

*Full Length Research Paper*

# Induction of remifentanil by bradycardia and hypotension through distinct biochemical mechanisms

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Remifentanil has been well known to cause much more significant bradycardia and hypotension as compared with other opioids. However, the underlying mechanism is still poorly understood. The present study hypothesized that remifentanil led to bradycardia and hypotension with distinct mechanisms and aimed to test this hypothesis. Testing the hypothesis would broaden our understanding the underlying mechanism of remifentanil and may provide some guidance in the use of remifentanil clinically. Mean arterial pressure and heart rate were measured before and at the indicated time after drug treatment in the anesthetized rabbits. In the vagotomy group, bilateral vagus in the neck was exposed and one section of the vagus severed respectively. Remifentanil caused slight but significant bradycardia immediately which recovered entirely within one minute. On the other hand, bolus injection of remifentanil led to severe hypotension which sustained for more than five minutes. Bilateral vagotomy could delay the recovery of remifentanil-mediated bradycardia but did not affect remifentanil-evoked hypotension significantly. Pretreatment with naloxone, a non-specific opioid receptor antagonist could entirely prevent the remifentanil-mediated hypotension but only partially reversed remifentanil-mediated bradycardia. Remifentanil induces bradycardia and hypotension with distinct physiological and biochemical mechanisms. Remifentanil-induced bradycardia is mediated by opioid receptor-dependent and -independent pathways; however, remifentanil-induced hypotension is only through opioid receptor-dependent pathway.

**Key words:** Remifentanil, bradycardia, hypotension, opioid receptor, hemodynamic effect.

## INTRODUCTION

Remifentanil is a potent opioid of the 4-anilidopiperidine type with a similar pharmacodynamic property to other opioids. The major difference in chemical structure of remifentanil with other opioids is that remifentanil contains methyl ester at N-acyl moiety which can be cleaved by widespread non-specific esterases, present ubiquitously in the tissue and plasma. Thus, as compared with other opioids, remifentanil has a unique pharmacokinetic characteristic with a very short half time and therefore widely used in clinical anesthesia (Beers

and Camporesi, 2004). However, it has been widely known that remifentanil has more severe hemodynamic consequences compared with other opioids (Elliott et al., 2000; Kazmaier et al., 2000; Wang et al., 1999). In spite of multiple mechanisms proposed including central vagal activation (Garofalo et al., 2008), direct negative chronotropic effect (Zaballos et al., 2009), peripheral vasodilation (Unlugenc et al., 2003), there is still not a consistent conclusion yet. For example, using echocardiography, a previous study suggested that the decreased blood pressure was caused by the reduction of heart rate (HR) (Chanavaz et al., 2005). However, another study showed that remifentanil decreased mean arterial pressure (MAP) in the patients with artificial heart

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in which the HR was fixed (Ouattara et al., 2004). In addition, it has also been reported that remifentanyl could lead to the vasodilation through the endothelium-released nitric oxide in the *in vitro* aorta and remifentanyl could decrease systemic vascular resistance thus resulting in the hypotension (Unlugenc et al., 2003). These findings suggest that remifentanyl induces bradycardia and hypotension through distinct physiological mechanisms. In addition, remifentanyl has more robust depressed cardiovascular effect than other  $\mu$  opioid agonists in similar analgesia doses. This suggests that remifentanyl may exert its cardiovascular effect through opioid receptor independent pathways. The present study thus hypothesized that remifentanyl induced bradycardia and hypotension through distinct mechanisms and aimed to test this hypothesis in the rabbit model. Testing the hypothesis would broaden our understanding the underlying mechanism of remifentanyl and may provide some guidance in the use of remifentanyl clinically.

## MATERIALS AND METHODS

### Animals

The study was carried out on male New Zealand white rabbits (2.5 to 3.5 kg) obtained from Central South University Animal Services (Changsha, China). A total of 64 rabbits were used in the different experimental protocols. In each protocol, eight rabbits were used for each group. The experimental protocol was approved by the Animal Care and Use Committee of Central South University and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of rabbits used and their suffering.

### Preparation

Animals were placed with polyethylene catheters in the ear vein for administration of drugs and saline. Anesthesia was induced by 25 mg/kg pentobarbital sodium and maintained by 10 mg/kg/h continuous infusion. Animals were paralyzed with 0.1 mg/kg vecuronium and infused continuously with 10 ml/kg/h Ringer's lactate solution to maintain the blood volume throughout the experiments. The animals were then tracheotomized and the lungs were ventilated with a small animal ventilator (Harvard Medical Apparatus, USA). Polyethylene catheters were placed in internal jugular artery for measurement of arterial pressure with a pressure transducer and sampling of arterial blood. HR was recorded from lead II of the electrocardiogram (Model, HP). Acid-base balance was maintained within normal limits (PaCO<sub>2</sub>, 35 to 45 mmHg, pH 7.35 to 7.45) by adjusting the tidal volume and frequency. The PaO<sub>2</sub> was maintained between 100 and 200 mmHg. Bilateral cervical vagotomy was performed by isolating and severing bilateral vagus nerve from the vagina carotica at the first tracheal ring (Farber, 1937).

### Experimental protocol

The study firstly compared the hemodynamic response of different doses of remifentanyl (Renfu pharmaceuticals, Hubei, China) with similar analgesic dose of fentanyl. Three different doses of remifentanyl (1.0, 2.5 and 5  $\mu$ g/kg) and 5  $\mu$ g/kg fentanyl rapidly were

administered to investigate the hemodynamic response. HR and MAP were recorded at 30 s, 1, 2, 3, 5 and 10 or 20 min after drug injection.

### Experimental protocol II

The present study also aimed to investigate the role of vagus in the hemodynamic response of remifentanyl. Animals were divided randomly into two groups, group I: remifentanyl only group (REM) and group II: vagotomy group (REM + Vag). 5  $\mu$ g/kg remifentanyl was administered to the different groups and HR and MAP were recorded in the indicated times after drug administration.

### Experimental protocol III

To investigate whether REM-induced hypotension and bradycardia was opioid receptor dependent, naloxone was injected before administration of remifentanyl. After 2 min, 5  $\mu$ g/kg remifentanyl was administered, and HR and MAP were recorded at different times after REM administration.

### Statistical analysis

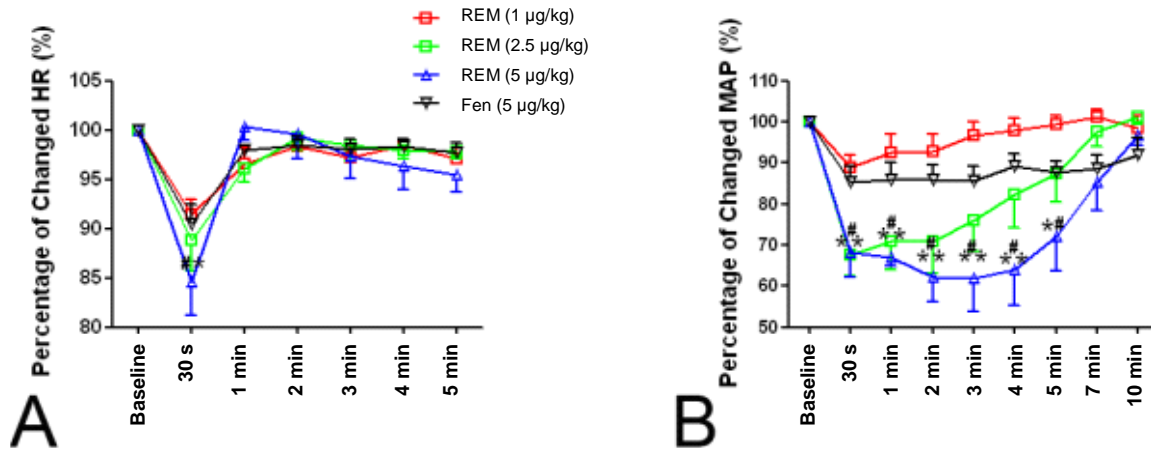
Statistical analysis was performed using software Prism 5.0 (Graphpad Software, San Diego, CA). Data are given as mean (95% confidence interval, CI). Differences between groups were compared using repeated measure analysis of variance (ANOVA) followed by post hoc Dunnett testing, Tukey testing or Bonferroni testing where appropriate. A value of  $P < 0.05$  was considered as significant difference.

## RESULTS

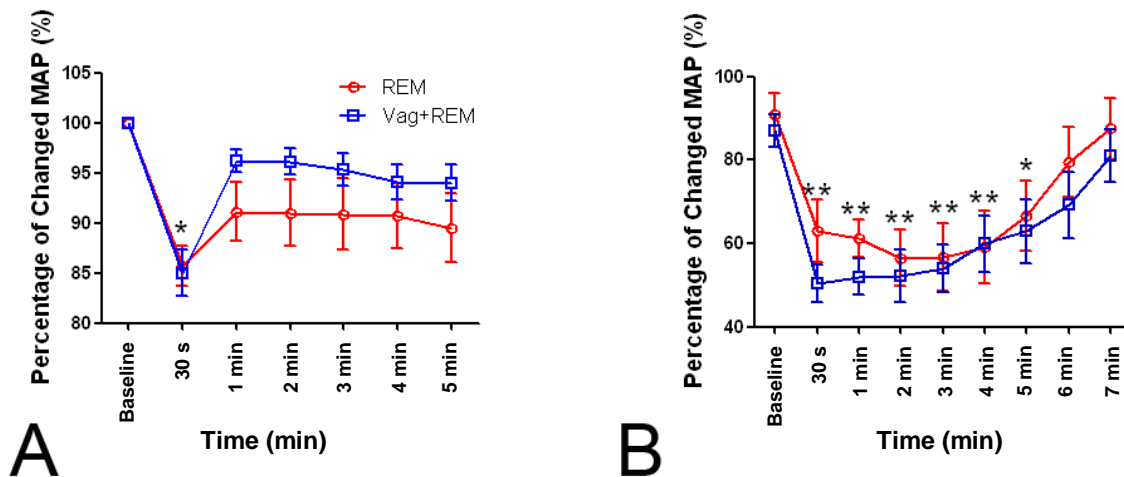
There is no significant difference among age and weight in different experimental groups of animals. No significant difference was observed among the basal HR and MAP in all groups of animals. The mean of baseline HR in all groups of animals is 265 per min [95% confidence interval (CI), 250 to 280.6]. Mean of baseline MAP is 87.31 mmHg (CI, 82.6 to 92.03 mmHg).

### Dose-dependent effect of remifentanyl on HR and MAP

Three different doses of remifentanyl (1, 2.5 and 5  $\mu$ g/kg) and 5  $\mu$ g/kg fentanyl were administered to compare the cardiovascular effect of remifentanyl with fentanyl. As shown in Figure 1A, 5  $\mu$ g/kg fentanyl only slightly decreases HR with 9.54% (CI, 6.45% to 11.25%) at 30 s after administration of drugs ( $p = 0.008$ ,  $n = 8$ ). After that, the decreased HR recovers and returns to the baseline level ( $p > 0.05$  vs baseline). Similarly, 1 and 2.5  $\mu$ g/kg remifentanyl also decreases HR around 8.19% (CI, 7.23 to 11.2%) and 11.19% (CI, 9.85 to 12.76%) respectively at 30 s after drug administration. The reduced HR returns to the baseline at 1 min and later time points. Bolus injection of 5  $\mu$ g/kg remifentanyl reduces HR with 15.47%



**Figure 1.** Time course changes (%) of HR (A) and MAP (B) after bolus injection of different doses (1, 2.5 and 5 µg/kg) of remifentanyl and 5 µg/kg fentanyl. REM, remifentanyl; Fen, Fentanyl. Values are mean ± SEM. \*, p < 0.05 vs Baseline, \*\*, p < 0.01 vs Baseline, #, p < 0.05 vs fentanyl group, n = 8 for each group.



**Figure 2.** Effect of bilateral vagotomy (REM + Vag) on the decreased HR (A) and MAP (B) after bolus injection of 5 µg/kg remifentanyl. Values are mean ± SEM. \*, p < 0.05 different experimental groups vs baseline; \*\*, p < 0.01 different groups vs baseline. N = 8 for each group.

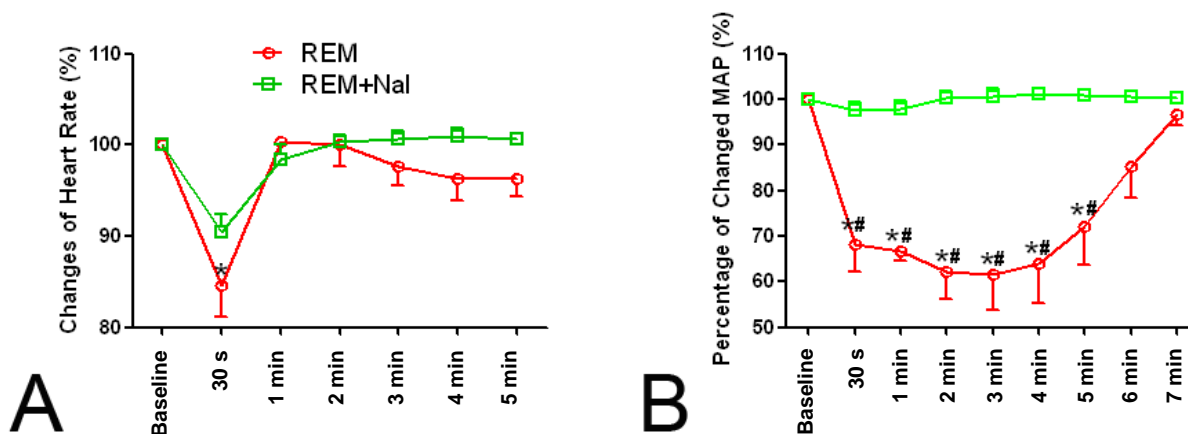
(CI, 13.24 to 18.35%) immediately as compared to baseline. Repeated measures ANOVA followed by Tukey test analysis shows that the 5 µg/kg remifentanyl produces more severe bradycardia than 5 µg/kg fentanyl ( $t_{2, 14} = 2.73, p = 0.025$ ) and 1 µg/kg remifentanyl ( $t_{2, 14} = 3.18, p = 0.012$ ). These data suggest that remifentanyl rapidly but transiently reduces HR in a dose-dependent manner.

As shown in Figure 1B, fentanyl (5 µg/kg) slightly reduces the MAP after drug administration ( $F_{8, 73} = 5.73, p = 0.0008$ ). However, 2.5 and 5 µg/kg remifentanyl dramatically reduces the MAP by more than 30% (CI, 32.3 to 37.6%) at 30 s after drug administration ( $F_{8, 71} = 5.818, p = 0.00009$ ). The decreased MAP responding to 5 µg/kg remifentanyl sustains for more than 5 min. At 5 min after 5 µg/kg administration, the MAP is still only around

72% (CI, 65.7 to 75.3%) of MAP at the basal level ( $p < 0.001$ , One way ANOVA followed by Dunnett test).

**Effect of vagotomy on remifentanyl-induced hypotension and bradycardia**

In an attempt to examine whether vagal activation is involved in the hypotension and bradycardia by remifentanyl, bilateral cervical vagotomy was performed. After 30 min for stabilization, the effects of 5 µg/kg remifentanyl on HR and MAP were examined. As shown in Figure 2, in the group with bilateral vagotomy, remifentanyl-induced bradycardia gradually returned to the baseline (Figure 2A and B). There was no significant difference of changes of MAP after vagotomy (Figure 2A and B) ( $F_{3, 24} = 0.24, p = 0.867$ , repeated measures).



**Figure 3.** Effect of naloxone (40  $\mu$ g) on the decreased HR (A) and MAP (B) responding to remifentanyl (5  $\mu$ g/kg) bolus injection. Values are mean  $\pm$  SEM. Naloxone totally prevented the decreased MAP (B) by remifentanyl administration but only partially attenuated the decreased HR (A). REM, remifentanyl \*,  $p < 0.05$  vs baseline; #,  $p < 0.05$  vs naloxone pretreatment group.  $N = 8$  for each group.

### Effect of naloxone on remifentanyl-induced decreased HR and MAP

To determine whether remifentanyl-induced hypotension and bradycardia was opioid receptor dependent, naloxone (40  $\mu$ g) was administered 2 min before remifentanyl was injected. As shown in Figure 3, in the groups with naloxone pretreatment, HR at 30 s after remifentanyl (5  $\mu$ g/kg) injection was 8.86% less than the basal level ( $t = 9.704$ ,  $p = 0.0006$  vs baseline, repeated measures one way ANOVA followed by Tukey test) (Figure 3A). However, in the group with naloxone pretreatment, MAP is not significantly changed at all time measured after remifentanyl bolus injection ( $F_{8, 56} = 1.153$ ,  $p = 0.34$ , repeated measures one way ANOVA followed by Tukey test) (Figure 3B). These findings suggest that naloxone completely prevents remifentanyl-evoked hypotension but only partially inhibits bradycardia.

### DISCUSSION

There are several interesting findings in the present study. First, remifentanyl-evoked bradycardia and hypotension are dissociated. In this regard, remifentanyl-evoked hypotension was more potent and sustained longer than remifentanyl-induced bradycardia. Second, vagus denervation by bilateral vagotomy did not prevent the hemodynamic changes in response to remifentanyl administration. Third, naloxone pretreatment completely prevented the decreased MAP whereas it only partially prevented bradycardia induced by remifentanyl.

In a similar model, a previous study reported that the maximum of decrease in HR in response to remifentanyl was approximately 15% and recovered within 1 min (Shinohara et al., 2000). In agreement with this, the

present study also showed that remifentanyl decreased HR mildly and shortly. However, inconsistent with the earlier study in which the time course of hypotension responding to remifentanyl was correlated with that of HR, remifentanyl-induced hypotension sustained more than 5 min when HR had been entirely recovered already. The discrepant response of blood pressure between the present study and earlier study may be due to different anesthetics or experimental manipulations. Notably, in this study, the anesthetic was barbiturates whereas urethane was used in the earlier study. In addition, in the present study, remifentanyl was injected faster in the present study than the earlier study. Despite this discrepancy, the present study suggested that there is temporal dissociation between hypotension and bradycardia in response to remifentanyl. In addition, in the present study, remifentanyl-induced hypotension was much more dramatically (~40% of baseline) higher than bradycardia (~15% of baseline). All these findings suggest that remifentanyl-induced hypotension and HR are due to different mechanisms. Though, a unifying hypothesis is preferred to explain the cardiovascular response of remifentanyl the results obtain indicate otherwise. Supporting this assumption, a recent clinical study also showed that atropine could prevent the decreased HR but not the fall of MAP (Maruyama et al., 2010).

Several physiological mechanisms were proposed to interpret remifentanyl-induced bradycardia including the central vagotomic activation, the negative chronotropic effect and direct effect on cardiac conduction (sinus node and atrioventricular node) (Fujii et al., 2011; Zaballos et al., 2009). In the present study, bilateral vagotomy did not reverse remifentanyl-induced bradycardia suggesting that central vagotomy may not contribute to the bradycardia induced by remifentanyl. This finding is consistent with a

clinical study which reported that the negative chronotropic effect, but not increased parasympathetic activity, contributed to remifentanil-induced bradycardia (Tirel et al., 2005) and supports the assumption that other effect of remifentanil such as direct effect of conduction system regulated remifentanil-evoked bradycardia. On the other hand, many studies have clearly demonstrated that remifentanil has direct vasodilator effect. For example, in the cultured human radial artery, remifentanil was shown to induce vasorelaxation in a concentration-dependent manner (Gursoy et al., 2006). In another clinical study, remifentanil still decreased MAP in the patients with artificial heart suggesting the direct effect on blood vessels but not mediated by the reduction of HR (Ouattara et al., 2004). In the present study, bilateral vagotomy did not attenuate the hypotension induced by remifentanil suggesting that the direct vasodilator effect by remifentanil contributes to the hypotension after remifentanil treatment.

The most interesting observation in the present study was that naloxone totally reversed hypotension but only marginally attenuated bradycardia after remifentanil administration. These data strongly indicate that remifentanil induces bradycardia and hypotension through distinct biochemical pathways. Surprisingly, the effect of naloxone on remifentanil-induced hypotension or bradycardia has never been tested in the intact animals or human beings despite the report in the baro-denervation rabbits. The fact that remifentanil-induced hypotension was entirely prevented by non specific opioid receptor antagonist naloxone implies that remifentanil-evoked hypotension was through opioid receptor-dependent pathway. However, naloxone only partially prevented bradycardia indicating that REM-induced bradycardia is also mediated by opioid receptor-independent pathway. Despite multiple physiological mechanisms, it seems undoubting that remifentanil exerts its pharmacological effect through opioid receptor from biochemical insights. This is because remifentanil is designed to possess an ester linkage which allows it metabolized by plasma and tissue non-specific esterases. Thus, the supposed key difference between remifentanil and fentanyl is the pharmacokinetics but not its molecular targets. However, after adding an ester linkage, remifentanil may also act on other receptors which cannot be inhibited by naloxone. The existence of unknown downstream signaling of remifentanil may also explain why remifentanil produces more potent bradycardia and hypotension than fentanyl. On the other hand, given that  $\mu$  opioid receptor is widely distributed in the endothelium of the blood vessels, remifentanil administration results in hypotension through acting on opioid receptor and dilating the blood vessels subsequently. Taken together, these findings suggest that opioid receptor-dependent pathway is responsible for remifentanil-induced hypotension but only partially contributes to bradycardia.

One limitation should be considered before assessing the clinical significance of the current study. The administration dose (5  $\mu\text{g}/\text{kg}$ ) was higher than that used in clinical practice. In addition, injection speed was faster than that in clinical anesthesia. Thus, the experimental protocol may not be applied in the clinical practice. However, in clinical anesthesia, lower dose of remifentanil may also exert more persistent and robust hypotension than bradycardia when used together with other anesthetics such as propofol or midazolam. Thus, other vasoactive drugs may be needed to prevent hypotension clinically.

In conclusion, the findings of the study suggest that remifentanil mediates bradycardia and hypotension through distinct physiological and biochemical mechanisms. Physiologically, central vagal activity may be involved in the induction of bradycardia and hypotension by remifentanil but not contribute to the later phase of hypotension. On the other hand, remifentanil-induced hypotension is mediated by opioid receptor-dependent pathway whereas bradycardia is mediated by opioid receptor-dependent and -independent pathways. The distinct biochemical mechanism may be able to explain the more potent cardiovascular consequence of remifentanil than other opioids. However, the findings in the animal study may be needed to be confirmed in the clinical study in the future study.

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