronic fluoxetine slightly depresses respiratory control at rest, but has minimal effects during exercise or with mild hypercapnia during rest or exercise in goats. On the other hand, it has been indicated that intracerebroventricular (ICV) 5-HT stimulates baseline ventilation and reverses the blunted hypercapnic ventilatory response in mice with near-complete absence of central serotonin neurons (25).

In light of these observations, the acute and chronic effects of fluoxetine on the hypercapnic ventilatory response change according to route of administration. Consequently, there is a controversy on whether fluoxetine has a role in the hypercapnic ventilatory response. In this study, we used acute fluoxetine to show the effect of endogene 5-HT. Taking this into consideration, acute fluoxetine was given *via* ICV into the lateral ventricules to investigate a direct central effects of fluoxetine by increasing 5-HT on neuronal network during normoxia and hypercapnia and to make sure that the determined dose of drugs reached to the lateral ventricles.

The aim of the present study was to investigate the effect of acute central fluoxetine on normoxic and hypercapnic respiratory response in the absence of peripheral chemoreceptors impulses. 5-HT_2 receptors play a critical role in respiratory rhythm generation (24) and modulation of respiratory motor neurons (7). For this reason, ICV ketanserin was used to clarify the effect of 5-HT_2 receptors on ventilatory responses induced by ICV fluoxetine and hypercapnia.

Materials and Methods

Animals and General Protocol

Experiments were carried out on 18 peripherally chemodenervated albino male rabbits weighing 2.4 ± 0.3 kg. All experimental protocols were performed in accordance with the National Institutes of Health guidelines and with the approval of the Istanbul University Animal Care and Use Ethics Committee. Test animals were divided into two groups: Fluoxetine (n = 9) and Ketanserin (n = 9). Animals were anesthetized with urethane (Sigma, St. Louis, MO, USA) (400 mg/kg i.v.) and alpha-chloralose (Sigma) (40 mg/kg i.v.). The onset of a surgical level of anesthesia was determined when they became flaccid and no longer exhibited either eye-blink or limb-withdrawal actions. Tracheotomy was done and the tracheal cannula connected to an inspiratory-expiratory valve was inserted into the trachea. The femoral vein and both femoral arteries were isolated. Catheters were inserted into both femoral arteries to obtain blood samples and to record the systemic arterial blood pressure (BP). The femoral vein was catheterized for

administration of saline solution. All rabbits were given heparin (500 U/kg i.v.) in order to prevent thrombosis which could occur before the experiment. Rectal temperature was monitored with a rectal thermistor and maintained at 37°C by a heat lamp. Animals were euthanized by i.v. injection of an overdose (500 mg/kg) of sodium pentobarbital that caused rapid and irreversible cardiac arrest.

Denervation of Peripheral Chemoreceptors

In order to denervate the carotid chemoreceptors, bifurcation regions of carotid artery were isolated bilaterally. N. caroticuses were cut. To denervate the aortic chemoreceptors, N. aorticuses were isolated and cut bilaterally in the middle cervical region. Anesthetized rabbit preparation responds with an increase in ventilation to i.v. NaCN before denervation and does not respond after (15, 16, 35, 36). For this reason, chemodenervation procedure was tested by the absence of ventilatory response to NaCN (40 µg/kg i.v.) injection.

ICV Catheter Placement

A cannula was placed intracerebroventricularly (ICV) to the left lateral ventricle with the stereotaxic method. Skulls of the animals were fixed to the stereotaxic device (Stoelting Co. Stellar Cat. No: 51 400; Wood Dale IL, Chicago, IL, USA) after which the scalp was incised at eye-level and the periosteum covering the bone was peeled. Skull was penetrated at 13 mm anterior to Lambda and at 2.5 mm to the left of the midline with a dentist's drill without damaging the dura mater. A screw was placed (to the point approximately 2 mm away from the hole) into the skull to fix the cannula after placement. The tip of the cannula, fixed to the stereotaxic device, was positioned opposite the hole in the skull. The cannula was placed in the left lateral ventricle at 9-mm depth and at an angle of 90°. Acrylic cement (Cold curing acrylic denture repair material powder + Panacryl self-cure acrylic repair material liquid, Zeist, Holland) was used for the fixation of the cannula. After the experiment, location of the cannula was verified. Methylene blue was injected through the ICV cannula after which craniotomy was done. Cerebrum was divided at the midline and methylene blue was seen in the lateral ventricle (16).

Drugs

Central injection of fluoxetine (Sigma) was made with a Hamilton syringe, at a dose of 10.12 mmol/kg, into the left lateral ventricle. This dose of fluoxetine was determined as choosing 7 mg/kg i.v. peripheral dose (3, 7, 10 mg/kg) by using a dose-response curve (38). For central doses, constant quantities on an average of 1/20 of the peripheral dose was used.

The dose of ketanserin (a selective 5-HT_2 receptor antagonist) (Sigma) was 0.25 mmol/kg ICV (12). The half-life of ketanserin is 2 to 3 h (29) and the half-life of fluoxetine is 48 to 72 h (2). These agents were dissolved in sterile isotonic saline solution. All solutions were freshly prepared on the day of the study. Injections of 0.1 ml were administered over one minute.

Recordings

Tidal volume (V_T) and respiratory frequency (f_R) of the rabbits were recorded with a Powerlab (16 SP, ADInstruments, Castle Hill, Australia) during air breathing and breathing of hypercapnic gas (7% CO_2 – 93% air) from the spirometer in each groups. Ventilatory response was tested by attaching an inspiratory-expiratory valve to the tracheal cannula, which in turn was connected *via* a threeway valve to a spirometer containing the hypercapnic gas mixture. By means of this arrangement, the animal was allowed to breathe through the inspiratory part of the valve either the atmospheric air or the hypercapnic gas mixture from the spirometer. For the recording of V_T and f_R, the expiratory part of the valve was connected to Respiratory Flow Head (MLT 10 L, ADInstruments, Castle Hill, Australia) attached a Spirometer Amplifier (ML 140, ADInstruments). For the recording of BP, right femoral arterial cannula was connected to a Quad Bridge Amplifier (MLT 0380/D, ADInstruments) by means of Reusable BP Transducer (MLT 0380/D, ADInstruments). BP was recorded to monitor whether BP changed in physiological limitation by the effect of experimental procedure. Respiratory minute volume (V_E) was calculated and recorded *via* Chart 5 software (ADInstruments).

PaO_2, $PaCO_2$ and pHa values were measured from arterial blood samples using a blood gas analyzer (Blood Gas Ciba Corning 860, Ramsey, MN, USA).

Experimental Procedure

The Fluoxetine Group: Animals were initially allowed to breathe room air (normoxia) for 15 min. The mean values of f_R, V_T and V_E during steady ventilation in room air breathing were taken as baseline values. Hypercapnia Phase: The animals were then switched to the hypercapnic gas mixture (7% CO_2 – 93% Air) for 3 min. Fluoxetine Phase: Consequent to normoxia phase (15 min), fluoxetine (10.12 mmol/kg) was slowly injected (1 min) *via* the ICV catheter while respiratory parameters were recorded during normoxia. Fluoxetine was administered only once

throughout the experiment. Fluoxetine + Hypercapnia phase: Following air breathing (15 min), in order to determine the effect of ICV fluoxetine injection on hypercapnic ventilatory response, the animals were exposed to the hypercapnic gas for 3 min while the effect of fluoxetine persisted.

The Ketanserin Group: Hypercapnia Phase: Consequent to normoxia phase (15 min), animals were exposed to hypercapnic gas mixture. Ketanserin Phase: Following air breathing (15 min), ICV Ketanserin (10 µg/kg) was slowly injected during normoxia. Ketanserin + Fluoxetine Phase: After blocking of 5-HT_2 receptors by ketanserin, fluoxetine was injected during normoxia. The half-life of ketanserin is 2-3 h (29). Therefore, fluoxetine injection was performed long before the half-life of ketanserin was over. Ketanserin was administered only once throughout the experiment. Ketanserin + Fluoxetine + Hypercapnia Phase: After air breathing (15 min), to study the effect of ketanserin on hypercapnic ventilatory response in the continued presence of fluoxetine, the rabbits were allowed to breathe hypercapnic gas. This phase was called the Ketanserin + Fluoxetine + Hypercapnia phase because the effects of both substances continued.

Statistical Analysis

The last 5 min of air breathing after the rabbits reached steady ventilation and all 3 minutes of hyercapnic gas exposure were marked and selected from the recordings. The selected data were analyzed with Chart 5 software of ADInstrument. Statistical significance of differences in f_R, V_T, V_E, PaO_2, $PaCO_2$ and pHa before and after ICV administration of fluoxetine and ketanserin during normoxia and hypercapnia was analyzed by the Wilcoxon-Matched Pairs test in the both groups. Furthermore, to express the ventilatory responses to CO_2, "CO_2 sensitivity Index" was calculated by the change in V_E (ml/min) divided by the change in arterial $PaCO_2$ (mmHg) for each individual animal and for each experimental phase. Mean values were analyzed by the Wilcoxon-Matched Pairs test. The percent changes of respiratory parameters in each phase were analyzed by one-way analysis of variance (ANOVA) and they were compared by the Bonferroni HSD test. The percent changes of respiratory parameters in both groups were compared by the Mann-Whitney U test. In all phases, $P < 0.05$ was considered statistically significant.

Results

The Fluoxetine Group

Effect of hypercapnia on ventilation (Hypercapnia Phase). Significant increases in f_R (from 21.90 ± 0.33 to 23.08 ± 0.47, $P < 0.05$), V_T (from 40.09 ± 0.67 to 58.17 ± 0.61, $P < 0.01$) and V_E (from 876.27 ± 4.66 to1340.36 ± 16.56, $P < 0.01$) were observed in peripherally chemodenervated rabbits during breathing of hypercapnic gas (Fig. 1A and Table 1).

Effect of ICV fluoxetine on normoxic ventilation (Fluoxetine Phase). Following air breathing (15 min), when ICV Fluoxetine (10.12 mmol/kg) was administered during normoxic ventilation, significant increase in V_T (from 40.01 ± 0.77 to 43.27 ± 0.90, $P < 0.01$) was observed (Fig. 1B and Table 1). There was no significant change in f_R. Consequently, the significant increase in V_E (from 919.42 ± 11.61 to 982.46 ± 14.83, $P < 0.01$) was mainly due to increase in V_T. The mean peak-response time of fluoxetine was calculated as 40 ± 0.5 seconds.

Effect of ICV fluoxetine on hypercapnic ventilatory responses (Fluoxetine + Hypercapnia Phase). When the animals were switched to hypercapnia f_R (from 23.91 ± 0.65 to 25.27 ± 0.92, $P < 0.05$), V_T (from 39.57 ± 0.61 to 64.07 ± 1.42, $P < 0.01$) and V_E (from 944.79 ± 24.39 to 1619.36 ± 73.38, $P < 0.01$) increased significantly while the effect of fluoxetine persisted (Fig. 1C and Table 1).

To indicate the effect of fluoxetine on hypercapnic ventilatory response, percent changes of the respiratory parameters in the Fluoxetine + Hypercapnia phase was compared with those of the Hypercapnia phase; the degrees of increases in V_T, V_E were found to be significantly higher than those of the Hypercapnia phase ($P < 0.01$ and $P < 0.05$, respectively) (Fig. 2).

CO_2 sensitivity Index in the Hypercapnia phase was found as 32.29 ± 1.27. There was an significantly increase in CO_2 sensitivity Index in the Fluoxetine + Hypercapnia phase (40.88 ± 3.20). CO_2 sensitivity Index increased by 27.62 ± 9.89%, $P < 0.01$.

The Ketanserin Group

Effect of hypercapnia on ventilation (Hypercapnia Phase). In the ketanserin group, following air breathing, significant increases in f_R (from 22.30 ± 0.29 to 24.01 ± 0.45, $P < 0.05$), V_T (from 39.23 ± 0.65 to 55.89 ± 0.73, $P < 0.01$) and V_E (from 874.82 ± 4.58 to 1342.91 ± 15.93, $P < 0.01$) were also observed during breathing of hypercapnic gas (Fig. 3A and Table 2).

Effect of ICV ketanserin on normoxic ventilation (Ketanserin Phase). After air breathing (15 min), ICV ketanserin administration significantly decreased f_R (from 23.74 ± 0.38 to 21.20 ± 0.39, $P < 0.01$), V_T (from 39.96 ± 0.72 to 37.62 ± 0.63, $P < 0.01$) and V_E (from 948.65 ± 22.89 to 797.61 ± 19.77, $P < 0.01$). This response was obtained in 1.5 ± 0.2 min after the injec-

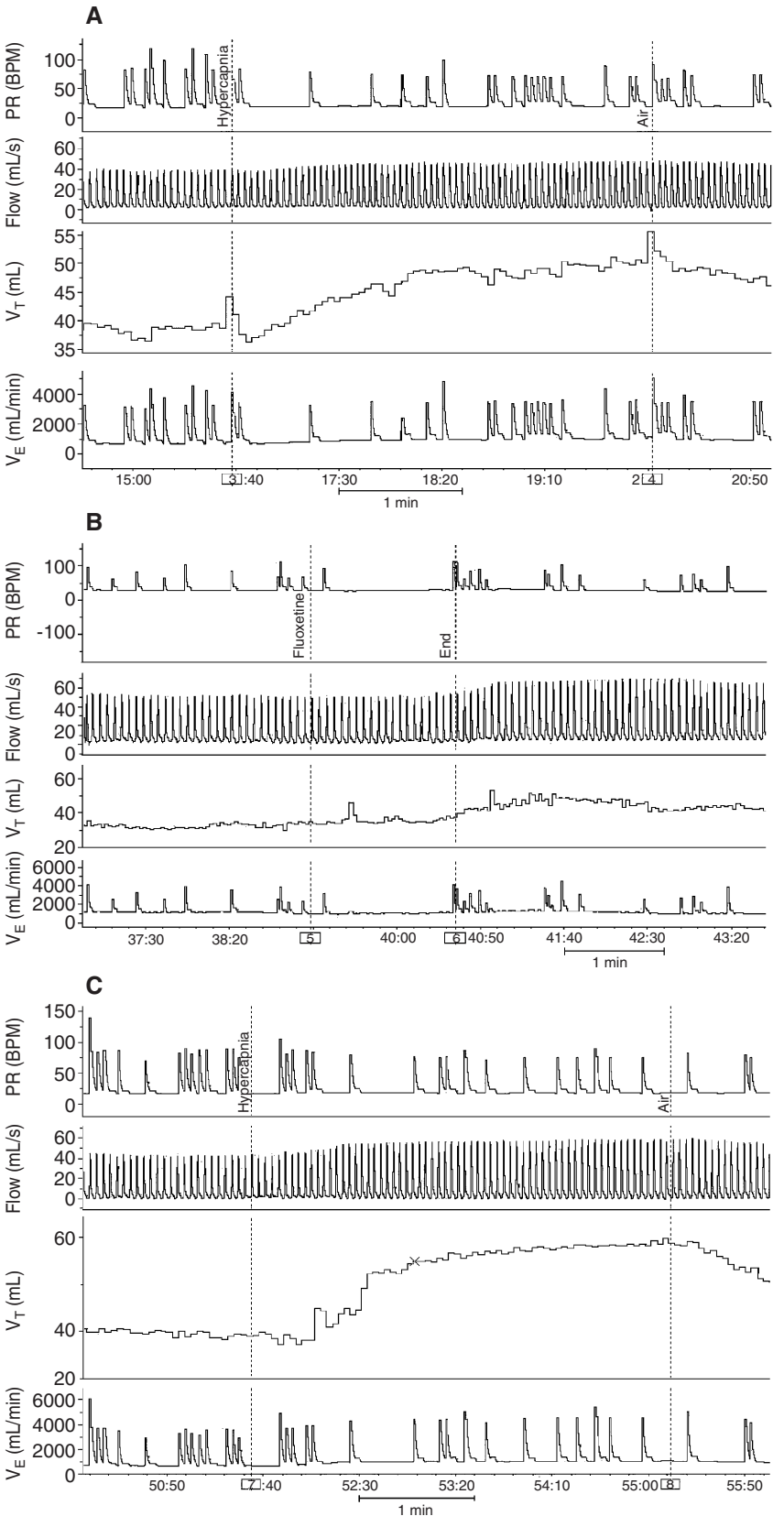

Fig. 1. Effects of Hypercapnia (7% CO_2 - air) (A), Fluoxetine (10.12 mmol/kg) and Fluoxetine + Hypercapnia (C) phases on respiratory parameters in peripherally chemodenervated rabbits. f_R, respiratory frequency; V_T, tidal volume; V_E, respiratory minute volume. Horizontal bar between two dotted lines indicates the ICV administrations of drugs (1 min) and exposure time to hypercapnic gas mixture.

Table 1. Effects of acute ICV fluoxetine (10.12 mmol/kg) on respiratory parameters (f/min, V_T, V_E) during normoxia and hypercapnia in peripherally chemodenervated rabbits

Experimental Phase n = 9	f /min		V_T (ml)		V_E (ml/min)	
	Baseline Value	Response	Baseline Value	Response	Baseline Value	Response
Fluoxetine Group						
Hypercapnia	21.90 ± 0.33	23.08 ± 0.47*	40.09 ± 0.67	58.17 ± 0.61**	876.27 ± 4.66	1340.36 ± 16.56**
Fluoxetine	23.02 ± 0.36	22.75 ± 0.37	40.01 ± 0.77	43.27 ± 0.90**	919.42 ± 11.61	982.46 ± 14.83**
Fluoxetine + Hypercapnia	23.91 ± 0.65	25.27 ± 0.92*	39.57 ± 0.61	64.07 ± 1.42**††	944.79 ± 24.39	1619.36 ± 73.38**†

Values shown are means ± SE. n = 9: the animal numbers in each groups. f/min; respiratory frequency, V_T; tidal volume, V_E; respiratory minute volume. Baseline value indicates the steady ventilation in air breathing. The response indicates the value obtained in experimental phase. Asterisks indicate statistical significance when the response is compared with the baseline value in each phase. *$P < 0.05$, **$P < 0.01$. The † symbol indicates statistical significance of the means of the respiratory response in Fluoxetine + Hypercapnia phase when compared with the Hypercapnia phase. †$P < 0.05$, ††$P < 0.01$.

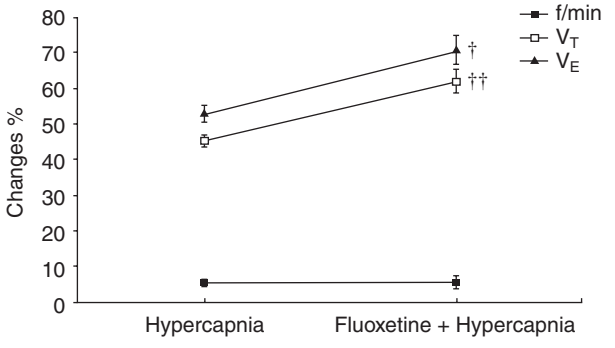

Fig. 2. Percent changes of respiratory parameters of the peripherally chemodenervated rabbits in the Hypercapnia and Fluoxetine + Hypercapnia phases. f/min; respiratory frequency, V_T; tidal volume, V_E; respiratory minute volume. The † symbol indicates statistical significance when percent changes in Fluoxetine + Hypercapnia was compared with that in Hypercapnia alone. †$P < 0.05$, ††$P < 0.01$.

tion (Fig. 3B and Table 2).

Effect of ICV fluoxetine on normoxic ventilation following ICV ketanserin (Ketanserin + Fluoxetine Phase). The injection of ICV Fluoxetine after ketanserin produced no change in respiratory parameters (Table 2). After that, the animals were allowed to breathe air (normoxia) for 15 min.

Effect of ICV ketanserin on hypercapnic ventilatory response in the presence of fluoxetine (Ketanserin + Fluoxetine + Hypercapnia Phase). After blocking 5-HT_2 receptors by ketanserin and after injection of fluoxetine, the rabbits were exposed to hypercapnic gas. Significant increases in f_R (from 22.02 ± 0.42 to 23.27 ± 0.53, $P < 0.01$), V_T (from 39.73 ± 0.70 to 47.82 ± 1.06, $P < 0.01$) and V_E (from 874.04 ± 17.81 to 1111.99 ± 29.68, $P < 0.01$) were observed (Fig. 3C and Table 2).

When the percent changes of the respiratory parameters in the Ketanserin + Fluoxetine + Hypercapnia phase were compared with those of the Hypercapnia phase, the degrees of increases in V_T and V_E were found to be less than those observed in the Hypercapnia phase ($P < 0.01$ and $P < 0.001$, respectively) (Fig. 4). Furthermore, increases in percentage values in V_T and V_E in the Ketanserin + Fluoxetine + Hypercapnia phase were lower than those during observed in the Fluoxetine + Hypercapnia phase ($P < 0.01$ and $P < 0.001$, respectively) (Fig. 5).

CO_2 sensitivity Index in the Hypercapnia phase was found to be 26.48 ± 1.37. There was a significantly decrease in CO_2 sensitivity Index in the Ketanserin + Fluoxetine + Hypercapnia phase (12.16 ± 0.96). CO_2 sensitivity Index decreased by 52.28 ± 9.89%, $P < 0.01$.

Changes of PaO_2, $PaCO_2$ and pHa Values

As shown in Table 3, variations in PaO_2, $PaCO_2$ and pHa values reflected the changes of the respiratory parameters during each experimental phase of both the Fluoxetine and Ketanserin groups.

Discussion

The results of the present study demonstrate that acute fluoxetine administration stimulates ventilation at rest, and enhances ventilatory response to hypercapnia in anesthetized and peripherally chemodenervated rabbits. Furthermore, ICV ketanserin prevents the stimulatory effect of ICV fluoxetine on normoxic ventilation and reduces the degree of hypercapnic ventilatory response.

Hypercapnia increases f_R, V_T and V_E by stimulat-

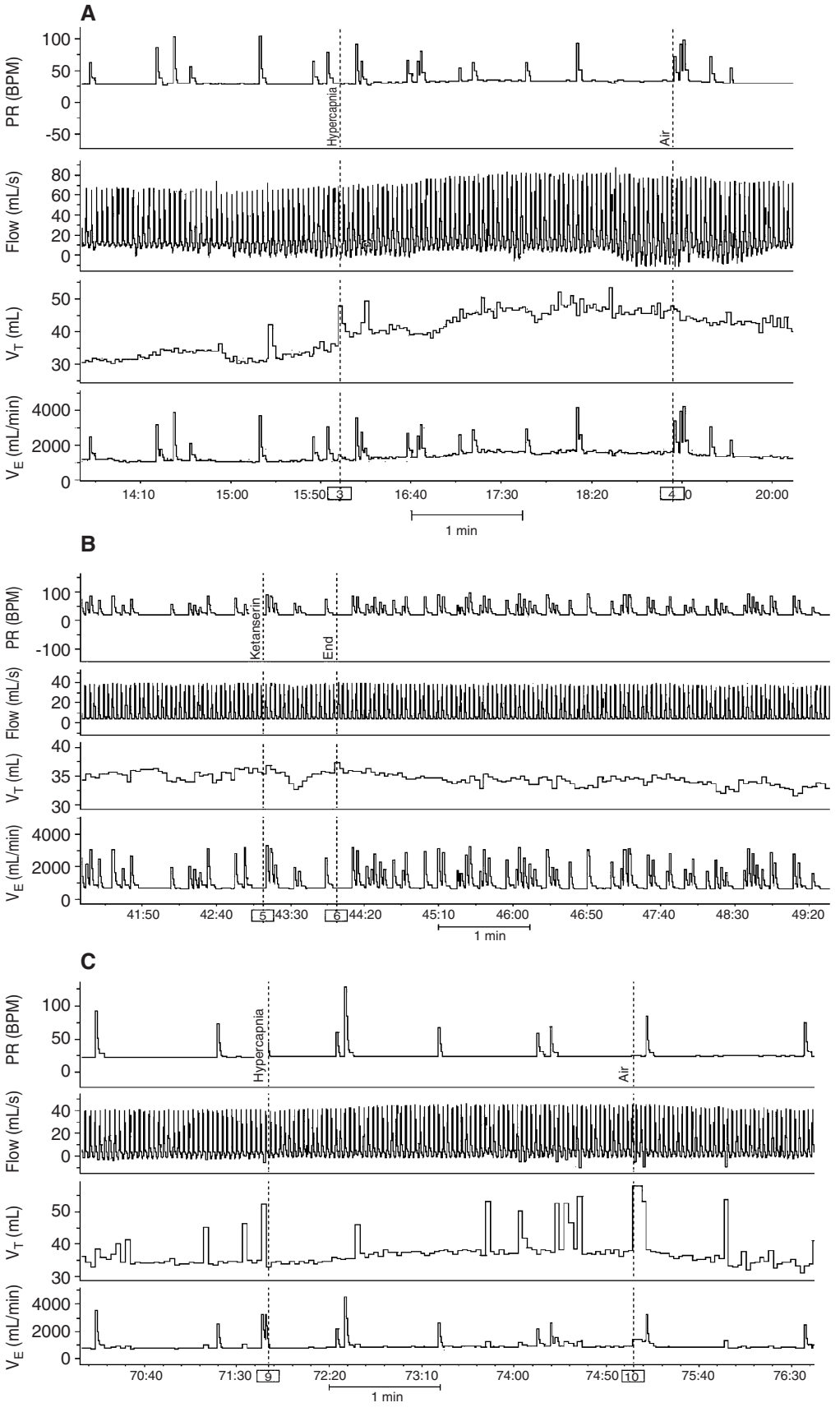

Fig. 3. Effects of Hypercapnia (7% CO_2 - air) (A), Ketanserin (0.25 mmol/kg) (B) and Ketanserin + Hypercapnia (C) phases on respiratory parameters in peripherally chemodenervated rabbits.

Table 2. Effect of ICV ketanserin (0.25 mmol/kg) and fluoxetin on respiratory parameters (f/min, V_T, V_E) during normoxia and hypercapnia in peripherally chemodenervated rabbits

Experimental Phase n = 9	f/min		V_T (ml)		V_E (ml/min)	
	Baseline Value	Response	Baseline Value	Response	Baseline Value	Response
Ketanserin Group						
Hypercapnia	22.30 ± 0.29	24.01 ± 0.45*	39.23 ± 0.65	55.89 ± 0.73**	874.82 ± 4.58	1342.91 ± 15.93**
Ketanserin	23.74 ± 0.38	21.20 ± 0.39**	39.96 ± 0.72	37.62 ± 0.63**	948.65 ± 22.89	797.61 ± 19.77**
Ketanserin + Fluoxetine	24.29 ± 0.46	24.39 ± 0.42	39.81 ± 0.78	39.78 ± 0.81	966.50 ± 23.80	970.19 ± 25.08
Ketanserin + Fluoxetine + Hypercapnia	22.02 ± 0.42	23.27 ± 0.53**	39.73 ± 0.70	47.82 ± 1.06**††	874.04 ± 17.81	1111.99 ± 29.68**†††

Values are means ± SE. n = 9. See footnote to Table 1. Asterisks indicate statistical significance when the response is compared with the baseline value in each phase. *$P < 0.05$, **$P < 0.01$. The † symbol indicates statistical significance of the means of the respiratory response in Fluoxetine + Hypercapnia phase when compared with the hypercapnia phase. ††$P < 0.01$, †††$P < 0.001$

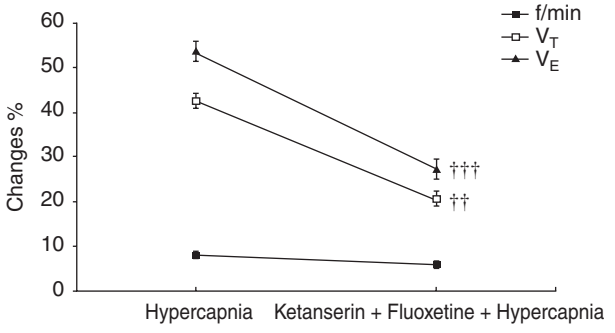

Fig. 4. Percent changes of respiratory parameters of the peripherally chemodenervated rabbits in Hypercapnia and Ketanserin + Fluoxetine + Hypercapnia phases. The † symbol indicates statistical significance when percent changes in Ketanserin + Fluoxetine + Hypercapnia were compared with that in Hypercapnia alone. ††$P < 0.01$, †††$P < 0.001$.

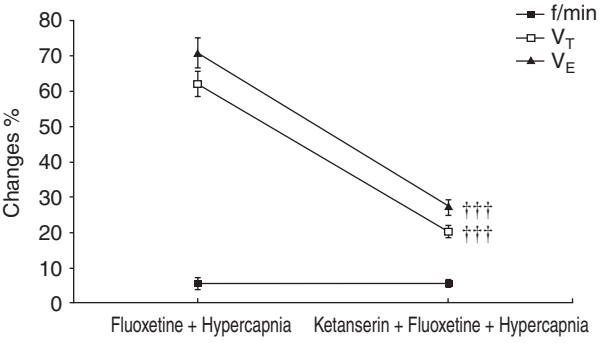

Fig. 5. Percent changes of respiratory parameters of the peripherally chemodenervated rabbits in Fluoxetine + Hypercapnia and Ketanserin + Fluoxetine + Hypercapnia phases. The † symbol indicates statistical significance when percent changes in Ketanserin + Fluoxetine + Hypercapnia were compared with that in Fluoxetine + Hypercapnia phase in both group. †††$P < 0.001$.

ing both peripheral and central chemoreceptors (35) and the ventilatory response to CO_2 is reduced in animals after denervation of peripheral chemoreceptors (9, 12, 13). There is evidence that supports the hypothesis that 5-HT neurons are respiratory CO_2 central chemoreceptors (26-28, 34, 39). However, there are also arguments made against to this hypothesis (17, 30, 37). The serotonergic neurons in the MR are capable of influencing ventilation during hypercapnia in fully conscious animals models during sleep and wakefulness (39). Acute ICV fluoxetine administration caused an elevation in ventilation due to an increase in V_T during normoxia without an effect on f_R. Fluoxetine inhibits 5-HT re-uptake at the axon terminals of serotonergic neurons, thus, elevating synaptic levels of 5-HT in several subcortical brain regions due to serotonin transporter blockade at serotonergic cell bodies (4, 8, 11). In our recent study, ICV injection of 5-HT (20 µg/kg) in anesthetized peripherally chemodenervated rabbits during normoxia caused an increase in ventilation due to an increase in V_T and it also reduced the degree of acute hypoxic ventilatory depression during hypoxia (15). We did not find an effect on f_R. *In vivo* studies have suggested that ICV 5-HT has either inhibitory or excitatory effect on f_R (22). The absence of elavation in f_R in the current study might be due to the administration of fluoxetine *via* ICV and the absence of peripheral chemoreceptor impulses. Peripheral chemoreceptors are active during normoxia (33, 36). Facilitatory input from peripheral chemoreceptors causes glutamate release in nTS which is the first central synapsis for primary afferents rising from peripheral chemoreceptors. Glutamate increases excitatory drive activating AMPA and NMDA

Table 3. Effect of ICV Fluoxetine and Ketanserin on PaO_2, $PaCO_2$ and pHa during normoxia and hypercapnia in peripherally chemodenervated rabbits in both groups

Experimental Phase n = 9	PaO_2 (mmHg)		$PaCO_2$ (mmHg)		pHa	
	Baseline Value	Response	Baseline Value	Response	Baseline Value	Response
Fluoxetine Group						
Hypercapnia	91.20 ± 2.30	106.08 ± 2.13***	39.79 ± 2.30	54.17 ± 3.10***	7.38 ± 0.04	7.25 ± 0.03***
Fluoxetine	93.34 ± 3.45	99.80 ± 4.20**	41.01 ± 2.90	36.27 ± 2.40**	7.38 ± 0.03	7.40 ± 0.06*
Fluoxetine + Hypercapnia	93.8 ± 2.9	105.9 ± 4.6***	39.6 ± 2.5	56.1 ± 2.6***	7.4 ± 0.04	7.3 ± 0.03***
Ketanserin Group						
Hypercapnia	93.40 ± 2.90	108.08 ± 2.13***	38.23 ± 3.60	55.89 ± 3.70***	7.38 ± 0.03	7.25 ± 0.02***
Ketanserin	92.30 ± 3.50	79.80 ± 3.20***	39.96 ± 3.60	46.32 ± 3.45***	7.38 ± 0.03	7.31 ± 0.03***
Ketanserin + Fluoxetine	95.34 ± 3.45	96.80 ± 4.20	41.01 ± 2.90	39.27 ± 2.40	7.38 ± 0.03	7.39 ± 0.06
Ketanserin + Fluoxetine + Hypercapnia	95.75 ± 2.70	102.87 ± 3.60***	39.54 ± 2.60	59.09 ± 2.30***	7.37 ± 0.04	7.27 ± 0.04***

Values are means ± SE. n = 9. Baseline value indicates the steady ventilation in air breathing. The response indicates the value obtained in experimental phase. Asterisks indicate statistical significance when the response is compared with the baseline value. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

receptors in respiratory neurons (16). Stimulation of nTS also stimulates raphe nuclei (19). This is why increase in ventilation during normoxia following acute ICV administration of fluoxetine depends on the increase in synaptic 5-HT concentration and the stimulator effect of 5-HT on respiratory network. Thus, increased central inspiratory activity and volume threshold of the inspiratory off-switch mechanism increase ventilation.

However, the increase in percent change values of V_T and V_E in the Fluoxetine + Hypercapnia Phase was found to be higher than that of the hypercapnia phase. This may have resulted from increased extracellular 5-HT concentration due to the effect of fluoxetine causing further increase in the hypercapnic ventilatory response by enhancing neuronal sensitivity to CO_2. The significant increased percent value of CO_2 sensitivity Index in the Fluoxetine + hypercapnia phase also supported this comment. The role of 5-HT neurons in response to hypercapnia is complex and depends upon species and impulses arising from carotid bodies (1, 9, 31). Wu *et al*. (40) indicated that 5-HT neurons increased their firing rate in response to increasing CO_2 in rat and mouse brain slices after blocking fast glutamate and GABA receptors. This chemosensitivity within the raphé is not due to input from another brain stem region. In our study, fluoxetine affected not only the medulla oblongata but also higher brain stem and other cerebral regions as it was administrated into the left lateral ventricule. Inputs from peripheral and/or suprapontine structures are important in the serotonergic control of the respiratory pontomedullary network (5). Suprapontine regions contain $5\text{-}HT_{1,2}$ receptors and their projections to medullary neurons can directly or indirectly effect the control of respiration (34). Peripheral afferents also have important influences on the triggering of $5\text{-}HT_{1,2}$ system in the nTS (5).

The argument that 5-HT neurons are not central chemoreceptors is based on the assertion that 5-HT neurons do not have a large response to CO_2 *in vivo* (17). This conclusion is based on recordings from rats under halothane anesthesia in which 5-HT neurons in the rostral ventrolateral medulla have no response to hypercapnia (30). Anesthesia is well known to have major effects on respiratory control. On the other hand, it has been suggested that RTN neurons respond to brain PCO_2 under hyperoxic conditions and chloralose-urethane anaesthesia, presumably *via* their intrinsic response to extracellular pH (37). In our previous study, ICV administration of 5-HT to rabbits under chloralose-urethane anaesthesia increased normoxic ventilation (15). The current research was also performed in rabbits anaesthetized with chloralose-urethane. Higher increase in respiration during the Hypercapnia + Fluoxetine phase indicates that rabbits under chloralose-urethane anaesthesia can respond to acute ICV Fluoxetine and consequently central 5-HT can increase central inspiratory activity. In this study, baseline values were

determined before each experimental phase in anesthetized rabbits while breathing room air. The response determined in experimental phases was compared to previous baseline values. For this reason, each response showed that the animals were able to respond despite anesthesia.

Furthermore, serotonergic function in the MR is important for ventilatory response to hypercapnia (28). Inhibition of MR neurons by focal microdialysis of the GABA receptor agonist muscimol (10 mM) significantly reduced ventilation by 17% in unanesthetized piglet model during a 5% CO_2 challenge but not in room air breathing (14). It was shown that daily focal fluoxetine microdialysis into the MR nuclei of the rat over a 15- day period could enhance ventilatory response to moderate hypercapnia due to an increase in V_T during both quite wakefulness and NREM sleep in an unanesthetized rat model, but systemic fluoxetine administration did not alter the ventilatory response to moderate hypercapnia (38). In contrast, it was observed in goats that chronic fluoxetine slightly depressed respiratory control at rest, but had minimal effects during exercise or with mild hypercapnia during rest or exercise (20).

Systemic 5-HT re-uptake inhibition with fluoxetine most likely affects several aspects of serotonergic function in the central nervous system. If regions are affected differentially, it is possible that serotonergic effects are offsetting such that no net changes in ventilation occur. In contrast, ICV administration of fluoxetine caused facilitating effect on normoxic and hypercapnic ventilatory responses. More recently, midline 5-HT neurons, which were specificaly lesioned in adult rats using the toxin saporin conjugated to an antibody against the 5-HT transporter, led to a decrease in the hypercapnic ventilatory response. In addition, injection of 8-hydroxy- 2 (di-n-propylamino) tetralin (8-OH-DPAT) to silence 5-HT neurons led to a decrease in the hypercapnic ventilatory response (39). On the other hand, near-complete 5-HT neuron loss in adult $Lmx1b^{f/f/p}$ mice did not affect baseline ventilation or hypoxic ventilatory response, but resulted in a 50% deficit in the hypercapnic ventilatory response. In addition, these data indicate that exogenous 5-HT stimulates baseline ventilation and reverses the blunted hypercapnic ventilatory response in $Lmx1b^{f/f/p}$ mice (25). These animal studies corroborate with our findings. Thus, several neurotransmiters or neuropeptides released by 5-HT neurons directly enhance the excitability of multiple subsets of neurons within the respiratory network.

While acute ICV fluoxetine caused an elevation in V_E during normoxia, ICV ketanserin caused a decrease in V_E due to a decrease in both f_R and V_T. Kanamura *et al*. (26) suggested that 5-HT_2 receptor activity in the dorso medial medulla (DMM) increased basal levels of airway dilation and ventilatory volume. These authors also suggested that 5-HT release acting on 5-HT_2 receptors in the DMM contributed to facilitation of respiratory motor and premotor neuron activity.

In our study, abolition of increases in V_T and V_E induced by the effect of ICV fluoxetine after ketanserin administration during normoxia suggested that ventilation probably increased by the activation of 5-HT_2 receptors. Ketanserin also has moderate affinity to 5-HT_7 receptors but it has greater affinity to 5-HT_2 receptors. Furthermore, ketanserin not only prevents the enhancing effect of fluoxetine on V_E during normoxia, but also reduces the degree of hypercapnic ventilation. The significant decrease in percent value of CO_2 sensitivity Index in the Ketanserin + Fluoxetine + hypercapnia phase also supported this comment. Kanamura *et al*. (26) also showed that hypercapnic responses of airway dilation and ventilatory augmentation were not suppressed by 5-HT_2 receptor antagonism in the DMM and suggested that 5-HT release acting on 5-HT_2 receptors in the DMM did not contribute to facilitation of sensitivity to CO_2 and pH. On the other hand, Cayetanot *et al*. (6) demonstrated that central respiratory modulation occurs *via* 5-$HT_{2A/2C}$ receptors induced by 5-HT. In our study, we confirm that ICV fluoxetine contributes to facilitation of sensitivity to CO_2. In addition blocking of 5-HT_2 receptors by ketanserin reduces the stimulator effect of fluoxetine and hypercapnia on respiratory neuronal network.

In conclusion, acute ICV fluoxetine caused both an increase in ventilation during normoxia as well as an increase in the magnitude of central hipercapnic ventilatory response. These facilitating effects of fluoxetine on the respiratory drive are likely to be mediated by central 5-HT_2 receptors and appear to be associated with an increase in 5-HT induced by acute ICV fluoxetine.

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