

# Reversal of Morphine-Induced Apnea in the Anesthetized Rat by Drugs that Activate 5-Hydroxytryptamine<sub>1A</sub> Receptors<sup>1</sup>

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## ABSTRACT

The purpose of our study was to test the hypothesis that 5-hydroxytryptamine (5-HT)<sub>1A</sub> receptor agonists counteract morphine-induced respiratory depression. Studies were conducted in anesthetized rats, and respiratory activity was monitored with diaphragm electromyography. Morphine was administered i.v. in doses that produce apnea. Once apnea was established, i.v. administration of the 5-HT<sub>1A</sub> receptor agonist drug 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) at 10 or 100  $\mu\text{g}/\text{kg}$  restored normal breathing in each animal ( $n = 24$ ). This antagonistic effect of 8-OH-DPAT on morphine-induced respiratory depression was observed in both spontaneously breathing and artificially

ventilated animals. Results obtained with 8-OH-DPAT were mimicked by buspirone (50  $\mu\text{g}/\text{kg}$  i.v.), another 5-HT<sub>1A</sub> receptor agonist drug. Pretreatment with 4-(2'-methoxyphenyl)-1-[2'-(2'-pyridinyl)-*p*-iodo-benzamido]ethyl]piperazine, an antagonist of 5-HT<sub>1A</sub> receptors, prevented 8-OH-DPAT from counteracting morphine-induced apnea. These results indicate that activation of central nervous system 5-HT<sub>1A</sub> receptors is an effective way of reversing morphine-induced respiratory depression. Most important, this is the third model of disturbed respiratory function in which drugs that stimulate 5-HT<sub>1A</sub> receptors have been shown to restore breathing to near-normal levels.

The brain serotonergic system has been implicated in the control and/or modulation of respiratory function in a number of studies. These data have been discussed in several review articles (e.g., Bianchi et al., 1995; McCrimmon et al., 1995) but no clear picture emerges as to whether the serotonergic system, specifically 5-hydroxytryptamine (5-HT)<sub>1A</sub> receptor activation, enhances or diminishes respiratory function. This issue can be highlighted by focusing on two published findings. One is the report by Garner et al. (1989) demonstrating that buspirone, a drug with 5-HT<sub>1A</sub> receptor agonist properties (Taylor, 1988), stimulates respiratory output primarily by increasing tidal volume when administered to anesthetized and unanesthetized decerebrate cats. The second is the report by Lalley et al. (1994a) demonstrating that 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), another 5-HT<sub>1A</sub> receptor agonist drug (Middlemiss and Fozard, 1983), inhibits respiratory output culminating in apnea when administered to anesthetized cats.

We became interested in this problem because of the findings of Lalley et al. (1994b) and Wilken et al. (1997). They

reported that 8-OH-DPAT and buspirone counteract respiratory disturbances (i.e., apneustic breathing) produced by hypoxia, pentobarbital, and antagonists of the *N*-methyl-D-aspartate receptor complex, specifically MK-801 (dizocilpine) and ketamine, in anesthetized cats. In addition, buspirone was found to reverse apneustic breathing in a pediatric patient after an operation to remove an astrocytoma located in the pons and medulla (Wilken et al., 1997). Consistent with an anti-apneustic effect of buspirone is the recent finding of Richter et al. (1999) demonstrating that microinjection of 8-OH-DPAT into the pre-Böttinger area of the ventrolateral medulla will counteract hypoxia-induced apneustic breathing in cats (Fig. 7 in Richter et al., 1999). In contrast, and in the same report, these investigators report that 5-HT-induced activation of 5-HT<sub>1A</sub> receptors contributes to hypoxia-evoked respiratory depression.

In our preliminary studies using the rat as the experimental animal model, we have found that 8-OH-DPAT and buspirone exert only positive effects on disturbances in respiratory function; that is, 8-OH-DPAT counteracted apnea produced by dizocilpine (Sahibzada et al., 1999), and 8-OH-DPAT and buspirone restored breathing to normal levels in animals subjected to spinal cord injury (Teng et al., 1999). To further delineate the respiratory conditions under which 5-HT<sub>1A</sub> receptor activation may be of benefit or detriment to

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**ABBREVIATIONS:** 5-HT, 5-hydroxytryptamine; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; dEMG, diaphragmatic electromyogram; iEMG, integrated diaphragmatic electromyogram; *p*-MPPI, 4-(2'-methoxyphenyl)-1-[2'-(2'-pyridinyl)-*p*-iodo-benzamido]ethyl]piperazine.

the organism, we examined the response to agonists of this receptor on another model of disturbed respiratory function: morphine-induced respiratory arrest produced in the rat. Our findings indicate that activation of 5-HT<sub>1A</sub> receptors restores breathing in rats with morphine-induced apnea.

## Materials and Methods

**General Procedures.** Results were obtained with the use of 36 Sprague-Dawley adult male rats (Taconic, Germantown, NY) weighing 270 to 420 g. Anesthesia was instituted with a 3 ml/kg i.p. injection of a cocktail containing urethane (800 mg) and  $\alpha$ -chloralose (60 mg) dissolved in 3 ml of 0.9% saline. The trachea was cannulated to provide access to the airway and for instituting artificial respiration when necessary. The left carotid artery was cannulated for blood pressure recording and for calculating the heart rate from the blood pressure trace. The right jugular and right femoral veins were cannulated for administration of drugs via the i.v. route. Blood pressure was recorded using a bridge amplifier connected to a MacLab (ADI Instruments, Milford, MA) data acquisition system. Rectal temperature was monitored and maintained at  $37 \pm 1^\circ\text{C}$  with an infrared heating lamp.

The endpoint used to detect a respiratory effect of the drugs studied was diaphragmatic electromyogram activity. The diaphragm electromyogram (dEMG) was obtained by making an incision below the chest cavity and inserting a hooked bipolar platinum-iridium electrode to the right half of the costal diaphragm. The electrode was coupled to a p511 AC preamplifier (Grass Instruments, Quincy, MA). The output signal of the amplifier was fed into an audiometer and displayed on a storage oscilloscope and computer monitor. Data were stored on computer (Apple Macintosh PowerPC connected to MacLab) for later viewing and analysis. The dEMG signal and the software-generated integration (iEMG) of the raw signal using a 100-ms time window were continuously recorded and stored for off-line analysis. Due to the low respiration rates in the present study, integration at a 100-ms time constant did not change the representation of the signal from that at a lower time constant (e.g., 10 or 20 ms).

The cervical vagus nerves were cut bilaterally in 12 of 24 animals used to determine the effect of 8-OH-DPAT on morphine-induced respiratory depression. The remaining 12 animals were not vagotomized. An additional 12 animals used for other studies were left with vagi intact. The purpose of performing vagotomy was to qualitatively assess whether peripheral receptors located in the lungs (J receptors), which send afferents through the vagus, played an important role in morphine-induced respiratory depression under our experimental conditions. Willette and Sapru (1982) reported that a significant portion of the effect of morphine on respiration in the rat is due to a peripheral action involving J receptors. Although the majority of animals were studied in a state of spontaneous breathing, some animals were studied under a condition of artificial respiration when receiving morphine. The purpose for this was to maintain arterial blood gases and pH constant. These animals were continuously artificially respired with room air by a volume ventilator (Harvard Apparatus, South Natick, MA). All animals were ventilated on entering into apnea. The respirator frequency setting was adjusted to approximate each animal's intrinsic respiratory rate as determined by the dEMG signal. Occasionally, ventilation at the intrinsic rate was found to decrease the dEMG signal strength; when this occurred, the ventilator rate was reduced slightly (usually no greater than 5 breaths/min) to increase the amplitude of the dEMG signal. Respiratory frequency observed in the anesthetized animals used for this study, although slower than those reported in the conscious rat (Baker et al., 1979), were similar to those reported in other studies using an anesthetized animal preparation (El-Bohy et al., 1998).

**Experimental Protocols.** In the study designed to test the effect of 8-OH-DPAT on morphine-induced respiratory depression (i.e., ap-

nea) in spontaneously breathing animals, four groups of six animals per group were evaluated. One group had their vagus nerves left intact and received 10  $\mu\text{g}/\text{kg}$  i.v. 8-OH-DPAT after morphine overdose. A second group underwent bilateral cervical vagotomy and received 10  $\mu\text{g}/\text{kg}$  i.v. 8-OH-DPAT after morphine overdose. Groups 3 and 4 both received 100  $\mu\text{g}/\text{kg}$  i.v. 8-OH-DPAT after morphine overdose, and one group had their vagus nerves left intact and the other group underwent bilateral cervical vagotomy. These two doses of 8-OH-DPAT were chosen because they approximate the two dose ranges of 8-OH-DPAT that Lalley et al. (1994a) described as exhibiting distinctly different effects on respiratory activity in anesthetized cats. The 10  $\mu\text{g}/\text{kg}$  i.v. dose fits in the dose range that shortens inspiratory duration and increases respiratory rate without directly affecting inspiratory drive in the cat. The 100  $\mu\text{g}/\text{kg}$  i.v. dose approximates the range of doses that directly abolish all inspiratory drive in the cat.

Stable baseline measurements of dEMG activity and arterial blood pressure were obtained for a 10-min period before the experiment was initiated. Next, for the majority of the experiments, i.v. morphine injections were administered using several different dosing regimens, with the goal of producing a stable degree of apnea. Morphine was administered using a constant i.v. infusion pump, and each dose was delivered over 24 to 36 s depending on the weight of the animal (see *Study Drugs*). Morphine was administered in doses of 4 mg/kg ( $n = 3$ ), 6 mg/kg ( $n = 19$ ), or 15 mg/kg ( $n = 2$ ) repeated once every 2 to 5 min until apnea was produced. No significant differences in total morphine dose administered were seen between any of the four treatment groups receiving 8-OH-DPAT. The mean dose of morphine administered was  $21.3 \pm 2.1$  mg/kg for all animals. Apnea was initially judged to occur when no respiratory activity was detected for longer than 20 s. During this initial apnea, blood pressure was observed to fall precipitously, and this necessitated placing animals on a ventilator. Animals were artificially respired for at least 1 min to restore blood pressure and blood gasses before being tested for evidence of a stable apnea. A stable apnea was considered to be present if removal of the animal from the ventilator for 20 to 30 s, during which time CO<sub>2</sub> would accumulate, did not restore dEMG activity. If spontaneous breathing returned with removal of the animal from the ventilator, another morphine bolus dose was administered, and this cycle was repeated until suspension of artificial respiration was ineffective in restoring respiratory activity. At this point, animals were artificially ventilated continuously, and i.v. 8-OH-DPAT in doses of either 10  $\mu\text{g}/\text{kg}$  ( $n = 12$ ) or 100  $\mu\text{g}/\text{kg}$  ( $n = 12$ ) were administered to attempt to restore respiratory activity. Artificial ventilation was removed when diaphragmatic activity was observed to return; animals were then allowed to breathe spontaneously and were followed for 15 to 20 min after the 8-OH-DPAT infusion.

The protocol just described was used in spontaneously breathing animals. In an additional group of rats ( $n = 5$ ), animals were placed on artificial respiration at the outset of the experiment, and artificial respiration was maintained for the duration of the study. Stable baseline values for amplitude and frequency of dEMG, the iEMG, and the arterial blood pressure were established over a 10-min observation period. Morphine in i.v. infusion doses delivered over 24 to 36 s at 4 mg/kg was administered at 2- to 5-min intervals until the dEMG signal was abolished. One animal received a single bolus dose of 12 mg/kg morphine, which produced apnea. In all cases, a waiting period of 2 min was used to ensure loss of the signal; at this point, 8-OH-DPAT in an i.v. dose of 10  $\mu\text{g}/\text{kg}$  was administered. The endpoint of the 8-OH-DPAT effect was restoration of the dEMG signal. In these studies, two of the five rats received buspirone in an i.v. dose of 50  $\mu\text{g}/\text{kg}$  instead of 8-OH-DPAT.

Other studies that we performed investigated the: 1) effects of 8-OH-DPAT alone on cardiorespiratory activity, 2) pretreatment with an antagonist of 5-HT<sub>1A</sub> receptors (the antagonist was also administered i.v. using a constant infusion pump such that a single dose was administered over 24–36 s) to determine whether the effect

of 8-OH-DPAT on morphine-induced respiratory depression could be prevented, and 3) effects of an antagonist of 5-HT<sub>1A</sub> receptors on cardiorespiratory activity. The protocols for these latter studies are described in *Results*. All experiments were carried out in accordance with the National Institutes of Health guidelines for the use of animals in research.

**Data Analysis.** All respiratory indices (dEMG, iEMG, and arterial blood pressure) were continuously recorded and stored on videotape and on computer. Data were analyzed off-line with an Apple Macintosh PowerPC using the MacLab (ADI Instruments) data acquisition system. Control or baseline values were obtained by averaging values during a 10-s period. Peak effects of each drug were obtained by noting the maximum change in cardiorespiratory activity that occurred at 30-s and 1-, 2-, 3-, 4-, 5-, and 10-min time points after the i.v. infusion of drug had ended. Values obtained at these time points were taken by averaging data during a period of 10 s. The EMG signal that was used for our calculation was obtained from the peak signal of the integrated EMG rather than the raw dEMG signal due to an occasional large amplitude single spike occurring in the raw signal, which would be unduly weighted by the data acquisition software analysis program. Values are given as means  $\pm$  S.E. Analysis of the change in iEMG amplitude from baseline (percent change) was accomplished using a Wilcoxon signed rank test. All other data were statistically analyzed using a one-way repeated measures ANOVA. Differences between groups were analyzed using a Student-Newman-Keuls test and were considered significant if  $P < .05$ .

**Study Drugs.** Urethane and  $\alpha$ -chloralose were purchased from Sigma Chemical Co. (St. Louis, MO). Urethane (800 mg) and  $\alpha$ -chloralose (60 mg) were dissolved in 3 ml of 0.9% saline. Morphine sulfate was purchased from Elkins-Sinn (a division of A. H. Robins Co., Richmond, VA) and dissolved in 0.9% saline as a 15 mg/ml solution. The 5-HT<sub>1A</sub> receptor agonist drugs 8-OH-DPAT and buspirone were purchased from Research Biochemicals Inc. (Natick, MA). Both drugs were dissolved in 0.9% saline and administered in doses of 10 or 100  $\mu$ g/kg for 8-OH-DPAT and 50  $\mu$ g/kg for buspirone. These doses were selected based on the previous reports that describe 5-HT<sub>1A</sub> agonist-induced reversal of apneustic breathing (Lalley et al., 1994a; Wilken et al., 1997). The 5-HT<sub>1A</sub> receptor antagonist 4-(2'-methoxyphenyl)-1-[2' [N-(2'-pyridinyl)-p-iodo-benzamido]ethyl]piperazine (*p*-MPPI) was also purchased from Research Biochemicals Inc. and was dissolved in 0.9% saline heated to 50–60°C to facilitate complete dissolution of the drug. The dose of administered *p*-MPPI was either 20 or 40  $\mu$ g/kg. This produced a *p*-MPPI/8-OH-DPAT ratio, on a  $\mu$ g/kg basis, similar to previous reports that demonstrated *p*-MPPI antagonism of 8-OH-DPAT effects (Allen et al., 1997; Shaikh et al., 1997; Wolff and Leander, 1997). All drugs, with the exception of the anesthetic agents (which were administered i.p.), were administered via polyethylene tubing connected to a 5-ml syringe driven by a Sage infusion pump. All i.v. drugs were infused at a constant rate of 0.69 ml/min. Drugs were prepared such that 1 ml of drug solution contained the dose necessary to achieve the desired dose in a 1.0-kg animal. Thus, the duration of drug infusion was the principal method used to achieve appropriate final dose in each animal and, depending on the animal's weight, varied between 24 and 36 s.

## Results

Two 5-HT<sub>1A</sub> receptor agonist drugs, 8-OH-DPAT and buspirone, were tested separately to determine their ability to reverse morphine-induced respiratory depression. To assess the effects of 8-OH-DPAT and buspirone on morphine-induced respiratory depression, we first examined the effect of morphine on cardiorespiratory function.

**Effect of Morphine on Respiration in Spontaneously Breathing Rats.** Morphine was administered i.v. to 24 spontaneously breathing animals using the dosing regimens de-

scribed earlier; the effects are given in Table 1. Half of the animals underwent bilateral cervical vagotomy. A representative experiment of the effect of morphine in a vagus nerve-intact animal appears in Fig. 1, A and B. The endpoint of a morphine effect with each dosing regimen was the occurrence of apnea. The dose of morphine to produce apnea did not differ significantly between the vagus nerve-intact and the vagotomized animals ( $P > .05$ ). The usual respiratory effect of morphine before the onset of apnea was a decrease in respiratory frequency ( $67 \pm 3$  to  $31 \pm 6$  breaths/min,  $P < .05$ , for vagus nerve-intact animals;  $51 \pm 2$  to  $27 \pm 6$  breaths/min,  $P < .05$ , for vagotomized animals) and a decrease in the iEMG (in arbitrary units:  $101 \pm 15$  to  $75 \pm 12$ ,  $P < .05$ , for vagus nerve-intact animals;  $110 \pm 14$  to  $80 \pm 12$ ,  $P < .05$ , for vagotomized animals). With the occurrence of apnea, all animals were placed on artificial respiration before the administration of 8-OH-DPAT (see *Materials and Methods*). Values for mean arterial pressure and heart rate were obtained and tabulated just before the administration of 8-OH-DPAT (Table 1). Both vagus nerve-intact and vagotomized animals exhibited a fall in mean arterial pressure after morphine administration, whereas only the vagus nerve-intact animals exhibited a statistically significant decrease in heart rate after morphine administration.

### Effects of 8-OH-DPAT on Morphine-Induced Respiratory Depression in Spontaneously Breathing Rats.

The 24 animals described earlier were placed on artificial respiration once life-threatening respiratory depression developed subsequent to morphine administration (see *Materials and Methods*). 8-OH-DPAT was administered i.v. in doses of either 10  $\mu$ g/kg ( $n = 12$ ) or 100  $\mu$ g/kg ( $n = 12$ ) over a period of 24 to 36 s. We found in our study that the 10 and 100  $\mu$ g/kg i.v. doses exerted the same effect, namely, reversal of morphine-induced apnea (Table 1 and Fig. 1, C–E). This occurred regardless of the status of the animal's vagus nerves. The time interval between the occurrence of morphine-induced apnea and the administration of 8-OH-DPAT was  $6.3 \pm 0.5$  min, during which the animal was continuously ventilated. Reversal of morphine-induced apnea occurred in half of the animals even before the continuous infusion of 8-OH-DPAT had been completed, and for all animals, it occurred within 2 min of completion of infusion. The time of the peak effect of 8-OH-DPAT on respiratory rate was similar in all animals and averaged  $2.6 \pm 0.3$  min after the completion of i.v. infusion. Generally, animals were followed for 15 to 20 min after the administration of 8-OH-DPAT, and within this time frame, reversal of morphine-induced apnea was still present in most animals. No important differences were noted between the effectiveness of 10 and 100  $\mu$ g/kg doses of 8-OH-DPAT. We also tested the effectiveness of 1  $\mu$ g/kg 8-OH-DPAT in four animals (data not shown) and found that only one of four animals exhibited a reversal of morphine-induced apnea when 1  $\mu$ g/kg 8-OH-DPAT was administered i.v.. We also noted that the recovery of respiration after 8-OH-DPAT infusion was more robust once the respirator was switched off (see, e.g., Figs. 1D and 4E). This increase in dEMG amplitude may have been due to the absence of a respiratory phase conflict between the ventilator and the spontaneous respiratory drive of the animal.

The efficacy of 8-OH-DPAT to restore morphine-induced cardiorespiratory depression to normal can be extracted from data in Table 1; 8-OH-DPAT normalized the amplitude of the



TABLE 1  
Effects of 8-OH-DPAT treatment on morphine-induced apnea and changes in cardiovascular function in spontaneously breathing and artificially ventilated rats

Group	State of Vagus	8-OH-DPAT Dose Received	Baseline				Morphine (before 8-OH-DPAT Infusion)				8-OH-DPAT (Peak Effect)			
			F	iEMG % baseline	MAP mm Hg	HR beats/min	F	iEMG % baseline	MAP mm Hg	HR beats/min	F	iEMG % baseline	MAP mm Hg	HR beats/min
<b>Spontaneously breathing</b>														
I (n = 6)	Intact	10 µg/kg	66 ± 3	100*	98 ± 5	396 ± 24	0*	-100*	58*	302* ± 44	49* ± 3	8** ± 16	78*** ± 5	289* ± 24
II (n = 6)	Cut	10 µg/kg	49 <sup>a</sup> ± 3	100*	102 ± 7	419 ± 25	0*	-100*	60* ± 9	350 ± 32	34*** ± 3	12** ± 4	73* ± 9	357 ± 31
III (n = 6)	Intact	100 µg/kg	68 ± 6	100*	89 ± 7	368 ± 25	0*	-100*	65 ± 4	299* ± 32	54*** ± 4	12** ± 22	78 ± 14	306* ± 24
IV (n = 6)	Cut	100 µg/kg	52 <sup>a</sup> ± 3	100*	83 ± 2	443 ± 10	0*	-100*	55* ± 6	412 ± 27	41*** ± 3	10** ± 12	58* ± 5	395 ± 17
<b>Ventilated animals</b>														
V (n = 3)	Intact	10 µg/kg	58 ± 5	100*	77 ± 8	419 ± 30	0*	-100*	78 ± 10	394 ± 51	38*** ± 6	48** ± 31	77 ± 4	398 ± 36

F, respiratory rate; iEMG (100-ms window) based on percent baseline of arbitrary units; MAP, mean arterial pressure; HR, heart rate.

<sup>a</sup> Baseline value of vagotomized animals was significantly different than corresponding values of vagus-intact groups.

\*  $P < .05$  versus control.

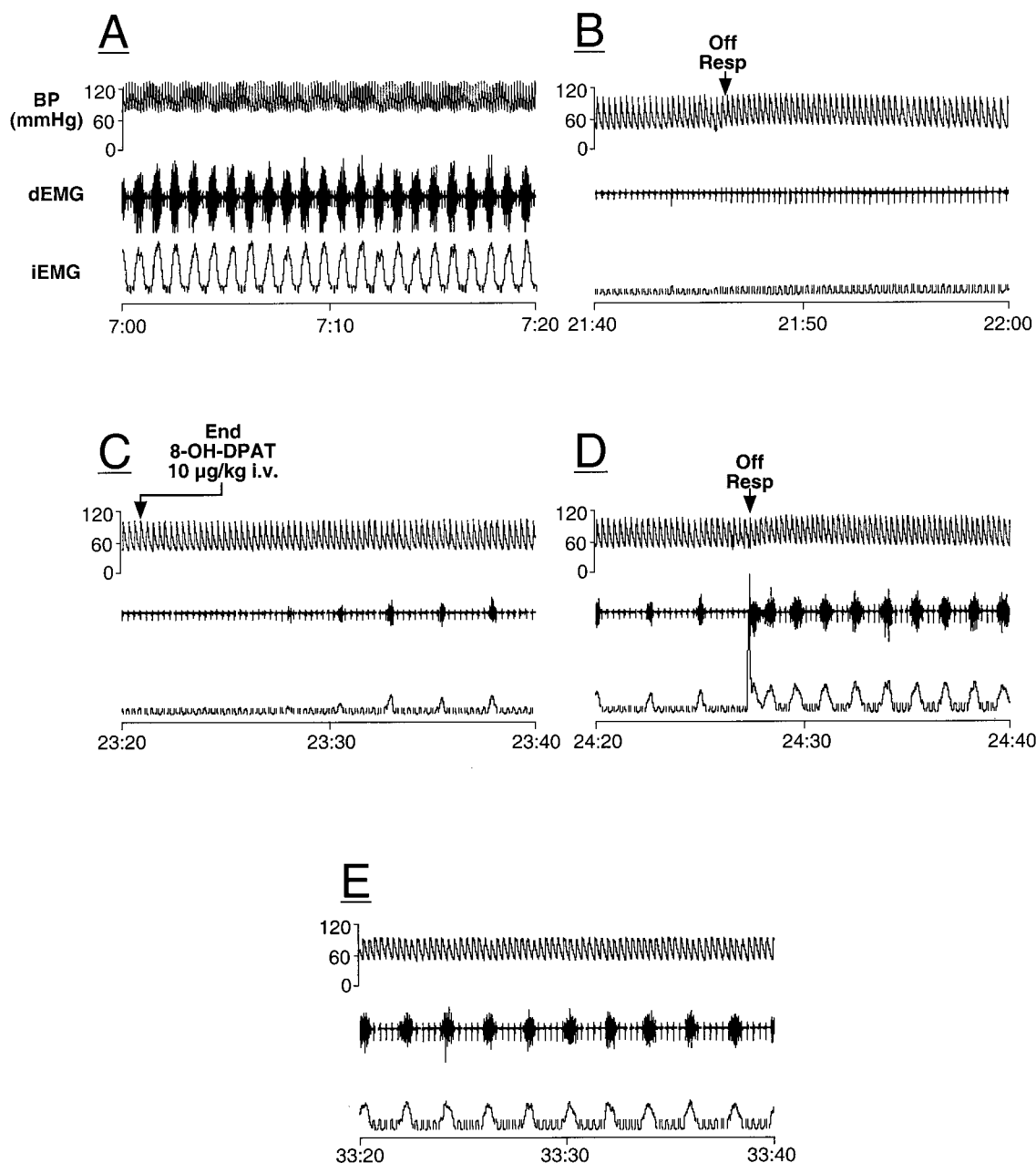
\*\*\*  $P < .05$  versus morphine.

iEMG signal. However, even though respiratory rate was close to baseline values, respiratory rate remained significantly below the baseline respiratory rates before morphine administration. A somewhat similar picture can be seen with the mean arterial blood pressure values. Morphine produced a decrease in mean arterial blood pressure and the administration of 8-OH-DPAT partially restored mean arterial pressure toward normal values in all groups. Heart rate values remained unchanged by the administration of 8-OH-DPAT. A representative experiment showing reversal of morphine-induced apnea by 8-OH-DPAT appears in Fig. 1.

**Effects of 8-OH-DPAT Per Se on Respiration in Spontaneously Breathing Rats.** 8-OH-DPAT doses that were found to be effective in reversing morphine-induced apnea were administered to three animals that had not received morphine to examine the cardiorespiratory effects of this 5-HT<sub>1A</sub> receptor agonist drug. The baseline respiratory rate, mean arterial blood pressure, and heart rate of animals receiving 8-OH-DPAT were  $62 \pm 4$  breaths/min,  $96 \pm 7$  mm Hg, and  $429 \pm 19$  beats/min, respectively. 8-OH-DPAT administered as either the 10 µg/kg i.v. dose or the 100 µg/kg i.v. dose had no significant effect ( $P > .05$ ) on respiratory rate, iEMG amplitude, mean arterial blood pressure, or heart rate (10 µg/kg:  $61 \pm 5$  breaths/min,  $93 \pm 9$  mm Hg, and  $405 \pm 8$  beats/min, respectively; 100 µg/kg:  $61 \pm 4$  breaths/min,  $86 \pm 12$  mm Hg,  $374 \pm 24$  beats/min, respectively). 8-OH-DPAT values were obtained 1 to 2 min after drug administration. This time point coincides with the time at which 8-OH-DPAT was found to exert its peak effect to reverse morphine-induced apnea.

**Effects of 8-OH-DPAT on Morphine-Induced Apnea in Artificially Respired Rats.** To further rule out CO<sub>2</sub> accumulation as a factor in reversing morphine-induced apnea, we performed three experiments similar to those described earlier except the animals were artificially ventilated throughout the experiment. In all three animals, a single i.v. dose of 4 mg/kg ( $n = 2$ ) or three i.v. doses of 4 mg/kg ( $n = 1$ ) morphine produced apnea as reflected by disappearance of the EMG signal (Fig. 2B). Within 2 min after the occurrence of apnea, the i.v. administration of 10 µg/kg 8-OH-DPAT restored breathing to normal in all three animals (i.e., EMG signal was restored and the amplitude of the signal was similar to the amplitude during the control period; Fig. 2C). Restoration occurred within 1 min of 8-OH-DPAT administration and was maintained until the end of the experiment. The tabulated data from these three experiments appear in Table 1 and indicate that values for respiratory frequency and iEMG amplitude after 8-OH-DPAT were not statistically different ( $P > .05$ ) from the corresponding values obtained before morphine administration. Also, mean blood pressure and heart rate were well maintained in these artificially ventilated animals throughout the duration of the experiment.

**Effects of Buspirone on Morphine-Induced Apnea in Artificially Ventilated Rats.** The effects of the 5-HT<sub>1A</sub> receptor agonist drug buspirone on morphine-induced apnea were studied in two animals placed on artificial respiration. Apnea was produced by administering three ( $n = 1$ ) or five ( $n = 1$ ) i.v. doses of 4 mg/kg morphine. Buspirone, administered i.v. in a dose of 50 µg/kg over 24 to 28 s, reversed morphine-induced apnea in both cases, and this reversal occurred within 1 min after administration. A representative

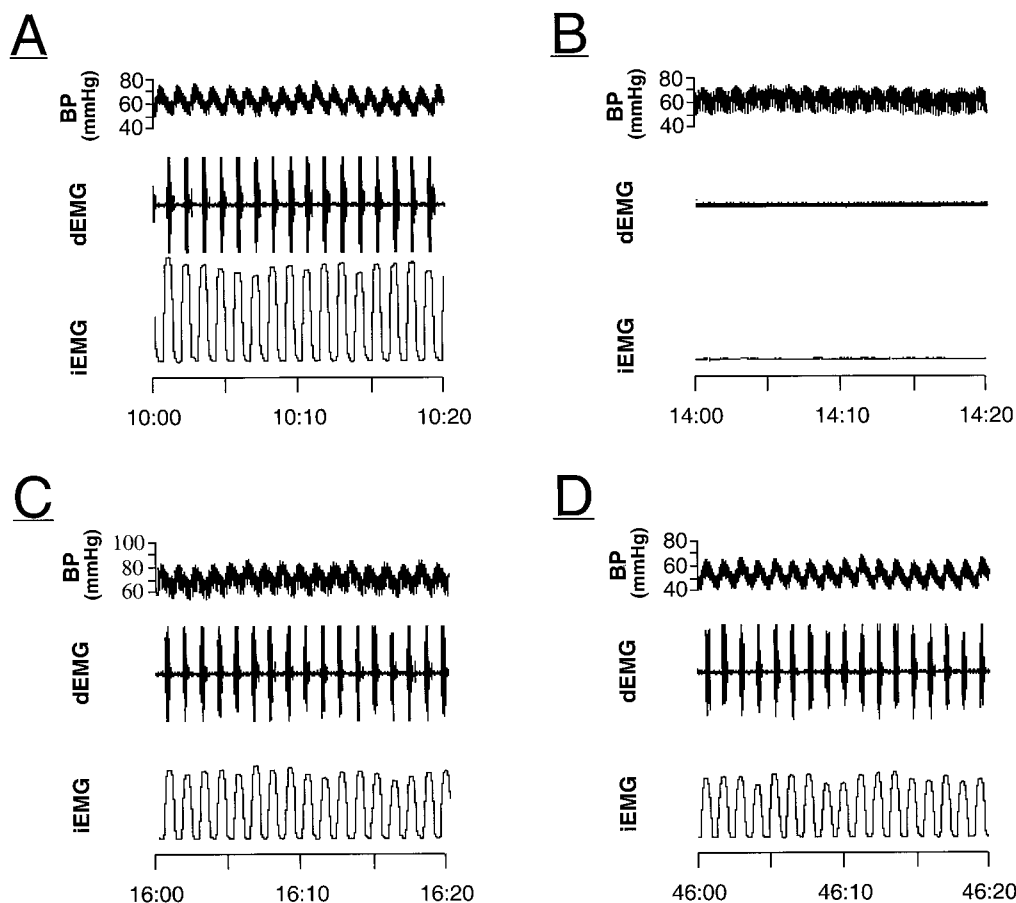


**Fig. 1.** Reversal of morphine-induced apnea by 10  $\mu\text{g}/\text{kg}$  8-OH-DPAT i.v. in a spontaneously breathing vagi-intact rat. A, control traces of blood pressure (BP), dEMG, and iEMG. B, trace obtained 2 min and 20 s after the third i.v. dose of 6 mg/kg morphine. Artificial respiration was instituted at the time of the onset of apnea. The animal was briefly removed from the ventilator as shown to determine whether  $\text{CO}_2$  accumulation would reverse apnea and restore respiratory rhythm (see *Materials and Methods*). Apnea was not reversed after 15 s off the ventilator, pressure was observed to drop markedly, and the animal was put back on artificial respiration (not shown). C, trace obtained at the conclusion of i.v. administered 8-OH-DPAT. Note recovery of breathing within 10 s after administration of the 5-HT<sub>1A</sub> agonist. D, the animal was taken off the ventilator as indicated by the notation "Off Resp" and allowed to breathe spontaneously for the duration of the experiment. E, cardiorespiratory traces obtained 10 min after 8-OH-DPAT administration demonstrating the continued effect of 8-OH-DPAT-induced respiratory recovery. Respiration was maintained at this level for the duration of the experiment (25 min). Horizontal lines at the bottom of each panel represent the time in minutes and seconds from the beginning of the experimental recording. Each panel represents a 20-s record of the above indices.

experiment illustrating the ability of buspirone to counteract morphine-induced apnea appears in Fig. 3. In contrast to the persistent effect of 8-OH-DPAT, the effect of buspirone began to diminish within 5 min after administration in both animals (Fig. 3D). However, repeat administration of 50  $\mu\text{g}/\text{kg}$  i.v. buspirone reestablished the antagonism of morphine-induced respiratory depression in both experiments.

#### Effects of *p*-MPPI Pretreatment on Capacity of 8-OH-DPAT to Reverse Morphine-Induced Respiratory De-

pression. Four experiments were performed in spontaneously breathing animals. The protocol used was as follows: morphine was first administered using the 6 mg/kg bolus dose regimen described in *Materials and Methods* until a stable apnea was present. Next, *p*-MPPI, an antagonist of 5-HT<sub>1A</sub> receptors (Kung et al., 1994), was administered i.v. at a dose of 40  $\mu\text{g}/\text{kg}$ . This was followed by the i.v. administration of 8-OH-DPAT in a dose of 10  $\mu\text{g}/\text{kg}$ . In three of the four experiments, 10  $\mu\text{g}/\text{kg}$  8-OH-DPAT i.v. failed to counteract



**Fig. 2.** Reversal of morphine-induced apnea by 10  $\mu\text{g}/\text{kg}$  8-OH-DPAT i.v. in an artificially respired vagi-intact rat. A, control traces for blood pressure (BP), dEMG, and iEMG. B, traces obtained 4 min after 4 mg/kg morphine i.v. produced apnea. C, traces obtained 1 min after 10  $\mu\text{g}/\text{kg}$  8-OH-DPAT i.v. was administered. Note the recovery of dEMG and iEMG signals. D, traces obtained 31 min after 8-OH-DPAT administration. Horizontal lines at the bottom of each panel represent the time in minutes and seconds from the beginning of the experimental recording. Each panel represents a 20-s record of the above indices.

morphine-induced apnea in animals pretreated with *p*-MPPI (note that the time interval between the administration of *p*-MPPI and 8-OH-DPAT was  $5.2 \pm 0.6$  min). This contrasts with 12 of 12 animals in which 10  $\mu\text{g}/\text{kg}$  8-OH-DPAT i.v. counteracted morphine-induced apnea in the absence of *p*-MPPI pretreatment (Table 1). One of the four animals did show reversal of morphine-induced apnea with the 10  $\mu\text{g}/\text{kg}$  i.v. dose of 8-OH-DPAT. In the three animals in which *p*-MPPI was administered and 10  $\mu\text{g}/\text{kg}$  8-OH-DPAT i.v. was found to be ineffective, subsequent administration of a 100  $\mu\text{g}/\text{kg}$  dose of 8-OH-DPAT did reverse morphine-induced apnea (Fig. 4E). The 100  $\mu\text{g}/\text{kg}$  i.v. dose of 8-OH-DPAT was administered  $9.8 \pm 0.9$  min after the 10  $\mu\text{g}/\text{kg}$  i.v. dose of 8-OH-DPAT had been administered.

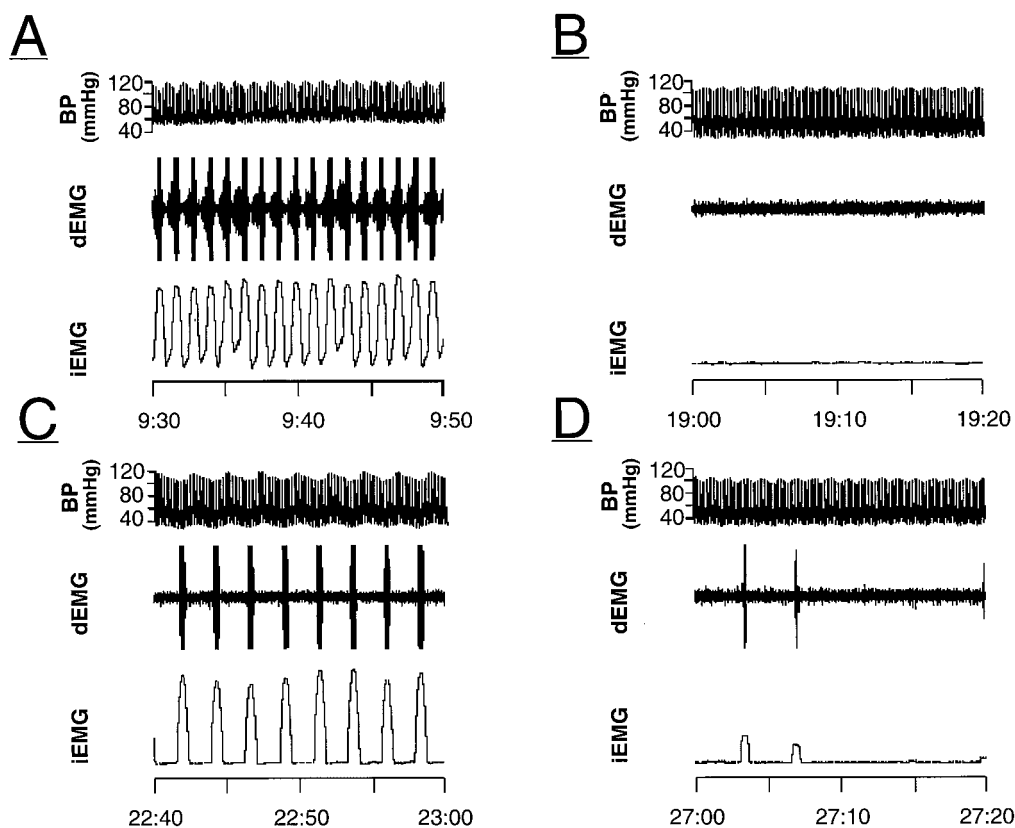
**Effect of *p*-MPPI in Spontaneously Breathing Animals.** The respiratory effects of *p*-MPPI alone were studied in three animals. The dose of 40  $\mu\text{g}/\text{kg}$  *p*-MPPI was administered i.v., and data were obtained at 5 min after this dose was administered. *p*-MPPI per se had no important effects on cardiorespiratory function. Baseline values for respiratory frequency, mean arterial blood pressure, and heart rate were  $72 \pm 6$  breaths/min,  $94 \pm 1$  mm Hg, and  $407 \pm 42$  beats/min, respectively. These values were not significantly altered within 5 min after the drug was administered ( $67 \pm 4$  breaths/min,  $100 \pm 3$  mm Hg, and  $420 \pm 38$  beats/min). The percentage change in amplitude of iEMG was  $+6 \pm 3\%$ . Thus, *p*-MPPI alone had no significant effect on cardiorespiratory function.

In general, we noted that the recovery of respiration after 8-OH-DPAT infusion was more robust once the respirator

was switched off (see, e.g., Figs. 1D and 4E). This increase in dEMG amplitude may have been due to the absence of a respiratory phase conflict between the ventilator and the spontaneous respiratory drive of the animal.

## Discussion

The purpose of our study was to address the question of whether activation of 5-HT<sub>1A</sub> receptors results in an improvement or deterioration of respiratory function. To answer this question, we evaluated the effects of 5-HT<sub>1A</sub> receptor agonists 8-OH-DPAT and buspirone on drug-induced respiratory depression using a model of morphine overdose in the anesthetized rat. The 5-HT<sub>1A</sub> receptor agonist drug 8-OH-DPAT administered i.v. counteracted apnea produced by i.v. morphine. Although the bulk of our data were obtained with 8-OH-DPAT, we also observed that buspirone, a partial agonist at 5-HT<sub>1A</sub> receptors (Taylor, 1988), also reversed apnea. Evidence that the positive effect of 8-OH-DPAT on depressed respiratory function was due to an action to stimulate 5-HT<sub>1A</sub> receptors was obtained using *p*-MPPI, a selective antagonist of the 5-HT<sub>1A</sub> receptor (Kung et al., 1994). Pretreatment of animals with *p*-MPPI i.v. prevented the 10  $\mu\text{g}/\text{kg}$  i.v. dose of 8-OH-DPAT from counteracting apnea produced by i.v. morphine. Not only did we find that 5-HT<sub>1A</sub> receptor agonists reverse apnea produced by morphine, but also our most recent data indicate that 8-OH-DPAT administered i.v. to rats reverses apnea caused by the antagonist of the *N*-methyl-D-aspartate receptor complex, dizocilpine (Sahibzada et al., 1999). In addition, we found that i.p. admin-



**Fig. 3.** Reversal of morphine-induced apnea by 50  $\mu\text{g}/\text{kg}$  buspirone i.v. in an artificially respired vagi-intact rat. A, control traces for blood pressure (BP), dEMG, and iEMG. B, traces taken 5 min after 12 mg/kg morphine i.v. in a single bolus dose produced apnea. C, traces obtained 1 min after buspirone was administered i.v. Note recovery of dEMG and iEMG signals. D, traces obtained 5.5 min after administration of buspirone. At this time, morphine-induced respiratory depression had been re-established. Horizontal lines at the bottom of each panel represent the time in minutes and seconds from the beginning of the experimental recording. Each panel represents a 20-s record of the above indices.

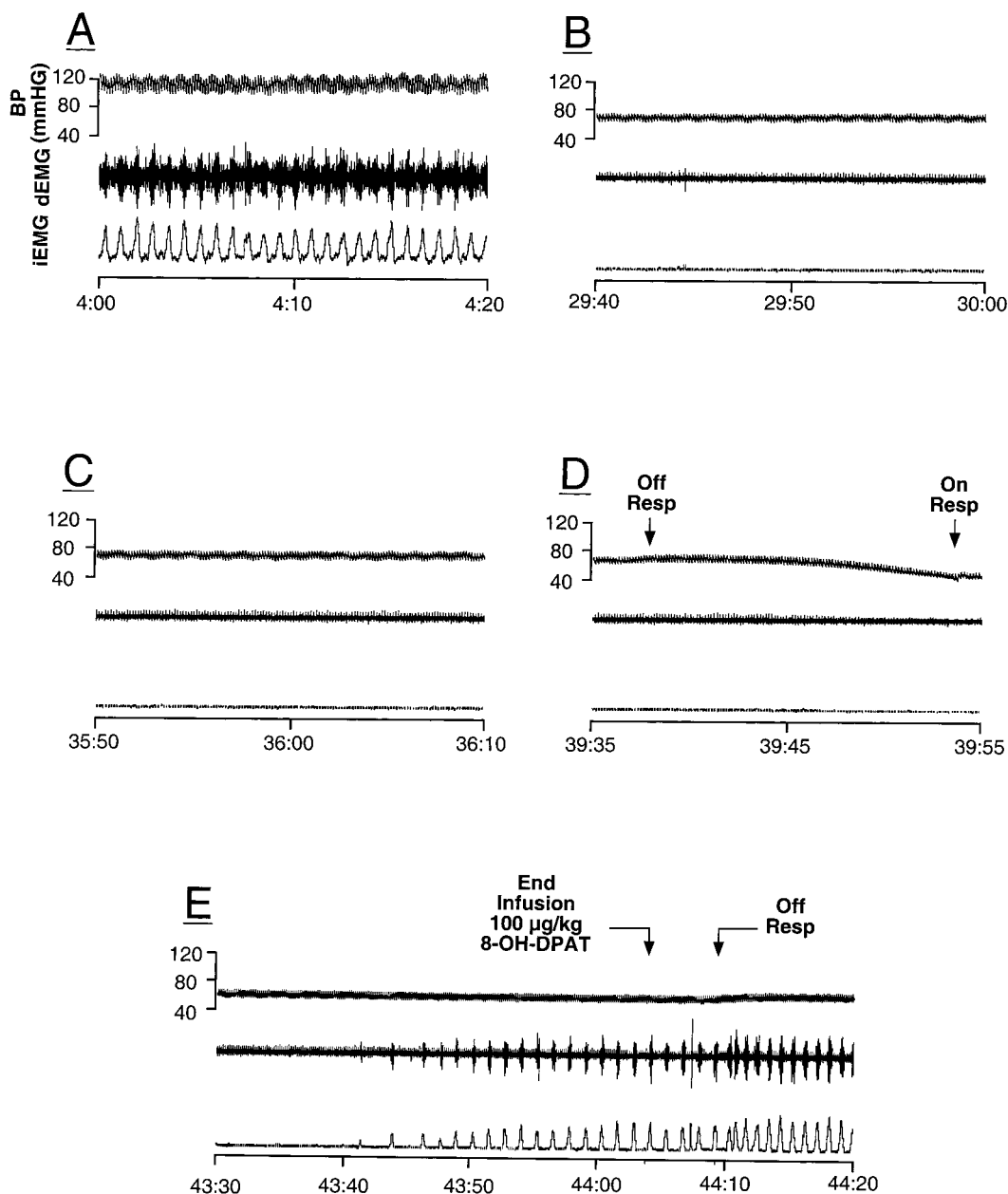
istration of either 8-OH-DPAT or buspirone will restore breathing to normal in rats with spinal cord injury and associated disturbances in respiratory function (Teng et al., 1999).

Our data are consistent with the view that the administration of drugs that activate 5-HT<sub>1A</sub> receptors are of potential benefit in situations where life-threatening respiratory depression is present. Our findings fit with those of others who have studied 5-HT<sub>1A</sub> agonists, specifically buspirone, and found a stimulatory respiratory effect of this compound in anesthetized and conscious experimental animals and humans (Garner et al., 1989; Mendelson et al., 1990, 1991). Garner et al. (1989) administered buspirone i.v. to anesthetized cats and reported that a dose of 0.32 mg/kg increased respiratory rate, tidal phrenic activity, and minute phrenic activity. Additionally, buspirone decreased the apneic threshold (determined by reduction in pCO<sub>2</sub> levels until breathing ceased) and shifted the CO<sub>2</sub> response curve to the left of the control CO<sub>2</sub> response curve. Mendelson et al. (1990) administered buspirone to conscious rats in doses of 10 and 20 mg/kg i.p. and monitored respiration by the "barometric technique." Both doses of buspirone were reported to increase respiratory rate, tidal volume, and minute ventilation. This group of investigators went on to study buspirone in humans with obstructive sleep apnea (Mendelson et al., 1991). They reported that buspirone decreased the number of apneas by one third in five patients.

Although Garner et al. (1989) and Mendelson et al. (1990) observed pronounced effects of buspirone in normal breathing animals, we did not observe alterations in respiration with 8-OH-DPAT unless respiratory depression was present. One possible explanation for this discrepancy is that our i.v.

drug doses were relatively low (i.e., 10 and 100  $\mu\text{g}/\text{kg}$  for 8-OH-DPAT and 50  $\mu\text{g}/\text{kg}$  for buspirone). In our most recent study of the effect of these drugs on respiratory function of normal conscious rats, we found that the i.p. administration of larger doses (i.e., 250  $\mu\text{g}/\text{kg}$  8-OH-DPAT and 500  $\mu\text{g}/\text{kg}$  buspirone) does produce significant respiratory stimulation (Teng et al., manuscript in preparation). Others have reported that 8-OH-DPAT will produce hypotension in anesthetized rats (e.g., Fozard et al., 1987). We assume that 8-OH-DPAT was administered as a rapid i.v. infusion in the study by Fozard et al., whereas we administered 8-OH-DPAT as a continuous i.v. infusion such that the dose of drug was administered over a period of 24 to 36 s. In addition, the mean blood pressures of the rats in the study by Fozard et al. were much higher than the mean blood pressures of our rats, but these investigators reported that 8-OH-DPAT lowered mean blood pressure to a range that coincided with the mean blood pressures in our study (i.e., 80–100 mm Hg).

In contrast to the findings described previously in which 5-HT<sub>1A</sub> receptor agonists produce a stimulatory effect on respiration and are useful in countering respiratory depression, other investigators have suggested that these drugs produce a depressant effect on respiration. Richter et al. (1996) provide evidence that indicate the predominant effect of local application of 5-HT on respiratory neurons is inhibition of activity and that this response is mediated through activation of 5-HT<sub>1A</sub> receptors. Subsequent to activation of the 5-HT<sub>1A</sub> receptor, there is augmentation of potassium conductances and inhibitory postsynaptic currents. In their most recent study using phrenic nerve recordings from anesthetized cats (Richter et al., 1999), 8-OH-DPAT was administered i.v. or microinjected into the pre-Bötzinger complex,



**Fig. 4.** *p*-MPPI (40 µg/kg i.v.) prevents restoration of respiratory activity by 10 µg/kg 8-OH-DPAT in a rat with morphine-induced apnea. A, control traces for blood pressure (BP), dEMG, and iEMG in a spontaneously breathing vagi-intact animal. B, traces obtained 8 min after a fifth and final bolus dose of morphine (total dose administered, 30 mg/kg) demonstrating the occurrence of apnea. Note at this time, the rat was artificially ventilated. Removal of the rat from the ventilator for periods of 20 s was ineffective in generating respiratory activity. C, traces obtained 3 min after i.v. administration of *p*-MPPI; no effect on cardiorespiratory function was observed. D, traces obtained 30 s after administration of 10 µg/kg 8-OH-DPAT demonstrating the lack of reversal of respiratory depression at a time when this dose of 8-OH-DPAT always reversed morphine-induced apnea in the absence of *p*-MPPI. Removal of artificial ventilation also failed to restore respiratory activity, and the animal was put back on the ventilator. E, traces obtained 20 s after beginning infusion of 100 µg/kg dose of 8-OH-DPAT. This higher dose was able to restore respiration even before the complete dose was infused, and the animal was removed from the ventilator and allowed to breathe spontaneously for the duration of the experiment with no recurrence of apnea observed. Horizontal lines at the bottom of each panel represent the time in minutes and seconds from the beginning of the experimental recording. A–D, a 20-s record of the above indices; E, 50-s record of the above indices.

an area of the ventral respiratory group that is considered to be essential for the generation of respiratory rhythm (Smith et al., 1991). Intravenous administration of 20 µg/kg 8-OH-DPAT produced apnea as registered as a total absence of neural discharge on the phrenic nerve recording. The same was true when 8-OH-DPAT was microinjected in an amount of 0.23 nmol unilaterally into the pre-Bötzinger complex.

Richter and colleagues (Lalley et al., 1994b; Wilken et al., 1997; Pierrefiche et al., 1998) made the important discovery

that drugs that act as 5-HT<sub>1A</sub> receptor agonists, such as 8-OH-DPAT and buspirone, can successfully abolish one type of respiratory disturbance, namely, apneustic breathing. Their explanation for this beneficial effect fits with their conclusion that 5-HT<sub>1A</sub> receptor agonists exert a depressant effect on respiratory neurons. In their view, an apneustic pattern of breathing can be caused by blockade of synaptic inhibition within the pre-Bötzinger complex (Pierrefiche et al., 1998), leading to inappropriate and sustained excitation



of respiratory neurons. The administration of a 5-HT<sub>1A</sub> receptor agonist would result in a postsynaptic inhibitory effect on respiratory neurons due to 5-HT<sub>1A</sub> receptor-induced activation of an outward potassium current that would act to hyperpolarize the membrane. This would counteract disinhibition-induced membrane depolarization of pre-Bötzinger neurons and restore rhythmic respiratory activity (Pierrefiche et al., 1998).

Thus, two diametrically opposed views exist for the effect of 5-HT<sub>1A</sub> receptor agonists on respiration: one highlighted by our present data and the data of Garner et al. (1989) and Mendelson et al. (1990 and 1991) indicating that these drugs are respiratory stimulants, and the other highlighted by the data of Richter and colleagues (Richter et al., 1996, 1999; Pierrefiche et al., 1998) indicating that these drugs are respiratory depressants. At the present time, it is not possible to explain these contradictory findings as being due to differences in species, anesthetic regimen, or drug dose studied. The same species and anesthetic regimen were used in two studies yielding opposite results (Garner et al., 1989; Richter et al., 1999). In the present study, we used the same dose range of 8-OH-DPAT as Richter et al. (1999).

A possible explanation for why 5-HT<sub>1A</sub> receptor agonists reverse morphine-induced respiratory depression can be found in data from an earlier study of Florez et al. (1972), in which an interaction between the serotonergic system and morphine was revealed. These investigators reported that pretreatment of cats with *p*-chlorophenylalanine, an inhibitor of 5-HT synthesis, counteracted morphine-induced depression of CO<sub>2</sub>-stimulated respiration and reduced morphine-induced respiratory depression observed during ventilation with normal levels of inspired CO<sub>2</sub>. Another way to inhibit the serotonergic system is by administering 5-HT<sub>1A</sub> receptor agonists. These drugs in the dose range used in our study have been demonstrated to stimulate 5-HT<sub>1A</sub> autoreceptors, and this effect completely inhibits the activity of serotonergic raphe nucleus discharge (Trulson and Arasteh, 1986; Jacobs and Azmitia, 1992; Veasey et al., 1995) and presumably the release of serotonin at target sites innervated by the raphe nuclei. Because brainstem raphe neurons innervate respiratory centers (Lindsey et al., 1998) and because stimulation of brainstem raphe neurons causes depression of respiration and apnea (Lalley et al., 1997), it logically follows that a drug such as 8-OH-DPAT would counteract respiratory depression because it inhibits raphe neurons. Buspirone also inhibits raphe neuron firing (Trulson and Arasteh, 1986) and would therefore exert the same profile of effects as 8-OH-DPAT. Evidence in support of this idea that 5-HT<sub>1A</sub> receptor agonists exert their beneficial effects on depressed breathing by inhibiting central serotonergic mechanisms are findings indicating that 8-OH-DPAT, buspirone, and pretreatment of animals with *p*-chlorophenylalanine all produce respiratory stimulation (Florez et al., 1972; Garner et al., 1989; Mendelson et al., 1990; Teng et al., 1999).

We propose that the beneficial respiratory effects of 5-HT<sub>1A</sub> receptor agonist drugs is due to an effect on somatodendritic 5-HT<sub>1A</sub> autoreceptors leading to the inhibition of central serotonergic neuronal discharge. In contrast, Richter et al. (1999) propose that the respiratory depressant effects of 5-HT<sub>1A</sub> receptor agonist drugs are due to an effect on postsynaptic 5-HT<sub>1A</sub> receptors leading to hyperpolarization of respiratory neurons. Further studies are needed to establish these proposed mechanisms and/or sites of action, and if

pursued, they may help to elucidate why both respiratory stimulation and respiratory depression have been reported for these drugs.

In summary, our data suggest that drugs that stimulate 5-HT<sub>1A</sub> receptors are effective in restoring disturbances in respiratory function to normal. Data were obtained with 8-OH-DPAT and buspirone reversal of morphine-induced apnea, but additional preliminary data of ours also show that these drugs will reverse dizocilpine-induced apnea (Sahibzada et al., 1999), and spinal cord injury-induced respiratory depression (Teng et al., 1999). Thus, the positive effect of 5-HT<sub>1A</sub> receptor agonists on disturbed respiratory function may be a general phenomenon and not limited to morphine overdose.

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