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Short communication

# Evidence for $\mu_1$-opioid receptor involvement in fentanyl-mediated respiratory depression

Shu-Wen Chen [a], Patricia A. Maguire [a], M. Frances Davies [a], Mark F. Beatty [b], Gilda H. Loew [a,*]

[a] *Molecular Research Institute, Palo Alto, CA, USA*
[b] *School of Pharmacy and Pharmacology, University of Bath, Bath, UK*

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**Abstract**

Several fentanyl analogs (Bagley et al., 1989, J. Med. Chem. 32, 663) were compared to fentanyl and morphine for their effects on respiratory depression as determined by arterial blood gas (pH, $pCO_2$ and $pO_2$) measurements. Fentanyl (0.1 mg/kg), morphine (10 mg/kg), #16 (1-phenethyl-4-[*N*-(pyridin-2-yl)-*N*-(methoxymethylcarbonyl)amino]piperidine, 1 mg/kg), #17 (1-phenethyl-4-[*N*-(pyridin-2-yl)-*N*-(2-furoyl)amino]piperidine, 0.5 mg/kg) and #29 (1-phenethyl-4-[*N*-(pyrimidin-2-yl)-*N*-(methoxy-methylcarbonyl)amino]piperidine, 10 mg/kg) produced significant respiratory depression in rats. Pretreatment with the $\mu_1$-opioid receptor selective antagonist, naloxonazine (10 mg/kg), blocked the respiratory effect of fentanyl and its analogs, but not that of morphine. The results suggest that the $\mu_1$-opioid receptor plays an important role in the respiratory effects of fentanyl and its analogs. Hence, the mechanism of fentanyl-induced respiratory depression appears to be distinct from that produced by morphine. The most likely explanation for this difference is the possible contribution of muscle rigidity and catalepsy to the observed changes in blood gas parameters caused by the fentanyl analogs, while the respiratory depression of morphine, measured by these same parameters, appears to be independent of its effect on muscle rigidity.

*Keywords:* $\mu_1$-Opioid receptor; Respiratory depression; Fentanyl; Morphine; Muscle rigidity

## 1. Introduction

The concept of two $\mu$-opioid receptors ($\mu_1$ and $\mu_2$) has been used to explain the multiple actions of some classic opiates such as morphine (Pasternak, 1986). This classification was suggested from studies with selective antagonists. The $\mu$-opioid receptor antagonist, $\beta$-funaltrexamine, produced dose-dependent reversal of morphine-induced analgesia and respiratory depression (Ward and Holaday, 1982; Ling et al., 1986). But prior treatment with the $\mu_1$-opioid receptor selective antagonist, naloxonazine, produced an attenuation of only the analgesic effect of morphine (Ling et al., 1986). It was suggested that $\beta$-funaltrexamine-sensitive effects could be classified as either naloxonazine-sensitive ($\mu_1$) and naloxonazine-insensitive effects ($\mu_2$) (Ling et al., 1983, 1985). Other $\mu_1$-opioid receptor-mediated effects are supraspinal analgesia and catalepsy, whereas respiratory depression, bradycardia and inhibition of gut motility and guinea pig ileal contractions are considered to be mediated by $\mu_2$-opioid receptors (Pasternak, 1986; Paul and Pasternak, 1988).

The results of the present study challenge this classification of opioid effects. Naloxonazine clearly blocked respiratory effects of fentanyl and some of its structurally related analogs. However, morphine respiratory depression was unaffected by this treatment. Thus, there is a significant $\mu_1$-opioid receptor component to fentanyl-induced respiratory depression that appears to be unique to this class of opioids.

## 2. Materials and methods

### 2.1. Materials

The fentanyl analogs (#16 – 1-phenethyl-4-[*N*-(pyridin-2-yl)-*N*-(methoxymethylcarbonyl)amino]piperidine;

* Corresponding author. Molecular Research Institute, 845 Page Mill Road, Palo Alto, CA 94304, USA. Tel.: +1 415 424 9924; fax: +1 415 424 9501.


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#17 – 1-phenethyl-4-[*N*-(pyridin-2-yl)-*N*-(2-furoyl)amino]piperidine; #29 – 1-phenethyl-4-[*N*-(pyrimidin-2-yl)-*N*-(methoxy-methylcarbonyl)amino]piperidine) were synthesized as described by Bagley et al. (1989). Morphine was kindly provided by NIDA. Naloxone and fentanyl were purchased from Sigma Chemical (St. Louis, MO, USA) and naloxonazine from Research Biochemicals (Natick, MA, USA). All the drugs were administered in saline except naloxonazine, which was solubilized in 20% cyclodextrin (Encapsin; American Maize, Indianapolis, IN, USA) in deionized water. Intramedic polyethylene cannulae tubing (PE50) and heparinized microhematocrit capillary tubes were purchased from Baxter Scientific Products (McGaw Park, IL, USA). Male Sprague-Dawley rats (220–320 g; Harlan Sprague Dawley, Indianapolis, IN, USA) were housed under 12-h light/dark conditions.

### 2.2. Procedures

The surgery was performed under metofane/oxygen anesthesia on the day before testing. The rats were surgically prepared with cannulae implanted in the carotid artery and jugular vein to permit sequential withdrawal of arterial blood and i.v. administration of drugs. All cannulae were kept patent with heparinized saline (10 U/ml). After surgery, the animals were housed individually and fed premoistened rat chow. During the study, arterial blood samples (100 $\mu$l) were drawn while minimally restraining the animal to avoid stress. The same animal was used to determine the effect of each drug prior to and again 24 h after naloxonazine treatment, so that each animal served as its own control. To determine the effect of each drug alone, on the first test day, the drug was administered through the jugular catheter and arterial blood samples were drawn before, 10 min, and in the case of morphine, 30 min, after injection. To determine the effect of naloxonazine, 10 mg/kg naloxonazine i.v. was administered to each animal, 3 h after the initial drug treatment. Subsequently, 24 h after naloxonazine administration, the effect of the drug on respiratory depression was assessed as described above. The pH, $pCO_2$ and $pO_2$ in each blood sample were measured in a Ciba Corning Model 178 Blood Gas Analyzer. In order to determine if drug treatment prior to the administration of naloxonazine influenced the subsequent determination of its effect on drug action, the evaluation of the drug effect after naloxonazine treatment was repeated in opioid-naive animals. This was done by injecting naloxonazine or saline i.v. immediately after surgery and determining the effect of morphine 24 h later.

### 2.3. Statistical analysis

A paired *t*-test was conducted to compare the difference before and after drug treatment for each measured blood gas parameter.

## 3. Results

Respiratory depression is observed as a decrease in pH and $pO_2$, and an increase in $pCO_2$ in arterial blood after drug administration. The post-drug measurements were compared to those before treatment. The effect of each drug was determined prior to and again 24 h after naloxonazine treatment so that each animal would serve as its own control.

In all three measurements of respiratory function, fentanyl (0.1 mg/kg), #16 (1 mg/kg), #17 (0.5 mg/kg), #29 (10 mg/kg) and morphine (10 mg/kg) demonstrated significant respiratory depression (Table 1). Treatment with naloxonazine (10 mg/kg) 24 h before drug administration, reversed the respiratory effects of fentanyl and its analogs in each of the three measures of respiratory function. However, the effect of morphine on the blood gas parameters was not significantly attenuated by naloxonazine treatment.

At the doses used in this study, all five compounds also produced muscle rigidity, but this effect was more severe for the fentanyls than for morphine. Naloxonazine reversed the muscle rigidity produced by all the compounds studied.

In order to assure that opioid treatment prior to naloxonazine administration did not bias the results, the morphine effect after naloxonazine treatment was repeated using opioid-naive animals. The results were indistinguish-

Table 1
Respiratory effects of morphine, fentanyl and its analogs and their antagonism by naloxonazine

| | Drug | | Drug + naloxonazine | |
|---|---|---|---|---|
| | Before | After | Before | After |
| *pH* | | | | |
| Fentanyl | 7.477 ± 0.03 | 7.162 ± 0.02 [b] | 7.436 ± 0.03 | 7.428 ± 0.02 |
| #16 | 7.478 ± 0.03 | 7.319 ± 0.06 [b] | 7.515 ± 0.01 | 7.499 ± 0.02 |
| #17 | 7.473 ± 0.02 | 7.290 ± 0.06 [b] | 7.475 ± 0.01 | 7.490 ± 0.02 |
| #29 | 7.472 ± 0.003 | 7.307 ± 0.03 [b] | 7.515 ± 0.01 | 7.412 ± 0.02 |
| Morphine | 7.478 ± 0.02 | 7.321 ± 0.03 [b] | 7.484 ± 0.02 | 7.387 ± 0.01 [a] |
| $pCO_2$ | | | | |
| Fentanyl | 38.7 ± 4.2 | 63.2 ± 3.5 [a] | 36.3 ± 1.6 | 36.7 ± 1.3 |
| #16 | 32.2 ± 2.3 | 42.0 ± 4.2 [a] | 31.2 ± 0.82 | 31.2 ± 2.7 |
| #17 | 30.3 ± 2.4 | 48.9 ± 6.4 [a] | 31.6 ± 1.4 | 30.0 ± 2.3 |
| #29 | 33.4 ± 3.4 | 52.7 ± 1.1 [b] | 31.2 ± 0.82 | 32.9 ± 0.50 |
| Morphine | 31.4 ± 2.4 | 46.9 ± 4.1 [b] | 33.6 ± 1.2 | 41.7 ± 2.0 [a] |
| $pO_2$ | | | | |
| Fentanyl | 76.7 ± 14.3 | 38.0 ± 7.6 [b] | 84.6 ± 18.4 | 86.7 ± 19.6 |
| #16 | 93.2 ± 1.2 | 89.3 ± 4.3 | 88.7 ± 6.5 | 100.3 ± 4.5 |
| #17 | 98.8 ± 12.2 | 69.1 ± 11.4 [a] | 88.6 ± 6.5 | 88.5 ± 10.2 |
| #29 | 97.1 ± 0.8 | 67.4 ± 2.0 [b] | 88.8 ± 6.5 | 73.8 ± 16.7 |
| Morphine | 96.2 ± 9.6 | 82.4 ± 4.8 [a] | 81.9 ± 7.5 | 67.5 ± 7.7 [b] |

Rats were tested i.v. with either drug alone or with naloxonazine (10 mg/kg) ($n = 3$–6) 24 h prior to drug treatment. Measurements were taken 10 min after drug administration. The $pCO_2$ and $pO_2$ are expressed in mm Hg. Comparisons are made before and after drug treatment (pair *t*-test).
Data: mean ± S.E.; [a] $P < 0.05$; [b] $P < 0.01$.

able from those presented in Table 1 (data not shown): morphine produced similar respiratory effects before and after this treatment.

## 4. Discussion

The results presented here clearly demonstrate the involvement of the $\mu_1$-opioid receptor in fentanyl-induced respiratory depression. This result is in contrast to morphine, whose respiratory effects are not altered by naloxonazine treatment. This disparity leads to the conclusion that morphine and fentanyl (and its analogs) produce their respiratory depressant effects through different mechanisms.

The inability of naloxonazine to reverse morphine-induced respiratory depression is in agreement with previous reports (Ling et al., 1983, 1985), and led to the inference that respiratory depression is mediated by $\mu_2$-opioid receptors (Ling et al., 1985). However, the results obtained for the fentanyls represent the first naloxonazine reversal of opioid-mediated respiratory depression observed, and present a challenge to this conclusion.

The $\mu_1$-opioid receptor has been implicated in respiratory stimulation, but not depression. Naloxonazine antagonized the respiratory stimulant effect of very low doses of dermorphin while potentiating the respiratory depressant effect induced by high doses of dermorphin (Paakkari et al., 1990). A similar effect of naloxonazine has been reported in the dermorphin analog Tyr-D-Arg-Phe-Sar (TAPS) (Paakkari et al., 1993). Both of these studies used respiratory rate as the measure of respiratory function. It has been suggested that experimental results using this parameter may be unreliable, as they can vary significantly when restraining or stressing the animals (Ward and Takemori, 1983). The statistically significant reversal by naloxonazine of the three blood gas parameters measured for all the fentanyls studied provide robust evidence that $\mu_1$-opioid receptors play an important role in their respiratory depressant action.

It is unlikely that the observed disparity between fentanyl and morphine in responding to naloxonazine is due to their receptor binding selectivity profiles between $\mu_1$- and $\mu_2$-opioid receptors. All of the compounds bind selectively to $\mu$-opioid receptors and display high affinity for both $\mu_1$- and $\mu_2$-opioid receptors. Morphine and fentanyl have been shown to have similar affinities at the rat $\mu_2$-opioid receptor and are 300- and 900-fold selective for the $\mu_1$- over $\mu_2$-opioid receptor, respectively (Chen et al., 1993). By contrast, the fentanyl analogs have similar high affinities for both $\mu_1$- and $\mu_2$-opioid receptors (Maguire et al., manuscript in preparation). These differences between fentanyl and its analogs in binding to $\mu_1$- and $\mu_2$-opioid receptors and the similarity in selectivity profile for $\mu_1$- and $\mu_2$-opioid receptors between morphine and fentanyl, make receptor binding selectivity an unlikely explanation for the observed differences in the contribution of $\mu_1$-opioid receptors to respiratory depression among the compounds included in the present study.

An alternate explanation may be the possible contribution of muscle rigidity and catalepsy to the observed changes in blood gas parameters for fentanyl but not for morphine. At the doses used in the present study, we have observed that fentanyl and its analogs produced more severe muscle rigidity than morphine and that naloxonazine reversed the muscle rigidity produced by all of the compounds studied. These results are consistent with previous observations of marked muscle rigidity of fentanyl and its analogs (Maguire et al., unpublished observations) and with the observation that naloxonazine reverses both rigidity and catalepsy induced by morphine (Paakkari et al., 1993) and alfentanil (Negus et al., 1994) as well as dermorphin and its analogs (Paakkari et al., 1993). Since naloxonazine reversed the muscle rigidity produced by all of the compounds studied, attenuated the respiratory depressant effect of fentanyl and its analogs but did not block morphine-induced respiratory depression, it seems likely that muscle rigidity or catalepsy are major factors in fentanyl-induced respiratory depression while morphine-induced respiratory depression proceeds by an alternative pathway that does not involve muscle rigidity.

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