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SYSTEMIC ADMINISTRATION OF SEROTONIN 2A/2C AGONIST IMPROVES UPPER AIRWAY STABILITY IN ZUCKER RATS

Toshiyuki Ogasa[1, 3], M.D., Ph.D., Andrew D. Ray[2], M.S., Charles P. Michlin[2], B.S., Gaspar A. Farkas[1, 2], Ph.D., Brydon J. B. Grant[1], M.D., and Ulysses J. Magalang[1, 3], M.D.

[1]Department of Medicine, Division of Pulmonary, Critical Care, and Sleep Medicine, [2]Department of Exercise and Nutrition Sciences, University at Buffalo, Buffalo, New York, and [3]Department of Medicine, Division of Pulmonary and Critical Care Medicine, The Ohio State University, Columbus, Ohio

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Address Correspondence and reprint request to:

Ulysses J. Magalang, M.D.

Division of Pulmonary and Critical Care Medicine

201 Davis Heart and Lung Research Institute

473 West 12th Avenue

Columbus, OH 43210

Tel: (614) 293-4925

Fax:(614) 293-4799

e-mail: magalang-1@medctr.osu.edu

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ABSTRACT

The effects of [±]-2,5-Dimethoxy-4-iodoaminophentamine, a serotonin$_{2A/2C}$ receptor agonist, on pharyngeal airflow mechanics were examined in isoflurane-anesthetized lean and obese Zucker rats. The pharyngeal pressure associated with flow limitation, maximum inspiratory flow, oronasal resistance, genioglossus muscle activity, and arterial blood pressure were measured before and after intravenous administration of the agonist. A robust activation of the genioglossus muscle in all lean and obese rats was associated with decreased upper airway collapsibility ($p<0.05$), unchanged maximum flow, and increased oronasal resistance ($p<0.05$) in both groups. The changes in upper airway mechanics and blood pressure after the drug were similar in lean and obese rats. The serotonin agonist had no effect on upper airway mechanics in a group of paralyzed (pancuronium bromide) rats, despite similar elevations in blood pressure. There was a smaller decrease ($P<0.05$) in upper airway collapsibility that was also associated with increased upstream resistance when the drug was administered after bilateral hypoglossal nerve transection. We conclude that systemic administration of a serotonin$_{2A/2C}$ receptor agonist improves upper airway collapsibility predominantly, but not exclusively, via stimulation of the hypoglossal nerves and also increases upstream resistance, at least in part, through activation of non-hypoglossal motoneuronal pools innervating the upper airway muscles.

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INTRODUCTION

Serotonin (5-HT) plays an important role in the control of upper airway (UA) dilator motoneurons and it modulates UA dilator muscle activity across sleep-wake states (1-3). The control of UA motoneurons by 5-HT is thought to be mediated predominantly via 5-HT_{2A} and 5-HT_{2C} receptor subtypes, although other types of receptors may also be implicated (1, 4, 5). Indeed, intravenous administration of ritanserin, a 5-$HT_{2A/2C}$ antagonist, to awake English bulldogs reduces UA dilator muscle activity and UA cross-sectional area (6).

The obese Zucker (Z.) rat (fa/fa), a genetic model of early-onset obesity, has a defective leptin receptor (7), and develops hyperphagia, hyperleptinemia, and hyperinsulinemia (8). These rats exhibit many of the same respiratory deficits as obese humans, including reduced lung volumes, reduced chest wall compliance, blunted ventilatory responses to hypercapnia and hypoxia, and narrowed UA (9, 10). Obese Z. rats develop morphologic and mechanical changes in respiratory muscle function that are consistent with a chronic overload ; the diaphragm becomes weak

while fiber hypertrophy is observed (11).

We have previously reported that the systemic administration of ritanserin had no effect on resting ventilation in older lean Z. rats, but decreased ventilation with a rise in oxygen consumption in older obese Z. rats (12). This effect of ritanserin on ventilation in older obese Z. rats was attributed to an increase in UA collapsibility associated with a decline in UA dilator muscle activity. These effects were qualitatively similar to those found when ritanserin was administered to English bulldogs (6), an animal model of sleep-disordered breathing with narrowed UA.

The effects of augmenting UA dilator muscle activity using 5-HT agonists on UA mechanics have not been determined. The consequences of activation of UA dilator muscles on UA pressure-flow relationships using serotonergic agents would be of interest since this knowledge may provide insight on how specific pharmacologic agents may be used to prevent UA collapse. We hypothesized that administration of a 5-$HT_{2A/2C}$ receptor agonist will increase UA dilator muscle activity, and that this increased activity would stabilize the UA. Since the UA is more collapsible in obese Z.

rats compared to lean Z. rats (12), we also hypothesized that the administration of a 5-$HT_{2A/2C}$ receptor agonist will improve the stability of the UA of obese Z. rats to levels comparable to those in leans. We examined the effects of the 5-$HT_{2A/2C}$ receptor agonist, [±]-2, 5-Dimethoxy-4-iodoaminophentamine (DOI), on pharyngeal airflow mechanics and genioglossus (GG) muscle activity in the isolated UA preparation in lean and age-matched obese Z. rats. Some of the results of these studies have been previously reported in the form of an abstract (13).

METHODS

A previously described UA preparation (12, 14) was used to determine the UA maximal inspiratory airflow ($\dot{V}_{Imax}$), the pharyngeal critical pressure (Pcrit), and oronasal resistance (Ron). Details of the methods are provided online. The Institutional Animal Care and Use Committee of the University at Buffalo approved the protocols.

Effects of Serotonin (5-HT)$_{2A/2C}$ agonist on UA mechanics (Protocol A): Eight lean and eight obese Z. rats were anesthetized, and the femoral vein and artery were cannulated. EMG electrodes were implanted into the GG muscle.

The trachea was cut, and an endotracheal tube was placed into the caudal tracheal stub. The animals were mechanically ventilated and continuously anesthetized with isoflurane maintaining end-tidal CO_2 at 5%. Five measurements of $\dot{V}_{lmax}$, Pcrit, and Ron were obtained five minutes after intravenous saline, and five minutes after DOI (0.5 mg/kg i.v., Sigma Chemical, St. Louis, MO). Average values were calculated from the five measurements. The magnitude of the changes in UA mechanics induced by DOI was calculated as the delta (Δ).

Effects of 5-HT$_{2A/2C}$ agonist on UA mechanics following neuromuscular paralysis (Protocol B): In four lean and four obese Z. rats, UA mechanics were measured before and following the administration of pancuronium bromide (2 mg/kg i.v.). Fifteen to 20 minutes following paralysis, UA mechanics were measured five minutes after the administration of DOI (0.5 mg/kg, i.v.).

Effects of 5-HT$_{2A/2C}$ agonist on UA mechanics after bilateral hypoglossal nerve transection (Protocol C): Fifteen to 20 minutes following bilateral hypoglossal nerve (cnXII) denervation, UA mechanics were measured five minutes after saline and five minutes after DOI (0.5 mg/kg, i.v.) in four lean and four obese Z. rats.

Statistical Analysis: Data was analyzed using SPSS (version 12.0) software (SPSS Inc., Chicago, IL). In Protocol A, the differences in UA mechanics, integrated EMG$_{GG}$, blood pressure (BP), and heart rate data were analyzed by two-way analysis of variance (ANOVA) with repeated measurements on one factor. The between subjects factor was lean vs. obese rats. The within subjects factor with repeated measurements was vehicle vs. DOI. An interaction term was included. For the paralysis experiments, a similar analysis was employed except the data for lean and obese animals were combined since no differences in response to DOI were seen in the first series of experiments. For the denervation experiments, the effects of DOI were analyzed using paired *t*-test. We compared the systolic and diastolic BP data between the three different Protocols using two-way repeated measures ANOVA

(between subjects factor: Protocol A vs. Protocol B vs. Protocol C, within subjects factor: pre-DOI vs. DOI) to determine if the hypertensive response to DOI was different between the three protocols. All data presented in the text, tables, and figures represent means ± SEM. We checked residuals for outliers and normal distribution. We tested for compound symmetry and made adjustments using the Greenhouse-Geisser correction method when appropriate. A $p < 0.05$ was considered statistically significant. If the overall F test showed statistical significance, a *post-hoc* *t*-test with Bonferroni's correction was used to determine where the differences lie.

RESULTS

Effects of 5-HT$_{2A/2C}$ agonist on isolated UA mechanics (Protocol A):

Obese Z. rats were significantly heavier than lean Z. rats ($p<0.001$). Control (pre-DOI) Pcrit was statistically greater in obese Z. rats (less negative or more collapsible) than that of lean Z. rats ($p=0.042$) (Table 1). The administration of DOI increased the stability of the UA airway (i.e., decreased Pcrit) without a change in

$\dot{V}_{Imax}$ due to a significant increase in Ron ($F(1,14)$= 17.6, 0.9, 19.1, $p<0.001$, $p=0.363$, $p<0.001$ for Pcrit, $\dot{V}_{Imax}$, and Ron, respectively). The significant effects of DOI on Pcrit and Ron did not depend on rat type; there was no significant interaction between the level of drug (control vs. DOI) and animal type (lean vs. obese). In lean Z. rats, DOI administration led to a significant decrease in Pcrit ($p=0.009$) and a significant increase in Ron ($p=0.007$), whereas $\dot{V}_{Imax}$ remained the same (ns). In obese Z. rats, DOI also significantly decreased Pcrit ($p=0.011$), increased Ron ($p=0.009$) and did not change $\dot{V}_{Imax}$ (ns) (Figure 1, Table 1). The Pcrit of obese Z. rats following DOI administration remained statistically greater (less negative) than the post-DOI Pcrit of lean Z. rats ($p=0.037$), but improved to similar levels as the baseline values of lean Z. rats (Table 1). The ΔPcrit, $\Delta\dot{V}_{Imax}$, and ΔRon after DOI were not significantly different (ns) between lean and obese Z. rats (Table 1). The measurement of UA dynamics was reproducible. The coefficients of variation of Pcrit, $\dot{V}_{Imax}$, and Ron within an animal during the control condition were 6.1 ± 1.4%, 1.3 ± 0.2%, 5.2 ± 1.2%, respectively and 3.5 ± 0.7%, 1.3 ± 0.2%, 3.2 ± 0.7% after DOI. A representative tracing depicting the effects of DOI infusion on $\dot{V}_I$, Pph and Php obtained in a lean Z. rat is illustrated in Figure 2.

DOI infusion altered the recruitment profile of the GG muscle. A representative tracing from one lean and one obese Z. rat is shown in Figure 3. During control (pre-DOI), clear phasic activity in the GG muscle was noted in five of eight lean and in six of eight obese Z. rats. All rats exhibited low levels of tonic GG muscle activity as depicted in Figure 3 between the phasic burst activation. Following DOI administration, all eight lean and all eight obese Z. rats exhibited a marked increase in tonic activation of the GG muscle such that no clear phasic activity was detected except in one lean rat. DOI significantly increased the integrated activity of the EMG_{GG} ($F(1,14)=14.0$, $p=0.002$). The integrated activity of the EMG_{GG} of lean Z. rats after DOI was 872 ± 316 % of control ($p=0.019$), and that of obese Z. was 878 ± 267 % of control ($p=0.019$). There was no statistical difference in the integrated EMG_{GG} activity between lean and obese Z. rats after DOI (ns).

DOI infusion increased both systolic ($F(1,14)=70.8$, $p<0.001$) and diastolic ($F(1,14)=37.0$, $p<0.001$) BP in all rats. During control measurements, systolic and diastolic BP were similar between lean and obese Z. rats (Table 1). DOI produced

increases in both systolic ($p<0.001$ in lean, $p<0.002$ in obese) and diastolic ($p<0.001$ in lean, $p=0.03$ in obese) BP in lean and obese Z. rats. Blood pressure remained elevated (Figure 3) for the remainder of the study (about 15-20 minutes). In contrast, the administration of DOI did not affect heart rate in either lean or obese Z. rats (Table 1).

Effects of 5-$HT_{2A/2C}$ agonist on UA mechanics following neuromuscular paralysis (Protocol B): Eight (four lean and four obese) Z. rats were studied to explore whether the effects of DOI were neuromuscular in origin and/or indirectly related to the DOI-induced increase in BP. Because the magnitude of the DOI-induced changes in UA mechanics were similar in lean and obese animals in Protocol A, we combined the data of the four lean and four obese animals in Protocol B. Figure 4 shows the combined mean values of the eight Z. rats during control condition, after neuromuscular paralysis, and after DOI administration. Complete paralysis with pancuronium resulted in an increase (less negative, more collapsible) Pcrit compared with control ($p<0.001$) that was associated with a decrease in Ron ($p<0.001$) and a

decrease in $\dot{V}_{Imax}$ (p=0.018). The effects of DOI on UA mechanics were abolished after neuromuscular paralysis. There was no significant change in Pcrit, $\dot{V}_{Imax}$, and Ron following DOI administration in all paralyzed Z. rats (all ns). DOI administration following paralysis resulted in significant elevations in systolic and diastolic BP in all eight Z rats (Figure 4) to similar levels observed following DOI infusion in our primary protocol.

Effects of 5-HT$_{2A/2C}$ agonist on UA mechanics after bilateral hypoglossal nerve transection (Protocol C): To determine the relative contribution of cnXII in mediating the effects of DOI, we studied the effects of DOI administration in additional eight (four lean and four obese) Z. rats following bilateral cnXII transection. We also analyzed the data of the lean and obese animals as one group in Protocol C. Figure 5 shows the effects of DOI on UA mechanics in eight cnXII denervated rats compared to the results obtained in intact animals in Protocol A (the data of lean and obese animals were combined). In cnXII denervated animals (Figure 5), there were significant reductions in $\dot{V}_{Imax}$ (p=0.007) and Pcrit (p=0.017), and increases in Ron (p=0.016) following DOI. The ΔPcrit (-0.8 ± 0.3 cmH_2O) in denervated animals was statistically smaller

compared to the ΔPcrit (-2.8 ± 0.6 cmH_2O) in animals with intact cnXII in Protocol A (unpaired *t*-test, p=0.042). CnXII denervation did not affect the $\Delta\dot{V}_{Imax}$ (-4.4 ± 1.2 ml/s in denervated vs. -1.8 ± 1.8 ml/s in intact animals, p=0.377), or ΔRon (23.2 ± 7.4 cmH_2O/l/s in denervated vs. 57.7 ± 12.8 cmH_2O/l/s in intact animals, p=0.083). The ΔRon in denervated animals tended to be smaller but the difference did not achieve statistical significance. The systolic BP significantly increased to 128 ± 12 mmHg (p=0.02) and diastolic BP increased to 83 ± 12 mmHg (p=0.014) after DOI. The administration of DOI did not affect heart rate. In all three protocols, systolic and diastolic BP increased to similar levels after DOI administration (Figures 4 and 5).

DISCUSSION

The major findings of this study are as follows: (*1*) systemic administration of a 5-$HT_{2A/2C}$ receptor agonist rendered the UA less collapsible in both lean and obese Z. rats, that was associated with an increased UA upstream resistance and unchanged $\dot{V}_{Imax}$; (*2*) the improvement in UA stability induced by a 5-$HT_{2A/2C}$ receptor agonist administration is neuromuscular in origin and predominantly, but not exclusively,

mediated by hypoglossal motoneurons; (*3*) non-hypoglossal motoneurons, at least in part, were responsible for mediating the increased Ron caused by the 5-$HT_{2A/2C}$ receptor agonist; (*4*) the magnitude of the changes in UA pressure-flow relationships induced by 5-$HT_{2A/2C}$ agonist is similar in lean and obese Z. rats, although UA collapsibilty in obese Z. rats improved to levels comparable to the baseline values noted in lean animals. To our knowledge, this is the first study that investigated the effects of serotonergic stimulation on UA mechanics in the isolated UA preparation.

Critique of Methodology of Isolated Upper Airway: Prior to discussing our results, several limitations of the isolated UA preparation should be addressed. First, it is well known that anesthesia depresses respiration and reduces the GG muscle activity. In the present study, the GG muscle exhibited faint activity at baseline (Figure 3), indicating that the level of anesthesia used did not totally suppress EMG_{GG}. However, the level of anesthesia and end-tidal CO_2 were maintained constant throughout the experimental protocol. Second, large negative suction pressures (-60 to -80 cmH_2O) were generated to achieve airflow limitation in our preparation. It is conceivable that these pressures could traumatize the UA (15), leading to alterations in collapsibility.

The UA is not actually exposed to such large negative pressure *in vivo* during normal physiological activity. However, repeated measurements of UA collapsibility were shown to be reproducible with low coefficients of variation. Third, the vascular tone and mucosal blood volume have been suggested to be potentially important non-muscular determinants of UA collapsibility. Wasicko et al. suggested that vasodilatation in cats increases UA collapsibility, while vasoconstriction tends to decrease airway collapsibility (16), although the degree of the effect on UA airflow mechanics, as well as effects in other animal species, remain unclear. We found that DOI induced a significant increase in BP but not heart rate in all Z. rats (Table1), suggesting that DOI increased peripheral vascular tone. Therefore, in eight additional Z. rats, we determined whether the DOI-induced increase in vascular tone affected UA mechanics. To isolate any potential secondary effect of the DOI-induced vasoconstriction on UA mechanics from the primary effects of DOI mediated through UA muscle activation, animals were paralyzed prior to assessing the impact of DOI on UA mechanics (Protocol B). Despite similar increases in BP in these eight animals compared to the BP changes noted in our primary study, Pcrit was unaffected in paralyzed rats by DOI (Figure 4). This suggests that neither the vasoconstriction nor

the increase of BP induced by DOI affected UA mechanics in our study.

Effects of DOI on UA mechanics: While ventilation may be normal during wakefulness in patients with obstructive sleep apnea (OSA), a sleep-induced reduction in UA dilator muscle activity results in collapse of an anatomically narrowed UA (17). It is thought that if UA dilator muscle activity can be maintained or augmented during sleep, then pharyngeal collapse may be prevented.

Serotonergic neurons exert an excitatory effect on UA dilator motoneurons (1, 18). Prior studies suggested that serotonergic agents may be effective in treating OSA (5, 6, 19-21). Indeed, administration of the serotonergic agents, trazodone and L-tryptophan, was effective in treating sleep-disordered breathing in the English bulldog and the effectiveness of this therapy was related to increased UA dilator muscle activity during sleep (19). Of the 15 different 5-HT receptor subtypes that have been identified so far (22), the specific type of receptor that mediates the excitatory effects of 5-HT in UA motoneurons is thought to be of the 5-HT_{2A} and 5-HT_{2C} variety (1, 4, 5). We reasoned that it would be important to know the specific effects of the

systemic administration of a 5-$HT_{2A/C}$ agonist on UA mechanics, especially since human studies involving selective serotonin reuptake inhibitors (SSRI) in the treatment of OSA have been disappointing (23, 24). We utilized a previously published isolated UA preparation (14). In this model of the UA as a Starling resistor system, $\dot{V}_{Imax}$ is modulated by Pcrit and by the resistance upstream to the flow-limiting site (FLS). We predicted that $\dot{V}_{Imax}$ would increase with decreasing Pcrit after the administration of DOI due to increased activity of the GG muscle. In cats, Schwartz et al. showed that the bilateral electrical stimulation of the cnXII increased $\dot{V}_{Imax}$ and decreased Pcrit, although this was offset by an increased upstream resistance which was thought to be due to narrowing of the upstream segment (14). This increase in upstream resistance was minor, but likely attenuated the increase in $\dot{V}_{Imax}$. In a preliminary study, we also found that bilateral supramaximal stimulation of the cnXII in Z. rats increased $\dot{V}_{Imax}$ due to a more negative Pcrit, although there also was a small increase in Ron (25).

In the present study, the administration of DOI resulted in a very significant increase in EMG_{GG} activity that was associated with a decrease in UA collapsibility in both lean

and obese Z. rats, as expected. There was a concomitant large increase in Ron, associated with an unchanged $\dot{V}_{Imax}$ overall (Table 1). Our results suggest that the stimulation of motoneurons in the cnXII nucleus mediated the improvement in UA collapsibility observed with DOI, since we saw large increases in tonic activity of the GG muscle, an UA muscle solely innervated by cnXII (26-28). The relatively unaltered UA dynamics after DOI administration with complete neuromuscular paralysis (Protocol B) supports the idea that the effect of DOI on UA collapsibility is neuromuscular in origin.

It is possible that DOI affected the reflex response to upper airway negative pressure (UANP), which may have altered UA collapsibility. The reflex response to UANP is an important aspect of cnXII motor control. The UA reflex increases cnXII neural output and genioglossal activity (29). However, Douse et al. showed that 5-HT at the hypoglossal motor nucleus caused a large increase in tonic activity that had no effect on the cnXII reflex response to UANP in cats (30). They speculated that the large increase in tonic activity obscured the reflex increase in phasic cnXII activity, and the progressive saturation of cnXII motor output may have led to a diminished

cnXII reflex response (30, 31). Therefore, it is unlikely that the reflex response to UANP played a major role to explain our results.

We also have to consider the effects of baroreceptor-mediated mechanisms on UA neuromuscular activity since DOI induced a significant increase in BP in all Z. rats (Table1). Increased BP increased the severity of UA airflow obstruction by increasing pharyngeal collapsibility (32, 33). Garpestad et al. found a decrease in EMG_{GG} activity during phenylephrine-induced hypertension (32). However, we found a large tonic activation of the GG muscle (Figure 3) with DOI. This large stimulation of cnXII nuclei activity may have offset any effects of the increased BP on UA collapsibility.

Serotonin is also known to affect UA muscle activity via peripheral mechanisms. It is possible that DOI affected the nodose ganglion since our animals were not vagotomized. Nodose ganglia, linking with the the vagi, are the relay station between sensory neurons in the respiratory organs and central axons transmitting to the nucleus of the solitary tract of the medulla (34). However, 5-HT_3 receptors are likely the relevant receptors in the nodose ganglia, and 5-HT_3 antagonist rather than 5-HT_3

agonist, increases cnXII activity (35, 36) through this mechanism. Therefore, we believe that the effects of DOI are not likely mediated through the nodose ganglia.

Role of cnXII in mediating the effects of DOI: Since DOI readily penetrates the blood brain barrier and was administered systemically, we cannot exclude that other motoneuronal pools were also affected and could be partly responsible for our results in animals with intact cnXII. Therefore, to explore the relative role of cnXII in mediating the effects of DOI, we performed additional experiments involving cnXII-denervated animals. Our results suggest that hypoglossal and non-hypoglossal motoneurons likely were involved in mediating the effects of DOI on UA mechanics. The fact that Pcrit decreased after DOI even in the denervated condition (Protocol C) suggests that the effect of DOI on Pcrit seen in intact animals is not solely mediated through stimulation of the GG muscle but likely through other pharyngeal muscles as well. Other UA muscles are known to modulate UA patency (37-39) and we speculate that they were also activated by DOI. However, the decline in Pcrit in denervated animals was smaller than in intact animals and therefore, the improvement in UA stability induced by DOI was attributed largely to activation of cnXII. These results are

consistent with prior studies that suggest that the GG muscle is the main UA dilator muscle responsible for UA patency (40).

The large increases in Ron suggest that the UA upstream segment could have narrowed after DOI administration. There are several possible reasons to explain such narrowing of the upstream segment after DOI. First, increased activity of the EMG_{GG} could result in a lower mean intraluminal pressure in the upstream segment, resulting in a decreased cross-sectional area. With a more negative Pcrit after DOI, the pressure at the FLS can decrease, resulting in a larger pressure gradient across the upstream segment, and as a consequence, a lower mean intraluminal pressure, causing a narrowing of this segment (14). However, alterations in UA airflow dynamics with cnXII stimulation has been associated with minor increases in Ron with cnXII electrical stimulation in cats (14). It is unlikely, therefore, that this mechanism would be solely responsible for the large increases in Ron observed in our experiments. Second, the lateral branch of cnXII innervates the styloglossus and hyoglossus muscles (26, 27). These tongue retractors could have been simultaneously stimulated after systemic administration of DOI. However, since Ron also increased following

transection of cnXII in Protocol C, activation of tongue retractors following DOI likely is not the only explanation for the increased Ron in intact animals. Third, narrowing of the upstream segment could be due to increased recruitment of pharyngeal constrictors due to DOI. The pharyngeal constrictor muscles are sail-like muscles forming the lateral and posterior walls of the pharyngeal airway (41) and are innervated by the pharyngeal branch of the vagus and the glossopharyngeal nerve (42, 43). Kuna et al. showed that stimulation of the pharyngeal branch of the vagus decreased cross-sectional area and decreased compliance of the velopharynx in an isolated UA in decerebrate cats (27, 37). Indeed, the nucleus ambiguous, wherein the pharyngeal branch of the vagus originates, is known to contain 5-$HT_{2A/2C}$ receptors (44). Since DOI was administered systemically in our experiments, we speculate that motoneurons in the nucleus ambiguous were stimulated by DOI, resulting in decreased upper airway cross-sectional area and decreased UA compliance. This may explain the decreased Pcrit and increased Ron in cnXII-denervated animals, since stimulation of the pharyngeal branch of the vagus by DOI could have affected UA cross-sectional area and stiffness.

Both electrical stimulation of cnXII and administration of serotonergic agents are being investigated as potential treatment strategies for OSA (23, 24, 45). In animals, electrical stimulation of cnXII has been reported to either have a minor (14) or no effect on Ron (46). This effect of cnXII electrical stimulation has been attributed to alterations in UA airflow dynamics rather than to a direct effect on UA muscles (14). Systemically administered 5-$HT_{2A/2C}$ agonist appears to exert a more complex effect on UA mechanics than isolated cnXII stimulation. Our results could have implications on the development of pharmacotherapy for OSA since both hypoglossal and non-hypoglossal motoneurons may possibly be modulated using serotonergic agents in this regard.

Obesity and UA Mechanics: In the present study, the Pcrit of obese Z. rats was significantly higher (less negative) compared to lean Z. rats. This finding is consistent with our previous report (12) and indicates that the UA of obese Z. rats is more collapsible compared with that of lean Z. rats. Obesity did not modify the response to DOI since there were no significant differences in UA mechanics between lean and obese Z. rats after DOI (Table 1). However, with increased recruitment of UA dilator

muscle activity after DOI, the UA collapsibilty of obese Z. rats improved to levels comparable to the baseline values noted in lean animals. This suggests that augmentation of UA dilator muscle activity could indeed be useful in preventing collapse of the UA.

In conclusion, systemic administration of a 5-$HT_{2A/2C}$ receptor agonist appears to have complex effects on UA mechanics. Although DOI rendered the UA less collapsible, an increased UA upstream resistance and an unchanged $\dot{V}_{Imax}$ accompanied this effect. The decrease in Pcrit is likely mediated primarily through hypoglossal motoneurons, while the increase in upstream resistance may be due to a narrowing of the upstream segment that may be partly due to pharyngeal constrictor stimulation. Obesity does not modify the UA response to the administration of a 5-$HT_{2A/2C}$ agonist, although UA collapsibility in obese Z. rats improved to levels that are comparable to the baseline values of lean animals after significant EMG_{GG} activation by this drug. We believe that simultaneous examinations of UA muscle activity and UA mechanics in the isolated UA preparation offer an attractive way of determining the effects of other pharmacotherapeutic candidates for the treatment of

OSA and help explain their potential mechanisms of action. Studies are also needed in unanesthetized animals to determine the effects of these agents on ventilation and recruitment of UA dilator muscles across sleep-wake states.

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FIGURE LEGENDS:

FIGURE 1.

Effects of DOI on isolated upper airway mechanics in individual lean (*open circles*) and obese (*closed square*) Zucker rats. Solid diagonal line represents the line of identity. If the data point falls above or below the line of identity, DOI infusion altered the parameter being plotted. DOI decreased Pcrit ($F(1,14)=17.6$, $p<0.001$) that was accompanied by an increased Ron ($F(1,14)=19.1$, $p<0.001$), and an unchanged $\dot{V}_{Imax}$ ($F(1,14)=0.9$, $p=0.363$). The significant effects of DOI on Pcrit and Ron did not depend on rat type.

FIGURE 2.

A representative tracing from a lean Zucker rat depicting inspiratory airflow ($\dot{V}_I$), pharyngeal pressure (Pph), and hypopharyngeal pressure (Php) in isolated upper airway preparation. Pph was measured at or immediately upstream to the flow-limiting site. As Pph was lowered, $\dot{V}_I$ rose and reached a maximum ($\dot{V}_{Imax}$) at the onset of inspiratory airflow limitation. Pcrit was defined as the nadir in Pph versus time curve. The administration of DOI caused a decrease in Pcrit, an index of upper airway

collapsibility.

FIGURE 3.

A. Representative tracings of the genioglossus muscle electromyogram (EMG_{GG}) and blood pressure in a lean Zucker rat. Intravenous administration of DOI (arrow) resulted in activation of the genioglossus muscle. DOI also increased blood pressure. These effects remained evident 15-20 minutes after DOI infusion. *B.* Representative tracings of the genioglossus muscle electromyogram (EMG_{GG}) in a lean (*upper*), and an obese (*lower*) Zucker rat five minutes after intravenous saline and five minutes after intravenous DOI showing the large tonic activation of the genioglossus muscle after the active drug in both rats.

FIGURE 4.

Effects of DOI on upper airway mechanics following neuromuscular paralysis. The combined data of Pcrit (*top panel*), $\dot{V}_{Imax}$ (*second panel*), Ron (*third panel*), and cardiovascular parameters (*bottom panel*) in four lean and four obese Zucker rats are shown during control condition, after pancuronium, and after DOI administration.

Pancuronium significantly increased Pcrit and decreased $\dot{V}_{Imax}$ and Ron. Following neuromuscular paralysis, DOI also significantly increased blood pressure, but the effects on upper airway mechanics were abolished. Neither pancuronium nor DOI changed heart rate.

§ $p<0.05$ significantly different from saline

* $p<0.05$ significantly different from pancuronium

FIGURE 5.

The combined data of Pcrit (*top panel*), $\dot{V}_{Imax}$ (*second panel*), Ron (*third panel*), and cardiovascular parameters (*bottom panel*) in eight (four lean and four obese) cnXII-denervated Zucker rats (Protocol C) are shown on the right panel during control condition (saline) and after DOI. The combined data of the sixteen (eight lean and eight obese) Zucker rats with intact cnXII in Protocol A are also shown on the left panel for comparison. In animals with transected cnXII, DOI significantly decreased Pcrit and $\dot{V}_{Imax}$, increased Ron and blood pressure, while there was no change in heart rate. The magnitude of the decrease in Pcrit in denervated animals was smaller ($p=0.042$) compared to the change in intact animals. The magnitude of the change in

Ron in denervated animals tended to be smaller than in intact animals but the difference did not achieve statistical significance ($p=0.083$).

§ $p<0.05$ significantly different from saline

Table 1

	$\dot{V}_{Imax}$ (ml/s)	Pcrit (cmH_2O)	Ron (cmH_2O/l/s)	SBP (mmHg)	DBP (mmHg)	HR (bpm)
Lean						
Control	60.3 ±5.6	-4.8 ± 0.9	85.2 ±16.1	69 ± 8	50 ± 6	254 ±17
DOI	56.7 ±4.7	-7.6 ± 1.0§	143.8 ±25.0§	149 ±18§	108 ±16§	261 ±17
Δ	-3.6 ±3.4	-2.8 ± 0.7*	58.7 ±16.4*			
Obese						
Control	46.2 ±4.4	-2.0 ± 0.4¶	44.4 ± 7.5	68 ±12	44 ±10	245 ± 8
DOI	46.1 ±5.1	-4.7 ± 1.2§¶	101.0 ±22.5§	121 ±15§	74 ±16§	252 ±11
Δ	0.1 ±2.0	-2.7 ± 1.1*	56.6 ±20.7*			

Values are mean ± SEM for eight rats in each group. $\dot{V}_{Imax}$, maximal inspiratory flow; Pcrit, pharyngeal critical pressure; Ron, oronasal resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; bpm, beats per minute; Δ, the magnitude of the changes induced by DOI.

§ $p<0.05$ significantly different from control

¶ $p<0.05$ significantly different from lean rats

* $p<0.05$ a significant change

Figure 1

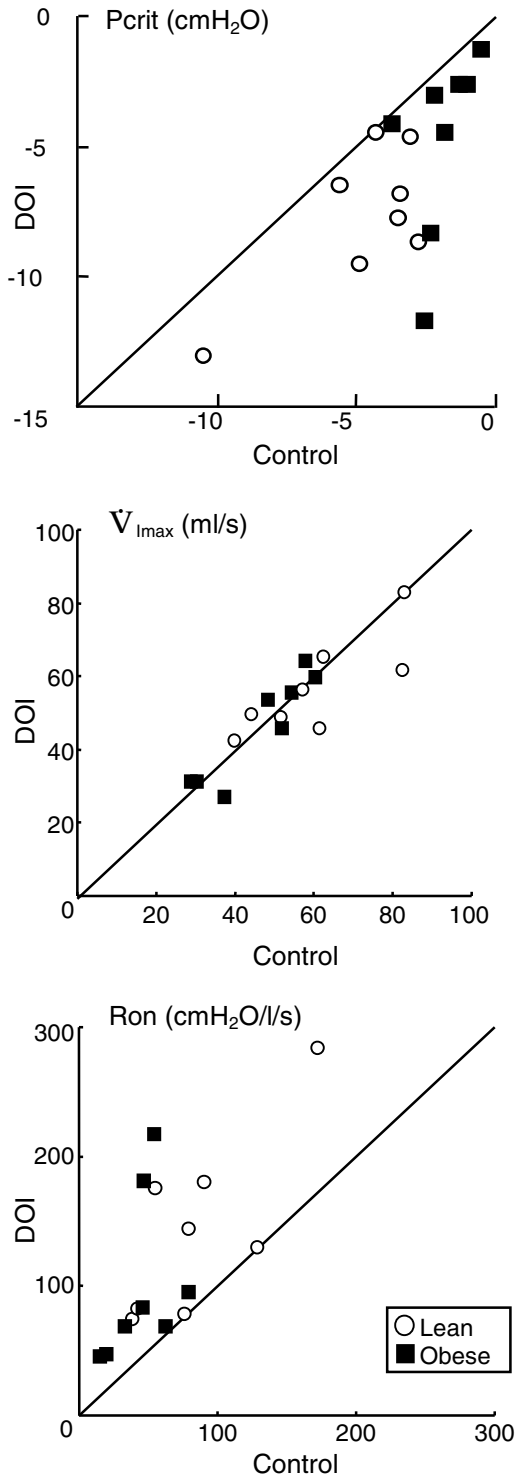

Pcrit (cmH2O)
0
-5
-10
-15
DOI
-10
-5
0
Control
V̇Imax (ml/s)
100
80
60
40
20
0
DOI
20
40
60
80
100
Control
Ron (cmH2O/l/s)
300
200
100
0
DOI
100
200
300
Control
Lean
Obese

Figure 2

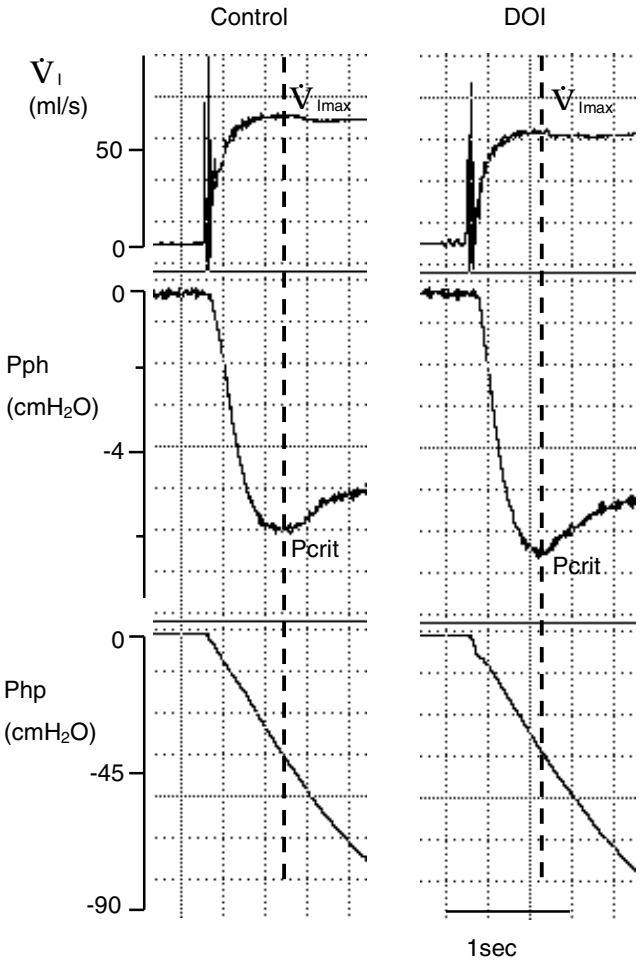

Control
DOI
$\dot{V}_I$ (ml/s)
$\dot{V}_{Imax}$
$\dot{V}_{Imax}$
50
0
0
Pph
(cmH_2O)
-4
Pcrit
Pcrit
0
Php
(cmH_2O)
-45
-90
1sec

Figure 3

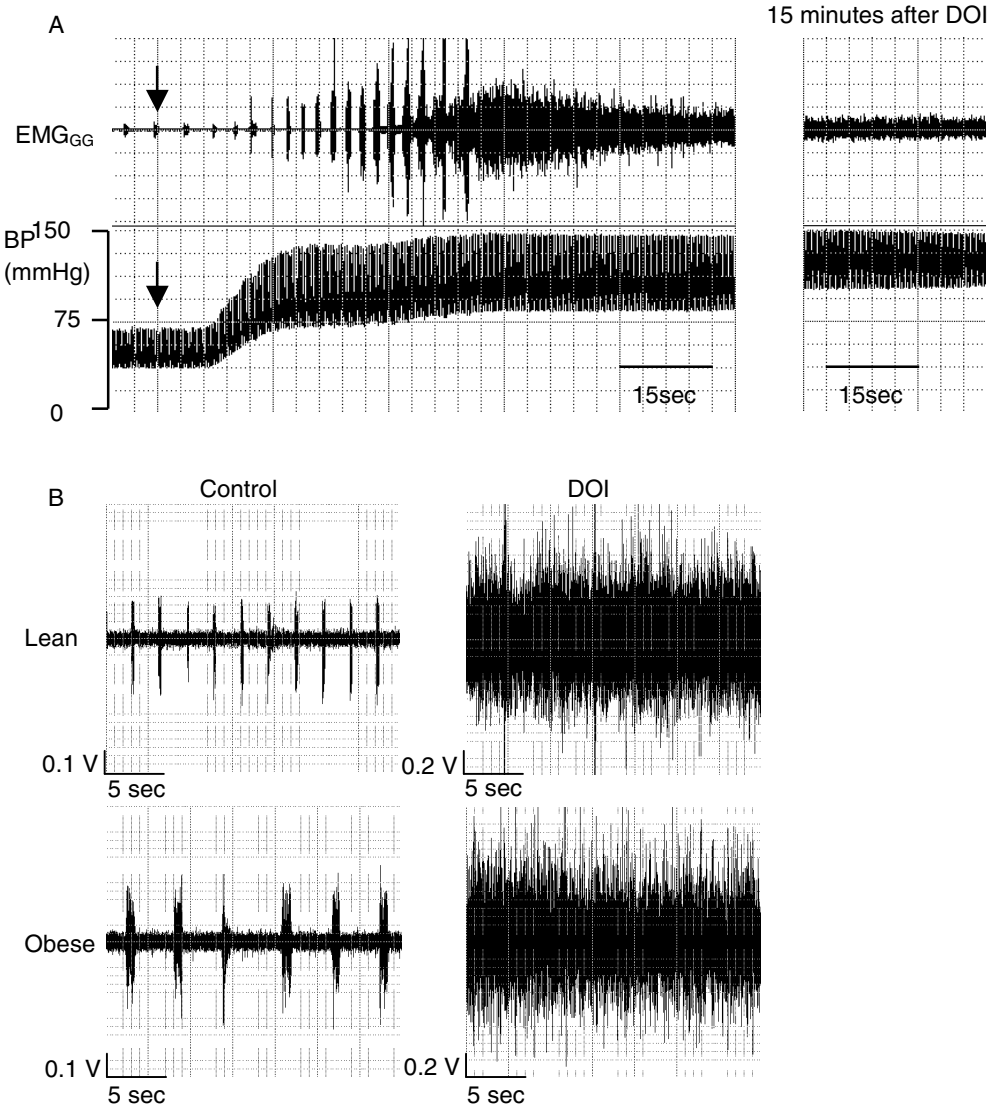

A
15 minutes after DOI
EMG$_{GG}$
BP
(mmHg)
150
75
0
15sec
15sec
B
Control
DOI
Lean
0.1 V
5 sec
0.2 V
5 sec
Obese
0.1 V
5 sec
0.2 V
5 sec

Figure 4

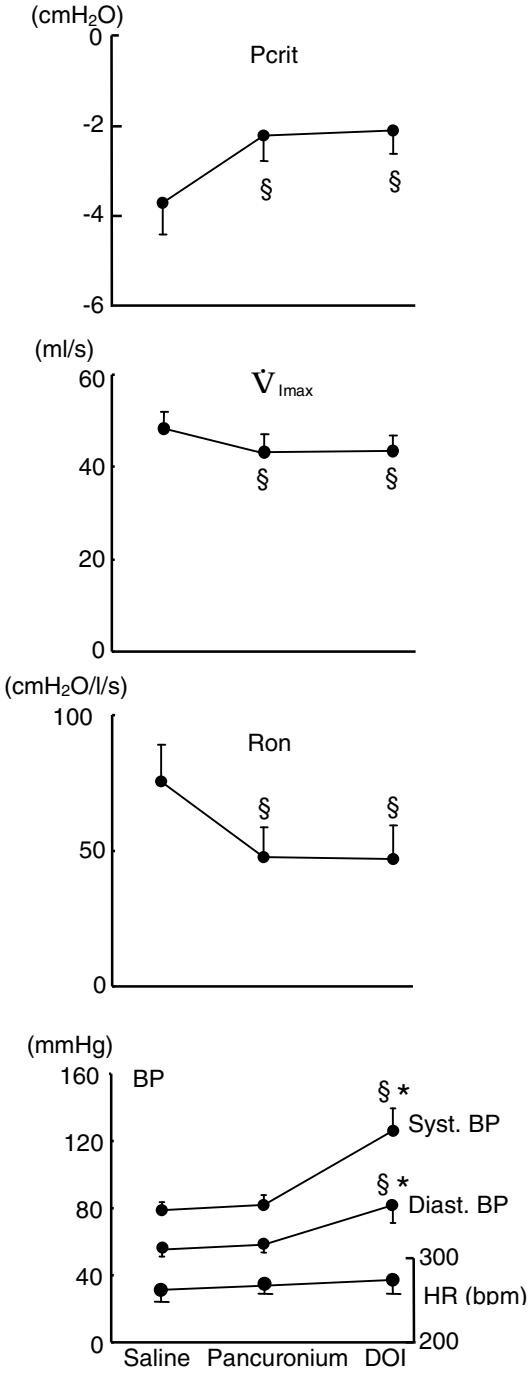
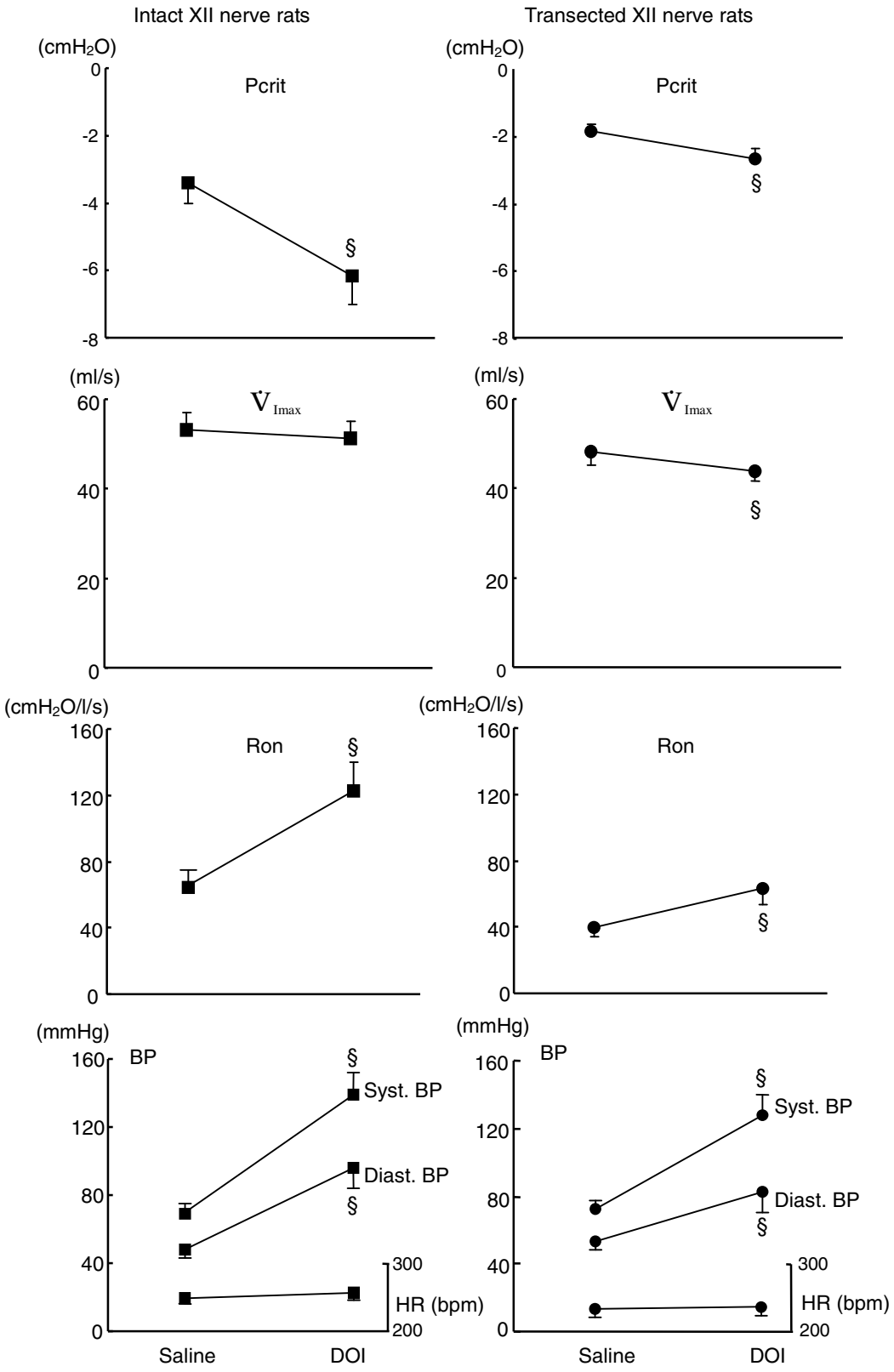

(cmH2O)
0
Pcrit
-2
-4
-6
§
§
(ml/s)
60
V̇Imax
40
20
0
§
§
(cmH2O/l/s)
100
Ron
50
0
§
§
(mmHg)
160
BP
120
80
40
0
§ *
Syst. BP
§ *
Diast. BP
300
HR (bpm)
200
Saline
Pancuronium
DOI

Figure 5


Intact XII nerve rats
Transected XII nerve rats
(cmH2O)
Pcrit
0
-2
-4
-6
-8
§
(ml/s)
V̇Imax
60
40
20
0
(cmH2O/l/s)
Ron
160
120
80
40
(mmHg)
BP
Syst. BP
Diast. BP
HR (bpm)
300
200
Saline
DOI

Appendix: Online-Only Supplement

SYSTEMIC ADMINISTRATION OF SEROTONIN 2A/2C AGONIST IMPROVES UPPER AIRWAY STABILITY IN ZUCKER RATS

Toshiyuki Ogasa, M.D., Ph.D., Andrew D. Ray, M.S., Charles P. Michlin, B.S., Gaspar A. Farkas, Ph.D., Brydon J.B. Grant M.D., and Ulysses J. Magalang, M.D.

METHODS

The study was performed in lean [Fa/?] (age: 10.3 ± 1.5 months, weight: 468 ± 14 g) and age-matched obese [fa/fa] adult male (age: 10.3 ± 1.2 months, weight: 660 ± 17 g) Zucker (Z.) rats (Vassar College, Poughkeepsie, NY). One lean and one obese Z. rat were housed per cage. Ambient temperature was maintained at 21 ºC, and an artificial 12-hour light-dark cycle was set. The rats were provided with standard laboratory chow (Ralston Purina, St-Louis, MO) and water *ad libitum*. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University at Buffalo.

An *in-situ* isolated upper airway (UA) preparation in anesthetized, mechanically ventilated animals was utilized to assess the effects of [±]-2,5-Dimethoxy-4-iodoaminophentamine (DOI) administration. As previously described (E1, E2), this airway preparation assesses the collapsibility of the pharyngeal airway by determining the maximal inspiratory airflow ($\dot{V}_{Imax}$) and the pharyngeal critical pressure (Pcrit) at the flow-limiting site (FLS).

Effects of Serotonin (5-HT)$_{2A/2C}$ agonist on UA mechanics (Protocol A): Eight lean and eight obese Z. rats were initially anesthetized with an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (5 mg/kg). Animals were placed supine on a circulating water heating pad and rectal temperature was monitored and maintained at 37 ºC. Atropine sulfate (0.4 mg/kg, i.m.) was administered to minimize airway secretions. A femoral vein was cannulated for the administration of drugs, while the femoral artery was cannulated to monitor systemic arterial blood pressure (BP) (P23XL; Statham Laboratories, Oxnard, CA).

The cervical trachea was cut, and the caudal tracheal stub was cannulated with an endotracheal tube. The animals were mechanically ventilated (Model 683; Harvard Apparatus, South Natick, MA) and continuously anesthetized with 2 - 3% isoflurane, balance oxygen. End-tidal CO_2 at the endotracheal tube was monitored continuously (Capstar-100; CWE, Inc., Ardmore, PA) and maintained at 5 % by adjusting the rate of the mechanical ventilator. A rigid cannula was then advanced into the rostral cut end of the trachea through the glottic structures, and tied in place at the level of the aryepiglottic folds. The position of this rigid cannula was confirmed at the completion

of each experiment. A mobile catheter (PE 50 tubing, 0.58 mm i.d.) with a side hole midway along its length was inserted through the rigid tracheal cannula into the pharynx and exited from one nostril. This catheter was used to measure the pharyngeal pressure (Pph) at different locations in the airway. A second catheter to record the hypopharyngeal pressure (Php) was introduced through the rigid tracheal cannula and positioned at the lower end of the UA. BP, Php and Pph were monitored with pressure transducers (P23XL; Statham Laboratories, Oxnard, CA). The inspiratory airflow ($\dot{V}_I$) through the isolated UA was measured using a pneumotachograph (No. 0.771; Fleisch, Switzerland) and a differential pressure transducer (Model MP 45-1, range ± 2 cmH_2O; Validyne Engineering Corp., Northridge, CA) placed in series between the rigid tracheal cannula and negative pressure source. The outputs of Pph, Php, $\dot{V}_I$, and BP were recorded with a data-acquisition system (WinDaq DI-720; DATAQ Instruments, Akron, OH) for real-time recordings and subsequent analysis.

$\dot{V}_{Imax}$ and Pcrit were measured as previously described (E1, E2). FLS was determined by monitoring the profile of $\dot{V}_I$ versus time and Pph versus time curves.

In brief, pressure at the downstream end of the UA was rapidly lowered by a negative pressure source. To localize the FLS, the catheter was slowly advanced cranially in the pharynx while monitoring Pph and $\dot{V}_I$. After localization of the FLS, the mobile catheter was fixed at or immediately upstream from this site to measure Pph, and was marked for later confirmation of the side-hole position. Pcrit was defined as the nadir in Pph at the onset of flow limitation. Resistance upstream to the FLS was defined as oronasal resistance (Ron). Ron was calculated as follows:

$$Ron = (Pon - Pcrit)/ \dot{V}_{Imax} \qquad (Eq.\ 1)$$

When oronasal pressure (Pon) remains 0 (atmospheric), therefore, *equation1* becomes

$$\dot{V}_{Imax} = -Pcrit/ Ron \qquad (Eq.\ 2)$$

During airflow limitation, $\dot{V}_{Imax}$ is modulated by the collapsibility (Pcrit) at the FLS and by the resistance of the upstream segment (Ron) (E2).

For each animal, five measurements were made to obtain $\dot{V}_{Imax}$ and Pcrit five minutes after intravenous administration of vehicle (saline) as a control, and again five minutes after the administration of DOI (0.5 mg/kg i.v., Sigma Chemical, St.

Louis, MO), a 5-$HT_{2A/2C}$ receptor agonist, that crosses the blood brain barrier. The dose was determined from preliminary experiments showing significant recruitment of UA dilator muscle activity. Average values were calculated from the five measurements following the administration of vehicle or DOI. We calculated the magnitude of the changes in UA dynamics induced by DOI as the delta (Δ). To determine whether repeated measurements alter the mechanics of the isolated UA, a preliminary study was performed. $\dot{V}_{Imax}$ and Pcrit were measured five times five minutes after an injection of saline and again five minutes after a second injection of saline, and it was found that the values were highly reproducible.

In order to assess the activation pattern of the genioglossus (GG) muscle, multistranded teflon-coated stainless steel wires (A5632, Cooner Wire Company, Chatsworth, CA) bared at their tips were inserted directly into the belly of the GG muscle in all animals. The electromyogram (EMG) of GG muscle (EMG_{GG}) signals were amplified, band-pass-filtered from 30 to 1,000 Hz (P511K, A.C. Pre-amplifier, Grass Instruments Co., Quincy, MA), and recorded at a sample rate of 1,000 Hz by a data-acquisition system (WinDaq DI-720; DATAQ Instruments, Akron, OH). The raw

EMG signal was rectified and a moving average was employed with a time constant of 100 milliseconds. To quantify the overall increase in GG activity, 30-second samples during control condition and after DOI (5 minutes after infusion) were compared. The raw GG signal was rectified and integrated and the value after DOI was expressed as a percent of control.

To explore potential mechanisms that may help explain the changes in upper airway dynamics with DOI, we performed additional experiments outlined in Protocols B and C.

Effects of 5-HT$_{2A/2C}$ agonist on UA mechanics following neuromuscular paralysis (Protocol B): Eight (four lean and four obese) Z. rats were studied to explore whether the effects of DOI were neuromuscular in origin and whether the observed DOI-induced increase in blood pressure were primarily responsible for alteration of UA mechanics. To isolate possible indirect effects arising from the DOI-induced hypertension on UA mechanics from the direct effects of DOI on UA muscle

activation, UA mechanics were determined in paralyzed rats prior to and following DOI administration.

The isolated UA was prepared as outlined previously. EMG electrodes were not inserted. Pcrit and $\dot{V}_{Imax}$ were measured before and following the bolus administration of pancuronium bromide (2 mg/kg i.v., Sigma Chemical, St. Louis, MO). The adequacy of paralysis was assessed by disconnecting the animal from the mechanical ventilator for 30 seconds, and by observing the lack of spontaneous breathing movements. The adequacy of anesthesia during paralysis was ensured by regularly checking that there was no significant response in systemic arterial pressure to toe pinch. Fifteen to 20 minutes following paralysis, UA mechanics were again measured five minutes after the administration of DOI (0.5 mg/kg, i.v.).

Effects of 5-HT$_{2A/2C}$ agonist on UA mechanics after bilateral hypoglossal nerve transection (Protocol C): To better define the role of the hypoglossal motoneurons in mediating alterations in UA mechanics observed in our primary protocol (Protocol A), the effect of DOI was assessed in eight additional (four lean and four obese) Z. rats

following complete bilateral transection of the hypoglossal nerve (cnXII).

The isolated UA was prepared as previously outlined. Both cnXII were dissected and the whole nerves were cut proximal to the bifurcation into lateral and medial branches. EMG electrodes were not inserted into the GG muscle. Fifteen to 20 min following cnXII denervation, Pcrit and $\dot{V}_{Imax}$ were evaluated five minutes after administration of saline control and five minutes following the systemic administration of DOI (0.5 mg/kg, i.v.).

Statistical Analysis: Data was analyzed using SPSS (version 12.0) statistical software (SPSS Inc., Chicago, IL). In Protocol A, the differences in UA mechanics, integrated EMG_{GG}, blood pressure, and heart rate data in lean and obese Z. rats following the administration of saline and DOI were analyzed by two-way analysis of variance (ANOVA) with repeated measurements on one factor. The between subjects factor was lean vs. obese rats. The within subjects factor with repeated measurements was vehicle vs. DOI. An interaction term was included. For Protocol B (paralysis experiments), a similar analysis was employed except the data for lean

and obese animals were combined since no differences in response to DOI were seen in the first series of experiments (Protocol A). For Protocol C (denervation experiments), the effects of DOI were analyzed using paired *t*-test. Finally, we compared the systolic and diastolic blood pressure data between the three different Protocols using two-way repeated measures ANOVA (between-subjects factor: Protocol A vs. Protocol B vs. Protocol C, within subjects factor: pre-DOI vs. DOI) and an interaction term was included to determine if the hypertensive response to DOI was different between the three protocols. All data presented in the text, tables, and figures represent means ± SEM. We checked residuals for outliers and normal distribution. We tested for compound symmetry and made adjustments using the Greenhouse-Geisser correction method when appropriate. A $p< 0.05$ was considered statistically significant. If the overall F test showed statistical significance, a *post-hoc* *t*-test with Bonferroni's correction for multiple comparisons was used to determine where the differences lie.

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