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Effects of opioid dependence and tobacco use on ventilatory response to progressive hypercapnia

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Abstract

Respiratory depression is a serious medical risk of opioid use. Most opioid abusers also smoke cigarettes, perhaps further compromising breathing. Differences in ventilatory response to nonhypoxic hypercapnia were studied in healthy volunteers with limited substance use (LU), tobacco smokers (SM), and opioid-dependent, methadone-maintained smokers (OD). The last two groups had similar current cigarette use and all groups were similar in gender and body mass index. Because previous data suggest that SM are sensitive to hypoxia but not hypercapnia, it was predicted that only the OD group would exhibit decreased carbon dioxide (CO_2) sensitivity. All subjects rebreathed CO_2 during three identical sessions (four trials per session). Fractional end-tidal (Fet) CO_2 levels during repeated 4-min exposures to progressive hypercapnia (6% to 10%) were similar across groups. Ventilatory response (breathing rate, tidal volume and minute volume) linearly increased with $FetCO_2$ concentration and did not differ significantly across sessions. Relative to the LU and SM groups (which did not significantly differ), the CO_2–minute volume and CO_2–breathing rate functions were significantly shifted rightward (decrease in intercept but not slope) for OD subjects. These data are consistent with the hypothesis that chronic opioid exposure and/or short-term methadone maintenance (but not tobacco or nicotine use) produces a specific decrease in CO_2 sensitivity, primarily through an inhibitory effect on respiratory frequency.

Keywords: Hypercapnia; Carbon dioxide; Respiratory depression; Ventilatory response; Opioid dependence; Methadone; Tobacco smoking; Nicotine

1. Introduction

Mu opioid agonists inhibit respiratory function; this toxic effect accounts for a significant proportion of emergency room visits and fatalities from heroin and other opioids (Warner-Smith et al., 2001; White and Irvine, 1999). Mechanisms underlying opioid-induced hypoventilation are not totally understood, but appear to involve diverse changes including muscarinic cholinergic-mediated reductions in firing rate of inspiratory neurons located in the ventral medulla and pons (Eguchi et al., 1987; Lydic et al., 1991; Takeda et al., 2001; Weinstock et al., 1980; Willette et al., 1987); increases in the duration of expiratory neuronal discharge (Laubie et al., 1985); and decreased chemosensitivity to hypercapnia (Schalefke, 1981), the magnitude of which is related to opioid intrinsic activity (Gerak et al., 1994; Liguori et al., 1996; Paronis and Woods, 1997b).

Researchers have often evaluated the respiratory depressant effect of opioids and the ability of treatment medications to block this adverse effect. Typically, breathing rate and/or oxygen saturation have been used to assess the ventilatory effects of opioids in human laboratory studies. Carbon dioxide (CO_2) sensitivity is an alternative measure of ventilatory drive that has been only infrequently used. This procedure assesses respiratory frequency (f), tidal volume (Vt) and minute volume ($VE = f \times Vt$) in relation to fractional end-tidal CO_2 ($FetCO_2$) concentration. This method (Read, 1967; Rebuck, 1976) requires subjects to breathe controlled amounts of CO_2 in air (under open-loop conditions) or rebreathe expired CO_2 (under closed-loop conditions) to induce hypercapnia, which stimulates medullary and pontine neurons to increase respiratory drive (White and Irvine, 1999). The extent to which opioids shift the CO_2-ventilatory response curve to the right or down (Bourke and Warley, 1989) is the operational definition of respiratory depression. This model provides information that is both dynamic (i.e., CO_2-dependent) and mechanistic (i.e., changes in the slope or intercept of ventilatory

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response measures), and may prove useful in human laboratory studies of drugs (or drug interactions) that influence respiratory function.

In opioid abusers, the acute administration of morphine shifts the CO_2–ventilatory response curve to the right (Jasinski et al., 1968). Chronic opioid exposure also influences ventilatory response. Fetuses (Richardson et al., 1984) and infants (Olsen and Lees, 1980) of methadone-maintained mothers (i.e., exposed to opioids in utero and perhaps continuing during breast-feeding) show decreased CO_2 sensitivity compared to nonexposed controls. In an inpatient study of former heroin addicts, Martin et al. (1968) found that relative to preaddiction baseline levels, CO_2 sensitivity decreased during chronic morphine administration, rebounded above baseline levels for 7 weeks after morphine withdrawal (cf. Paronis and Woods, 1997a), and decreased below baseline levels for 30 weeks postwithdrawal. CO_2 sensitivity has also been shown to vary as a function of methadone treatment duration. Patients who were maintained on methadone for less than 2 months showed significant decreased CO_2 sensitivity compared to patients receiving methadone for 8 or more months (Santiago et al., 1977) and a healthy control group that was administered acute doses of methadone (Santiago et al., 1980). These human data suggest that tolerance may develop to decreased CO_2 sensitivity during chronic opioid exposure, although some rhesus monkey data suggest otherwise (Paronis and Woods, 1997b). This issue is clinically relevant due to the risk of respiratory toxicity during medication induction, arising from illicit opioid use during medication maintenance or, as noted above, in relation to its impact on the developing embryo and infant.

An overlooked variable in these previous studies is that the majority of opioid-dependent individuals smoke tobacco cigarettes. Heroin abusers smoke cigarettes at rates that exceed 85%—more than three times the rate in the general population (Campbell et al., 1995; Frosch et al., 1998; Navaratnam and Foong, 1990; Stark and Campbell, 1993). The tobacco (or nicotine) use of opioid-dependent participants in previous studies of ventilatory response is unknown. Therefore, it is not clear to what degree tobacco use may have influenced results of prior CO_2-sensitivity studies. Unfortunately, the high prevalence of smoking among opioid abusers (comorbidity rates are about 95% in the Detroit area; Ebenbichler and Greenwald, 2002) makes it exceptionally difficult to recruit nonsmoking opioid abusers for research studies. Acute cigarette smoking differentially decreases sensitivity to hypoxia but not hypercapnia (Yamamoto et al., 1985), and smokers have been shown to differ in hypoxic but not hypercapnic sensitivity relative to nonsmokers (Kawakami et al., 1982). In one study of chronic in utero tobacco exposure, infants of mothers who smoked cigarettes during pregnancy showed deficient hypoxic awakening but no significant differences from nonsmoking controls in hypercapnia-induced awakening (Lewis and Bosque, 1995). Thus, available data suggest that tobacco-exposed humans are more sensitive to hypoxia than hypercapnia, but this has not been tested in an opioid-dependent population. In short, it remains possible that cigarette smoking could interact with opioid dependence to further decrease CO_2 sensitivity. If so, this would be an important medical risk to these individuals.

Another limitation of previous ventilatory response studies with drug abusers is that the influence of hypoxia (which tends to occur with hypercapnia during CO_2 rebreathing) was not generally controlled. Therefore, it is not possible to determine whether hypercapnia, hypoxia, or their interaction affected the results of those studies. An important methodological feature of the present study is that hypoxia was avoided using two procedures. First, hyperoxic air was added before each CO_2 rebreathing trial. Second, because CO_2 and O_2 were continuously monitored, the (relatively few) trials on which hypoxia occurred resulted in excluding the ventilatory response data from analyses.

The present study had three aims. First, to validate the CO_2 rebreathing method in our laboratory, we determined whether OD smokers would exhibit decreased CO_2 sensitivity (in the absence of hypoxia) relative to a control group with limited lifetime substance use. The OD participants were maintained on methadone for a relatively brief period (to minimize tolerance) and tested beginning 30 min after the daily dose (to maximize this opioid agonist effect). Second, to establish the specificity of opioid-induced decreases in CO_2 sensitivity (due to chronic heroin abuse and/or methadone maintenance), we evaluated a third experimental group (smokers; SM) to control for effects of current tobacco use and other selected variables (e.g., body mass index and gender). Third, we investigated the source of this reduction in CO_2-stimulated breathing, i.e., whether changes in breathing rate or tidal volume uniquely accounted for changes in minute ventilation, and whether there were specific changes in the intercept versus slope of the CO_2–minute ventilatory response function. The OD group was predicted to show a significantly lower slope or intercept in the FetCO_2-ventilatory response (VE, *f*, or Vt) regression lines during progressive hypercapnia relative to the SM and LU groups. There was no hypothesis concerning differences between the SM and LU groups. There were no predicted differences across test sessions, based on the assumption that this measurement technique and subjects' responding would be reliable.

2. Methods

2.1. Participants

The Wayne State University Human Investigation Committee (Institutional Review Board) approved this protocol. Volunteers ranging in age from 18 to 50 years were recruited from the Detroit area by newspaper ads and word of mouth. We attempted to test equal numbers of males and females in

each of the three experimental groups (OD, SM, and LU). All volunteers provided a medical history, blood and urine samples for routine laboratory testing, and they received a physical examination. Those selected had no chronic health problems (e.g., no pulmonary, cardiovascular, neurological, or systemic disorders) and were not taking prescribed medications (except for methadone in the methadone group) or using nicotine-replacement products. An experienced clinician interviewed all volunteers using the Structured Clinical Interview for DSM-IV (SCID; First et al., 1996). Volunteers were excluded if they reported a current Axis I psychiatric disorder, drug-dependence disorders (except opioids in the methadone group), or were cognitively impaired (estimated IQ <80) on the Shipley (1967) Institute of Living Scale. Subjects in the smoking groups were not required to be nicotine dependent but, rather, were matched for intensity of current cigarette use. During screening, OD participants were required to submit a urine sample that was positive for opioids or methadone (>200 ng/ml). Urine specimens from all volunteers were required to be negative for cocaine metabolites (<250 ng/ml), benzodiazepines, and barbiturates (<200 ng/ml) and—for the SM and LU control groups—free of opioids and methadone (<200 ng/ml). All participants were required to provide an expired breath sample that was alcohol free (<.002%). Smokers were required to submit a carbon monoxide (CO) sample that was positive (>8 ppm); CO samples from nonsmokers had to be negative (<8 ppm). Participants provided written informed consent and were paid for their time.

2.2. Study design

This study used a mixed-model design to determine the effects of opioid dependence and tobacco use (group effects) on ventilatory response to progressive hypercapnia during three sessions (repeated measures). The groups were LU controls, SM, OD smokers. Within-subject factors were session (3 levels) and trial time (13 bins of 20 s each; baseline, equilibration, and 11 postequilibration), as described below.

2.3. General procedures

On each test session day, participants first provided a urine specimen (which, for methadone-maintained participants, was visually monitored by a research assistant) and expired air samples to measure recent alcohol (breathalyzer; all were alcohol free, <.002%) and cigarette use (CO). Volunteers in the methadone group were administered their daily dose 30 min before the first trial of each session (i.e., testing occurred during peak opioid agonist effects). Smoking cigarettes and drinking caffeine-containing beverages were restricted starting 30 min before testing and throughout the 2-h session. Volunteers did not report distress or withdrawal symptoms from nicotine or caffeine restriction. Breath alcohol tests conducted before each session were required to be negative. Test sessions were scheduled at the same time of day for each participant, although time of day (morning vs. afternoon) varied across individuals. There was no evidence that responding varied as a function of time of testing; this variable is not discussed further.

2.4. CO_2 sensitivity

2.4.1. Apparatus

Ventilatory response to progressive hypercapnia was measured using a CO_2 rebreathing technique (Read, 1967; Rebuck, 1976). The participant wore a nose clip and breathed through a rubber mouthpiece (28-mm inside diameter) connected to an electronic mass flow sensor and Vmax 29c cardiopulmonary module (Sensor Medics, Yorba Linda, CA). The opposite (open) side of the mass flow sensor was connected via a large-bore (34.2-mm inside diameter), short-length (50-cm) tube to a three-way stopcock valve fitted on a 7-l nondiffusing gas-collection bag (Hans Rudolph, Kansas City, MO). The three-way valve could be switched open (to admit room air) and closed (to admit gas mixtures contained in the gas-collection bag).

The mass flow sensor automatically recorded ventilatory response measures (minute volume, VE; breathing rate, *f*; tidal volume, Vt) and $FetCO_2$ and $FetO_2$ concentrations on each breath cycle. The computer module automatically phase-aligned gas concentrations to the inspiratory and expiratory segments of each breath cycle. Menu-driven software was used to set program parameters and to monitor, store, manage, and graph data. Before each session, the system was calibrated to confirm accurate measurement of air volume changes (using a 3-l artificial lung) and certified gas mixtures (Sensor Medics). To maintain a healthy breathing environment, two micropore air filters (Collins Medical, Braintree, MA), located on either side of the mass flow sensor, were routinely changed to protect the participant against bacterial and particulate deposits. The mass flow sensor and other parts of the apparatus exposed to air exchange were bathed in bacteriocide solution after each session.

2.4.2. Procedure

Each participant completed three test sessions, scheduled on separate days close in time (i.e., usually within 1 week). In each session, there were four trials separated by intertrial intervals of 30 min. Previous data suggested that this interval would allow sufficient recovery time, so that ventilatory response on consecutive trials was not influenced (Gozal et al., 1995). During each 4-min test, the participant stood. At the start of each trial, the participant first breathed room air (valve open) for at least 20 s until a normal and stable respiratory rhythm was obtained (baseline); this was monitored in real time using a graphical data display. Then the experimenter closed the three-way valve to expose the participant to the gas-collection bag, which was filled beforehand with a certified mixture of 48% O_2 (to

prevent hypoxia during rebreathing), 5% CO_2, and balanced N_2 (Nellcor Puritan Bennett, Livonia, MI). As the participant breathed into this closed-loop system over 4 min, CO_2 concentration increased linearly with time (see below). Participants' $FetCO_2$ levels equilibrated with the system gas mixture during the first 20–30 s. The relationship between $FetCO_2$ level and minute ventilation (VE; respiratory frequency [*f*] × tidal volume [Vt]) was plotted over the course of the test in 20-s bins (excluding baseline and equilibration).

2.5. Data analysis

Each participant's $FetCO_2$–VE trial data were first plotted and studied for outlying (i.e., nonlinear) values and within-session trends. Using a menu-driven software algorithm, breath-by-breath data were then collapsed into 20-s averages, yielding 13 bins per trial (i.e., 20-s normocapnic baseline followed by 4 min of CO_2 rebreathing). Trial data had to meet three criteria to be considered valid and, thus, included in the analyses.

First, the 20-s average $FetCO_2$ values had to increase monotonically across time bins. If $FetCO_2$ level does not steadily increase, then it is reasonable to suspect equipment or operator error (e.g., open-loop conditions due to a perforated gas bag, a loose hose, or open valve). Alternatively, the participant may be disengaging from the closed-loop system (e.g., subjects who are anxious, salivate excessively, or whose nose clip does not hold produce erratic data), signs that the experimenter can observe. In the range of 6% to 10% $FetCO_2$, hypercapnia-induced ventilatory response is expected to be linear (Cunningham et al., 1986; Duffin et al., 2000). Therefore, it is statistically valid and useful to constrain the data to meet linearity assumptions for calculating the regression function on each trial.

Second, peak $FetCO_2$ values were required to exceed 7.5% with change in $FetCO_2$ greater than 1%, resulting in at least five postequilibration data bins. Because at least 3 points are needed to fit a line, a somewhat arbitrary decision was made to require at least 5 points to improve the precision of the curve fit (i.e., because one of the study aims was to determine whether opioid dependence alters the slope or the intercept). Furthermore, the typical onset of CO_2-induced hyperventilation occurred at $FetCO_2 = 6.5\%$. Thus, it was decided that the minimum 5 points for the regression line should extend from at least $FetCO_2 = 6.5\%$ to 7.5% to provide a meaningful physiological increment. In the obtained data, it was rare that such a small range of $FetCO_2$ values was observed.

Third, end-tidal expired oxygen concentration ($FetO_2$) averages for all 20-s bins had to exceed 14%. This rule was designed to exclude hypoxia-influenced data (which only occurred infrequently at the end of trials, i.e., under maximum hypercapnia). Because most previous studies with drug abusers have examined ventilatory response under conditions of combined hypercapnia and hypoxia, another goal of the present study was to exclude the possibility that hypoxia might independently or synergistically (with hypercapnia) influence group differences in hyperventilation (see Duffin et al., 2000).

Initial analyses indicated that ventilatory response was reliable across trials within session. Therefore, to simplify data analyses, trial data were averaged so that each subject's session average responses served as the units of analysis. First, we plotted and calculated the positive slope and intercept (at $FetCO_2 = 6.5\%$, which was empirically derived from this data set; see Lorinc et al., 1991) of each $FetCO_2$–VE regression line over the available bins (i.e., excluding baseline, equilibration and data that did not meet criteria above) to define each session-average function. Identical functions were plotted and calculated for $FetCO_2$–*f* and $FetCO_2$–Vt functions. Second, mixed-model analyses of variance (ANOVAs) were performed to evaluate group- and session-related differences in the slopes and intercepts of the regression lines.

A Group × Bin (13) × Session (3) ANOVA on the $FetCO_2$ values was used to determine whether the groups differed in CO_2 exposure. This manipulation check was performed to assure that the groups were exposed to the same levels of CO_2 stimulation during the tests. A Group × Bin × Session ANOVA on the VE values was used to determine whether the groups differed in minute ventilation during testing. Session effects and interactions in each of the above ANOVAs were studied to determine whether CO_2 sensitivity was reliable across test days. Huynh–Feldt

Table 1
Demographic characteristics (means and standard deviations)

Measure	LU, $n = 10$	SM, $n = 10$	OD, $n = 10$	Group comparison
Gender	5m, 5f	5m, 5f	7m, 3f	ns
Ethnicity	1w, 5b, 4o	6w, 4b	2w, 8b	
Age (years)	28 (5)	24 (5)	44 (3)	OD>(SM = LU)
Weight (lb)	157 (39)	171 (35)	164 (36)	ns
Body mass index	24 (4)	26 (4)	25 (7)	ns
Methadone dose (mg)	–	–	63 (13)	–
Cigarette use				
Lifetime years	0 (0)	8.9 (6.2)	28.5 (5.4)	OD>SM>LU
Past 30 days	0 (0)	29.8 (0.6)	29.0 (3.2)	(OD = SM)>LU
No, cigarettes per day	0 (0)	14.4 (7.3)	17.8 (7.1)	(OD = SM)>LU
Alcohol past 30 days	1.9 (2.4)	7.0 (5.0)	6.4 (8.2)	ns
Marijuana past 30 days	0.3 (0.7)	7.5 (10.2)	0.5 (1.6)	SM>(OD = LU)
Cocaine past 30 days	0 (0)	0 (0)	1.1 (1.9)	OD>(SM = LU)
Stimulant past 30 days	0 (0)	0.5 (0.8)	0 (0)	ns
Opiate past 30 days	0 (0)	2.5 (5.4)	30 (0)	OD>(SM = LU)

Abbreviations: m = male, f = female; w = white, b = black, o = other (Hispanic and Asian American); ns = no significant difference.

Table 2
Baseline ventilation measures (means and standard deviations)

Measure	LU, $n=10$	SM, $n=10$	OD, $n=10$	Group comparison
$FetCO_2$ (%)	4.7 (0.5)	4.9 (0.6)	5.0 (0.6)	ns
VE (l/min)	16.8 (4.7)	21.2 (6.6)	18.2 (3.6)	$P<.07$
f (breaths/min)	17.5 (5.3)	18.2 (6.7)	14.5 (2.2)	ns
Vt (l/breath)	1.02 (0.3)	1.32 (0.2)	1.28 (0.6)	ns

corrected significance levels were used for all repeated measures terms. The rejection region for all significance tests was set at $P<.05$.

3. Results

Thirty individuals (10 per group) completed the study. Demographic characteristics of the participants are presented in Table 1. The gender composition, average weight, and body mass index of the groups did not significantly differ. The OD and SM groups did not significantly differ in daily cigarette use. However, OD subjects were significantly older than participants in the other groups, and had a longer history of cigarette use than the SM group. Daily methadone maintenance doses ranged from 50 to 95 mg (median = 60 mg).

Prior to rebreathing (trial baseline), the groups did not significantly differ in average (i.e., across trials and sessions) $FetCO_2$ levels or breathing measures. Mean ($\pm$ S.D.) values for these baseline measures are presented in Table 2.

As expected, CO_2 rebreathing produced time-dependent linear increases in $FetCO_2$ (i.e., progressive hypercapnia) that did not significantly differ across sessions or groups. $FetCO_2$ concentrations typically ranged from 6% to 10% across subjects and sessions. The onset of $FetCO_2$-induced

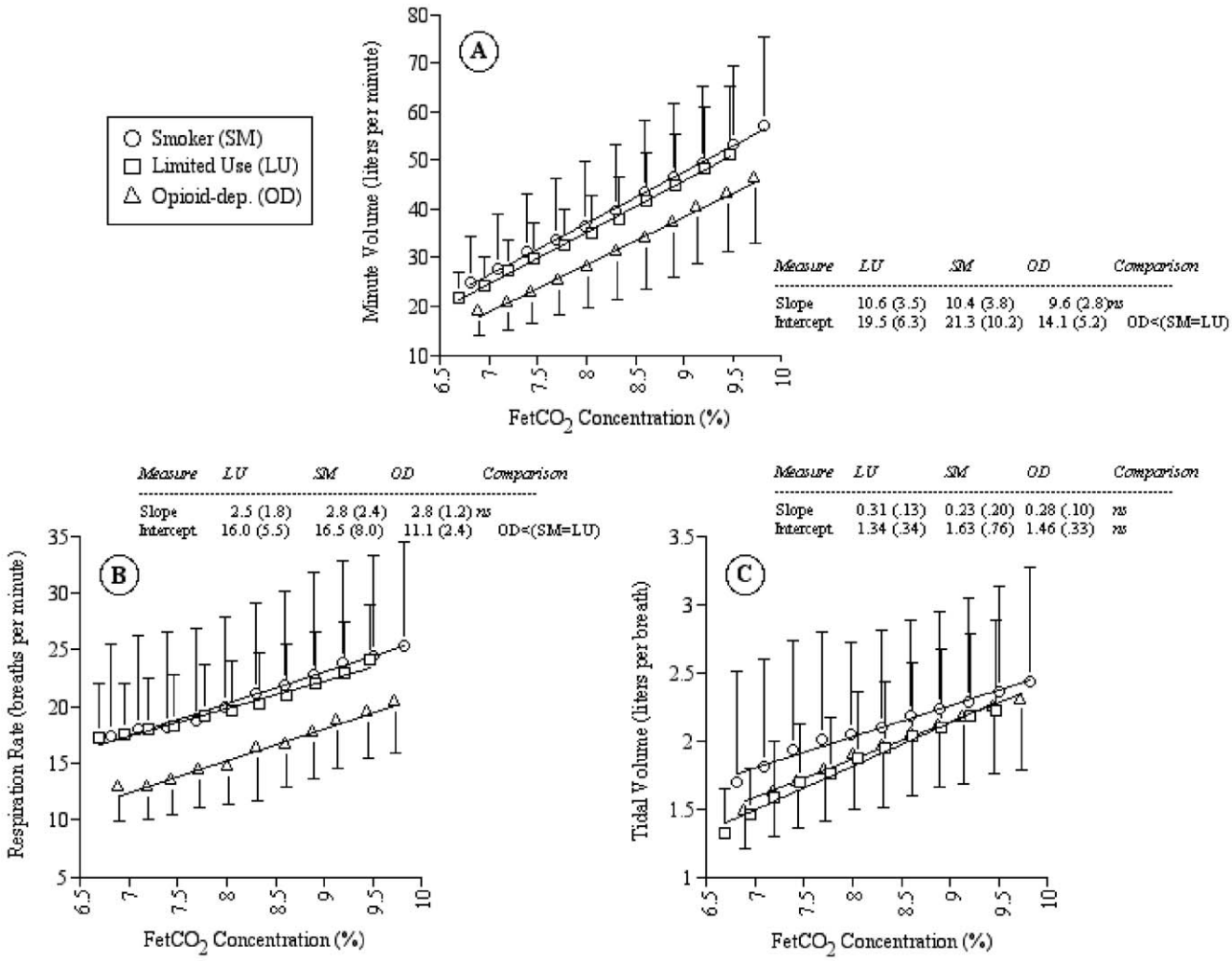

Fig. 1. Effect of progressive hypercapnia on mean (+1 S.D.) minute ventilation (panel A), respiration rate (panel B), and tidal volume (panel C) for the LU, SM, and OD groups; $n=10$ per group. Least squares regressions ($r^2>.975$ for all lines here) were computed to obtain slopes and intercept values at $FetCO_2=6.5\%$. As indicated by the means (S.D.s) in the table inserts, planned comparisons found group differences only for the minute ventilation and breathing rate intercepts, with the OD group showing a significantly ($P<.05$) smaller ventilatory response than the SM and LU groups (which did not differ).

increases in ventilatory response was typically observed to occur at about 6.5%. Therefore, least-squares regression lines were computed to obtain slopes and intercept values at $FetCO_2 = 6.5\%$ (see Fig. 1).

Fig. 1 (panel A) shows that the $FetCO_2$–VE function for OD subjects was shifted to the right—but not down—relative to the other two experimental groups. The planned comparisons showed significant differences between the OD group and the LU group, $F(1,18) = 4.72$, $P < .025$, and SM group, $F(1,18) = 4.62$, $P < .025$. Intercepts of the $FetCO_2$–VE function did not significantly differ for the SM and LU groups. There were no significant group differences in slopes of the $FetCO_2$–VE function.

Fig. 1 (panel B) shows that the $FetCO_2$–f function for OD subjects was shifted to the right—but not down—relative to the other two groups. Planned comparisons showed significant differences between the OD group and the LU group, $F(1,18) = 10.16$, $P < .0005$, and SM group, $F(1,18) = 4.39$, $P < .025$. Intercepts of the $FetCO_2$–f function did not significantly differ for the SM and LU groups. There were no significant group differences in slopes of the $FetCO_2$–f function.

Fig. 1 (panel C) shows that the $FetCO_2$–Vt function for SM group was shifted slightly to the left of the other two groups. However, the planned comparisons indicated that the intercepts and slopes of the regression lines did not significantly differ between any of the three groups.

Demographic characteristics (see Table 1) were correlated with the mean (across trials and sessions) intercept and slope values of the CO_2–ventilatory response functions. This was intended to assess whether certain individual factors might account for variability in hypercapnic response. With the exception of age (which was confounded with group membership; see Table 1), none of these factors (i.e., gender, age, body mass index, weight) was significantly related to ventilatory response. Within the OD group, which was small in size ($n = 10$), the rather limited variations in methadone dose across subjects did not significantly correlate with intercept or slope values.

The number of cigarettes per day was significantly ($P < .05$) correlated with the $FetCO_2$–breathing rate intercept in the entire sample ($r = -.41$, $df = 28$) and in just the two smoking groups ($r = -.48$; $df = 18$), but not other ventilatory response parameters. That is, higher levels of current smoking were associated with smaller breathing rate increases during hypercapnia. Analysis of covariance was conducted to determine whether the group difference in breathing rate intercept (see Fig. 1B) would be affected by including this factor. The group main effect remained statistically significant in the overall analysis with the covariate (which, consistent with the zero-order correlation, was significant, $F = 4.46$, $P < .05$). Using the a priori directional (one-tailed $P < .10$) tests, pairwise comparisons of the adjusted means indicated that the OD group $FetCO_2$–breathing rate intercept remained significantly lower than the LU and SM group intercepts.

4. Discussion

The present study was designed to examine whether previously observed decreases in CO_2 sensitivity among OD individuals might be explained, in part, by the tobacco use that is highly prevalent in this population. Because we had not used this methodology before in our laboratory, the first aim was to demonstrate diminished CO_2 sensitivity for OD participants relative to a control group with limited lifetime substance use (LU). Our confirmation of this effect is therefore consistent with previous data using individuals chronically maintained on morphine (Martin et al., 1968) and methadone (Santiago et al., 1977).

The second aim was to determine whether reduced hypercapnic ventilatory drive is specific to chronic opioid exposure. A group that principally smoked cigarettes with relatively limited exposure to other drugs (SM) was matched to the OD group on current cigarette use (in the absence of nicotine-replacement products), and all three groups were similar in weight, body mass index, and gender. Additionally, the present study controlled for the influence of hypoxia on ventilatory response, a factor that appears to have been disregarded in previous hypercapnia studies with opioid abusers. Consistent with our hypothesis, the SM group differed significantly from the OD smokers, but not from the LU controls. These results are consistent with growing evidence showing that smokers (Kawakami et al., 1982; Yamamoto et al., 1985) and infants exposed to tobacco in utero (Lewis and Bosque, 1995) appear to be sensitive to hypoxia rather than hypercapnia. Taken together, this pattern of data suggests that opioid and tobacco exposure may alter ventilatory response through different chemosensitivity mechanisms. Because tobacco cigarettes contain numerous chemical compounds, it remains possible that some of these agents (if isolated) could be shown to affect CO_2 sensitivity. This issue lies beyond the scope of this study but it is worth noting that nicotine did not significantly decrease ventilatory response to hypercapnia in rhesus monkeys (Howell, 1995).

The present results should be interpreted cautiously due to a limitation of this study design. Despite matching the groups on several relevant variables, the OD group was significantly older than the SM and LU groups (which were similar in age). Thus, it is possible that the decreased CO_2 sensitivity of these OD individuals could be partly explained by advancing age. The respiratory physiology literature suggests that, among healthy elderly subjects, arterial gas exchange (e.g., CO_2 tension) during rest and exertion remains normal. Rather, aging tends to diminish respiratory reserve via several factors (e.g., loss of pulmonary tissue; and decreases in surface area, chest wall compliance, respiratory muscle strength, and expiratory flow rates) that may indirectly reduce hypercapnic ventilatory response (Janssens et al., 1999). In four large-scale studies that included elderly subjects to evaluate the effect of age on

decreasing hypercapnic ventilatory response in healthy subjects, results were mixed. Specifically, those studies found no significant age effect on hypercapnic ventilatory response (Kawakami et al., 1981; $n=127$), a significant effect (Molho et al., 1986; $n=105$), a modest effect mediated through decreased vital capacity (Jones et al., 1993; $n=181$), or an effect only in males (Sin et al., 2000; $n=176$). Because of these mixed effects and the different focus of the present study, it was not a priority to match groups on age. On the one hand, elderly subjects were excluded from this study. On the other hand, the small sample and the lack of vital capacity testing in this study could have masked a potential effect of age on hypercapnic ventilatory response. Additional data analyses, using age as a covariate, found that the group difference in ventilatory response was attenuated and no longer significant. Consistent with the literature above, this suggests that age was a modulator (but not a mediator) of the OD group's hypercapnic ventilatory response.

A related limitation of this study is that—despite similar current levels of cigarette use—the (older) methadone-maintained group reported a significantly longer history of tobacco use than the (younger) smoking group. Thus, it is possible that the reduced hypercapnic sensitivity of the OD group could be partly explained by a longer lifetime period of tobacco use. However, this alternative account appears unlikely because ventilatory response of the SM group did not significantly differ from the (age-matched) LU control group. Clinical experience suggests that many opioid dependent individuals are sporadic tobacco users (''chippers''), and subjects in the OD group smoked 7 to 25 cigarettes daily. Consistent with this natural variability in tobacco use, it was decided a priori to treat daily cigarette use as a continuous (dimensional) rather than a categorical (i.e., dependent or not) variable. Subjects in the two smoking groups were therefore not required to meet criteria for nicotine dependence but instead were matched on intensity of current smoking. Because the number of cigarettes smoked by these individuals was moderate (averaging slightly less than one pack per day), the present study cannot exclude the possibility that higher levels of tobacco use and/or dependence could significantly influence hypercapnic ventilatory response. To further examine the possibility that individual differences in tobacco use might affect hypercapnic ventilatory response, covariance analyses were conducted. The only significant relationship found was between daily cigarette use (within the range presented by subjects in this study) and the $FetCO_2$–breathing rate intercept. However, the group difference in this measure did remain statistically significant after adjusting for this factor.

There were also some minor differences in other substance use across the experimental groups. The SM group reported more past-30-day marijuana use than both other groups; however, this group difference was mostly due to frequent use by two subjects. Although this is a limitation of the study, ventilatory response data for this group did not change when these two subjects were removed and the analysis was repeated. A similar story emerged for the slightly but statistically higher amount of past-30-day cocaine use in the OD group. Again, ventilatory response data for this group did not change when two subjects who used cocaine (3 and 5 times in the past 30 days) were removed.

Minute volume (the measure of CO_2 sensitivity in this study) integrates changes in breathing rate and tidal volume. Minute ventilation is a reliable, sensitive and, typically, the primary index of drug-induced changes in hypercapnic ventilatory response in animals (Gerak et al., 1994; Howell, 1995; Liguori et al., 1996; Paronis and Woods, 1997a,b; Willette et al., 1987) and humans (Jasinski et al., 1968; Kawakami et al., 1982; Martin et al., 1968; Pianosi et al., 1994; Santiago et al., 1977; Yamamoto et al., 1985). The final aim of this study was to determine whether decreased CO_2 sensitivity in the OD group was attributable to decreased breathing rate or decreased tidal volume, and whether the observed group difference arose from a shift in the intercept or slope of the rebreathing curve. The results unambiguously demonstrated that CO_2-induced decreases in minute ventilation were attributable to a reduction in hypercapnia-stimulated changes in breathing rate and not tidal volume. Thus, OD subjects appear to respond to the physiological burden of hypercapnia via smaller magnitude CO_2-related increases in respiratory frequency. This may relate to electrophysiological data showing that the primary effects of opioids are to inhibit the firing of chemosensitive inspiratory neurons (Eguchi et al., 1987; Lydic et al., 1991; Takeda et al., 2001; Willette et al., 1987), resulting in ''respiratory inertia.'' Furthermore, the nature of this rebreathing curve shift was specifically attributable to a change in the intercepts rather than slopes of the CO_2/ventilatory response (VE or *f*) functions (i.e., an additive, not multiplicative, effect). This is consistent with an earlier report by Jasinski et al. (1968) showing that acute morphine administration shifts the CO_2/ventilatory response curve rightward. These observations suggest the working hypothesis that mu opioid exposure may, in part, alter respiratory drive by decreasing the potency of CO_2 to elicit normal ventilatory response.

Although decreased responsiveness to hypercapnia was not correlated with methadone dose in the OD group, this is not surprising. Clinical research has showed that intersubject differences in methadone maintenance dose (or plasma levels of the drug) are generally not well correlated with pharmacodynamic responses (e.g., Berkowitz, 1976; Torrens et al., 1998). Thus, with the small group size and limited range of methadone doses in the present study, it is not surprising that *between-subject* correlations of methadone maintenance dose with ventilatory response were not significant. While hypercapnic ventilatory response was measured in OD subjects shortly after methadone administration, the acute impact of a daily dose within the same

methadone-stabilized individuals was not measured in the present study. That is, it would be necessary to examine this issue by conducting CO_2 sensitivity tests at peak methadone effect (as done in the present study) and trough level (24 h postmethadone or even longer intervals after dose omission). Some data suggest that, *within subjects* across the 24-h dosing cycle, it is possible to obtain stronger correlations between plasma methadone levels and pharmacological effects such as pupil diameter and withdrawal symptoms (e.g., Dyer et al., 1999; Hiltunen et al., 1995).

In conclusion, the present findings suggest that the decreased CO_2 sensitivity displayed by OD individuals is unlikely to be explained by CO_2 production, current moderate cigarette use, body mass index, or gender. However, possible modulatory effects of age or lifetime duration of cigarette use cannot be ruled out as contributing factors. The depressed ventilatory response of OD subjects also cannot be explained by health problems (because medical disorders excluded participants in all three groups), group differences in baseline breathing measures, or hypoxia (which, unlike previous studies with drug abusers, was controlled in this procedure). Finally, the data point to a specific reduction in the intercept of the CO_2–breathing rate function. The procedure used in this study is capable of supplying more detailed information concerning mechanisms of respiratory depression compared to standard measures of breathing rate or oxygen saturation under baseline conditions (i.e., in the absence of chemosensitivity challenge procedures). If these findings were replicated and extended (e.g., ruling out long-term cigarette use and age), this technique may be able to exclude alternative interpretations of respiratory depressant effects that occur with chronic opioid exposure. The procedure may also be useful for evaluating medications that can antagonize the ventilatory depressant effects of abused opioids (e.g., Elmalem et al., 1991; Gerak et al., 1994; Kishioka et al., 2000; Liguori et al., 1996).

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