# Antiarrhythmic Action of Naloxone

## Suppression of Picrotoxin-Induced Cardiac Arrhythmias in the Rat

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SUMMARY

The antiarrhythmic properties of the opiate antagonist naloxone have been reported in a variety of models of arrhythmia. To determine the generality and the possible central involvement of its antiarrhythmic activity, the effects of naloxone were assessed against cardiac arrhythmias induced by intravenous bolus injections of picrotoxin. Naloxone at doses of 0.33 and 1 mg/kg significantly reduced the incidence and severity of picrotoxin-induced arrhythmias in a dose-related manner, without alteration of blood pressure and heart rate. The results demonstrate the antiarrhythmic efficacy of naloxone in an additional animal model. They further suggest that the antiarrhythmic actions of naloxone may be mediated by the central nervous system via both the autonomic and GABAergic pathways.

**Key Words:**
Naloxone  Endogenous opioid peptide  Picrotoxin  Arrhythmia

THERE is substantial evidence supporting the hypothesis that endogenous opioid peptides (EOP) are involved in cardiac arrhythmogenesis (for a review, see Ref. 1). The antiarrhythmic effects of opiate antagonists have been verified in several experimental models of arrhythmias (for a review, see Ref. 2). The pure opiate antagonist naloxone has also been shown to inhibit arrhythmias resulting from (1) coronary artery occlusion in rats[3] and dogs,[4] (2) ouabain in guinea pigs,[5],[6] (3) adrenaline[7] or chloroform-hypoxia[8] in rats and (4) ischemia-reperfusion in isolated rat hearts.[9],[10] These findings indicate that naloxone possesses antiarrhythmic properties. On the other hand, Bergey and Beil[11] observed no antiarrhythmic action of naloxone in the anesthetized pig subjected to coronary arterial ligation. This study tested the antiarrhythmic activity of naloxone against picrotoxin-induced centrogenic arrhythmias in the rat.

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## Materials and Methods

Sprague-Dawley rats of either sex (350 to 400 gm) were anesthetized with pentobarbitone sodium (60 mg/kg) intraperitoneally. They were tracheotomized, intubated and artificially ventilated with a respiratory rate synchronized with that of the rat (60–80 stokes per minute, 1 ml/100 gm body weight). The animals were immobilized with pancuronium bromide, initially 0.08 mg/kg followed by 0.02 mg/kg intravenously every 20 40 min. The femoral artery and vein were cannulated for the measurement of blood pressure and heart rate with a Statham pressure transducer and a Biotechnometer (Gould), and for the administration of drugs, respectively. Electrocardiograms were recorded from lead II limb leads, using the Lifepak ECG Monitor (Physio-Control Corp., USA).

Twenty-seven animals were divided equally into 3 groups. Group I received saline prior to picrotoxin administration. Groups II and III received naloxone at the doses of 0.33 and 1 mg/kg in 0.2 ml, respectively, administered 10 min before the bolus injection of picrotoxin. The doses of naloxone were selected from previous studies and/or preliminary experiments. These doses were sufficient to antagonize both opioid receptors in the central or peripheral tissues, particularly for direct actions on the heart.[3),7),9),12),20)]

Picrotoxin (Sigma Chemical Co., USA) was dissolved in 10% ethanol in normal saline, and placed in a syringe wrapped with aluminium foil. It was given as an intravenous bolus at a dose of 20 mg/kg to induce cardiac arrhythmias. All lights in the room were turned off during the injection of picrotoxin.

The rat was allowed to equilibrate for 15 min. Afterwards, either naloxone (Sigma Chemical Co., USA) dissolved in normal saline, or saline was administered intravenously. The blood pressure, heart rate and electrocardiogram were monitored before and after the bolus injection of picrotoxin continuously throughout the experimental period.

To assess the incidence and severity of cardiac arrhythmias and to enable quantitative comparison, an arrhythmia scoring system (modified from Curtis and Walker[13)]) was used. Each rat was given one score, representing the most severe type of arrhythmia observed during the entire experimental period. The details of the scoring system were: score 0=no arrhythmia; score 1=occasional ventricular premature contraction (VPC); score 2=frequent VPC when there were 3 or more VPCs occurring within 1 min; score 3=ventricular tachycardia (VT; 1–2 episodes); score 4=VT (3–5 episodes); score 5=VT (>5 episodes); score 6=ventricular fibrillation (VF; 1–2 episodes); score 7=VF (3–5 episodes) and score 8=VF (>5 episodes).

The chi-square test was used to analyze the difference in the incidence of arrhythmias between control and naloxone-pretreated groups. Student's t-test was used to test the difference in arrhythmia score and in onset of arrhythmia between control and treated groups. The differences in mean arterial pressure and heart rate before and after picrotoxin administration were analyzed by paired comparisons. Analysis of variance for split-plot design was used to compare the difference in time courses of changes in mean arterial pressure and heart rate between the groups treated with saline or with naloxone. A p value of less than 0.05 was considered as statistically significant.

## Results

Figure 1 and Table I show the representative electrocardiograms and the effects of naloxone on picrotoxin-induced arrhythmias, respectively. The bolus injection of picrotoxin invariably produced immediate atrioventricular (AV) blocks, VPC and/or VT. The arrhythmia persisted throughout the 50 min experimental period. Of 9 rats in the control group, 9 showed AV block and VPC and 5 showed VT, with onset of arrhythmias at 1, 4 and 11.6 min, respectively. The mean arrhythmia score was 3.56. Pretreatment with naloxone significantly reduced the incidence and severity of picrotoxin-induced arrhythmias in a dose-related manner. Thus, of 9 rats receiving 0.33 mg/kg naloxone, 9 showed AV block and VPC and 3 showed VT, with onset of arrhythmias at 2.78, 9.56 and 11.67 min, respectively. The mean arrhythmia score was 2. Of 9 rats receiving 1 mg/kg naloxone, all 9 showed

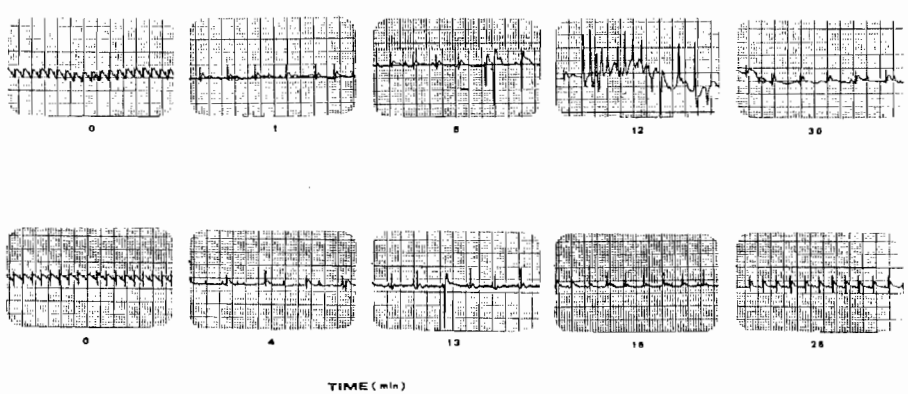


Fig. 1. Representative electrocardiograms following administration of picrotoxin in the rat. (C) Effects of pretreatment with saline. Atrioventricular block, ventricular premature contraction and ventricular tachycardia were observed after 1.5 and 12 min, respectively. (N) Effects of pretreatment with 1 mg/kg naloxone. Atrioventricular block and ventricular premature contraction appeared after 4 and 13 min, respectively.

Table I. Effects of Naloxone on Picrotoxin-Induced Arrhythmias in the Rat

| | Arrhythmia | | AV Block | | VPC | | VT | |
|---|---|---|---|---|---|---|---|---|
| | N | Score | n | Onset (min) | n | Onset (min) | n | Onset (min) |
| Control | 9 | 3.56±0.5 | 9 | 1 ±0.2 | 9 | 4 ±0.73 | 5 | 11.6 ±2.16 |
| Naloxone (0.33 mg/kg) | 9 | 2 ±0.37* | 9 | 2.78±0.55** | 9 | 9.56±0.77*** | 3 | 11.67±2.19 |
| Naloxone (1 mg/kg) | 9 | 1.78±0.32** | 9 | 4.56±2.07 | 9 | 12.67±3.82* | 1# | 10 |

Values are mean±SEM; N and n are number of rats. AV Block=Atrioventricular block; VPC=ventricular premature contraction; VT=ventricular tachycardia. *,**,*** Significantly different to the corresponding control group at the levels $p<0.05$, 0.01 and 0.001 by Student's t-test, respectively. # Significant difference to the corresponding control group at the level $p<0.05$ by chi-square test.

AV block and VPC, and 1 showed VT, with onset of arrhythmias at 4.56, 12.67 and 10 min, respectively. The mean arrhythmia score was 1.78.

Figures 2 and 3 show the effects of naloxone on the changes in mean arterial blood pressure (MAP) and heart rate (HR) following a bolus injection of picrotoxin. The bolus injection of picrotoxin caused a significant increase in MAP and a decrease in HR. The HR before and maximal bradycardia (at 50 min) after picrotoxin administration were 360±4.57 and 244±4.20 beats per minute, respectively ($p<0.001$). Hypertension lasted for about 25 min and was superceded by a significant decrease in MAP.

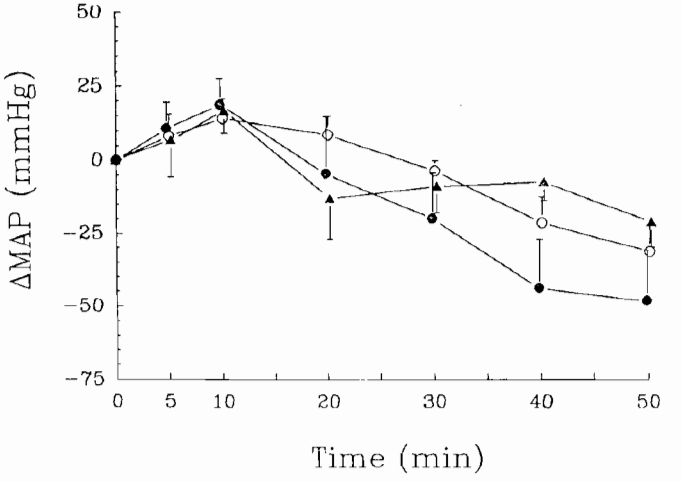


Fig. 2. Effects of naloxone on the change in mean arterial blood pressure ($\Delta$MAP) following administration of picrotoxin. Values are the mean and s.e.m. (vertical bars) for 9 animals. (○) saline; (●) naloxone 0.33 mg/kg; (▲) naloxone 1 mg/kg. There is no statistical difference between the saline- and naloxone-treated groups.

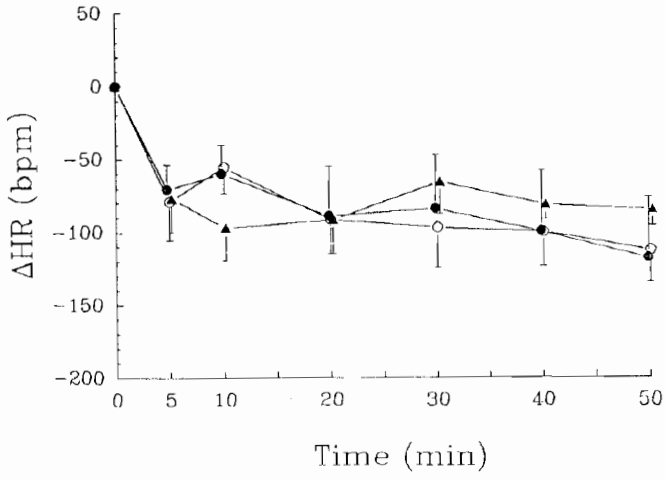


Fig. 3. Effects of naloxone on the change in heart rate (ΔHR) in beats per minute (bpm) following administration of picrotoxin. Values are the mean and s.e.m. (vertical bars) for 9 animals. (○) saline; (●) naloxone 0.33 mg/kg; (▲) naloxone 1 mg/kg. There is no statistical difference between the saline- and naloxone-treated groups.

The MAP before, and maximal hypertension (at 10 min) and hypotension (at 50 min) after picrotoxin administration were $120\pm3.24$, $135\pm5.29$ ($p<0.05$) and $88\pm10.31$ mmHg ($p<0.01$), respectively. These cardiovascular changes were unaffected by naloxone pretreatment. Therefore, the antiarrhythmic doses of naloxone had no significant effects on the picrotoxin-induced alteration of MAP or HR.

## Discussion

Picrotoxin has long been used as a tool to induce cardiac arrhythmias by enhancing central sympathetic outflow to the heart and increasing release of adrenal catecholamines.[14),15)] In this study naloxone reduced the incidence and severity of picrotoxin-induced arrhythmias in the rat. Naloxone has previously been shown to block cardiac arrhythmias induced by myocardial ischemia and reperfusion in the rat and dog, chloroform hypoxia in the young rat, digitalis intoxication in the guinea pig and adrenaline administration in the rat.[3)–10)] The results of the present study are consistent with these previous findings, suggesting the general efficacy of naloxone as an antiarrhythmic agent. Since EOP can induce arrhythmias,[16),17)] these findings suggest a possible involvement of EOP in cardiac arrhythmogenesis due to myocardial ischemia, respiratory arrest, digitalis intoxication, adrenaline or, as shown in this study, picrotoxin. Thus, it is possible that the effects of

naloxone reflect blockade of opiate receptors in the central nervous system. However, the possibility of a direct effect of naloxone on the heart cannot be excluded. The failure of naloxone to inhibit coronary-ligation arrhythmia in the pig[11] may be due to a species difference, such as less collateral flow in the pig. This species difference has a precedent: Benfey et al[18] observed that during coronary artery occlusion and reperfusion, prazosin and propranolol had antiarrhythmic effects in the dog, but not in the pig.

Lee et al[11] reported that cardiac arrhythmias induced by bolus injection of picrotoxin (4 mg/kg) could last for 15–24 min. In the present study, a 20 mg/kg dose of picrotoxin produced consistent and prolonged arrhythmias, which lasted for the entire experimental period of 50 min in all control cases. Therefore, a spontaneous conversion to normal rhythm seems implausible. Moreover, both decreased MAP and HR and bradyarrhythmias were observed in all experimental groups. These changes may have been mediated primarily by the cardiac vagus nerve. DeMicco et al[19] reported two phases of effects after 2 mg/kg picrotoxin administration. The early phase consisted of transient hypotension, bradycardia and bradyarrhythmias which was replaced by an increase in MAP and HR and tachyarrhythmias. In this study, we observed hypotension, hypertension, bradycardia, bradyarrhythmias and tachyarrhythmias following picrotoxin administration but not tachycardia. The discrepancy between the two studies might be due to differences in the doses of picrotoxin employed (20 mg/kg versus 2 mg/kg).

The antiarrhythmic effect of naloxone may result from the influence of the central nervous system on the opioid receptors, vagal reflex, as well as from the receptors located in the heart.[11,19] Brasch[20] has demonstrated that naloxone increased both the cardiac action potential duration and the functional refractory period, thus rendering the heart less vulnerable to cardiac arrhythmias. He suggested that naloxone exerted a negative chronotropic effect due to the inhibition of the time-dependent membrane potassium outward current. Others reported prolongation of the conductance in the frog node of Ranvier,[21] and rat heart,[7] which suggested inhibition of the inward sodium or calcium currents. In addition, naloxone has a significant influence on the electrophysiological properties of the proximal part of the heart conduction system. It has been reported to lengthen the sinoatrial, intra-atrial and atrioventricular node conduction times, and to prolong the atrial and atrioventricular node effective refractory periods.[22]

In this study, naloxone produced an antiarrhythmic effect without affecting MAP or HR, suggesting that the blocking effect of naloxone against picrotoxin-induced arrhythmias is not secondary to changes in MAP or HR. It also suggests that antiarrhythmic doses of naloxone may not alter hemo-

dynamics. Since naloxone is a relatively innocuous and short-acting drug[23] with a similar antiarrhythmic potency as the prototype antiarrhythmic agents such as propranolol, quinidine and lidocaine,[24] it is a likely candidate for clinical applications. Further studies are needed to determine the therapeutic potential of naloxone for treatment of cardiac arrhythmias.

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