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# CARDIAC ANTIARRHYTHMIC EVALUATION OF NALOXONE WITH OR WITHOUT PROPRANOLOL USING A MODIFIED CHLOROFORM-HYPOXIA SCREENING TEST IN THE RAT

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SUMMARY

1. The effects of naloxone and propranolol on cardiac arrhythmias and durations from respiratory arrest to ventricular asystole and cardiac standstill were studied in unanaesthetized young rats induced to suffer respiratory arrest and exhibit ventricular fibrillation (VF) by a modified chloroform hypoxia technique.

2. Both naloxone and propranolol reduced the incidence of VF dose dependently, indicating that they have antiarrhythmic effects. Addition of naloxone to propranolol shifted the dose-response curve of propranolol to the left significantly, indicating an additive effect of the two drugs in their antiarrhythmic activity.

3. Both naloxone and propranolol prolonged the duration from respiratory arrest to ventricular asystole. However, joint administration of both did not further prolong this duration indicating an absence of additive effects.

4. Naloxone prolonged the duration from respiratory arrest to cardiac standstill, indicating that naloxone prolonged the survival time. In contrast, propranolol did not produce the same effect.

5. That naloxone both produced antiarrhythmic effect and prolonged the survival time whereas propranolol only corrected cardiac arrhythmias suggests that the antiarrhythmic effect of naloxone may not result in prolongation of survival time and that different mechanisms may be involved in the antiarrhythmic effect.

**Key words: antiarrhythmic evaluation, naloxone, propranolol, respiratory arrest, survival time, ventricular fibrillation.**

## INTRODUCTION

Naloxone has been shown to inhibit early arrhythmias resulting from acute coronary ligation in anaesthetized and conscious rats (Fagbemi *et al.* 1982). This antiarrhythmic effect of naloxone was found to be absent in anaesthetized pigs after acute coronary occlusion (Bergey & Beil 1983). It is the aim of this study to access the effect of naloxone on chloroform-hypoxia-induced cardiac arrhythmias in unanaesthetized young rats.

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Since propranolol, a β-blocker is a well known antiarrhythmic agent and is widely used clinically in the prevention and treatment of cardiac arrhythmias, it is of interest to compare the antiarrhythmic effect of naloxone and propranolol and to study whether joint administration of naloxone and propranolol produces more beneficial effects on correction of cardiac arrhythmias and prolongation of time of survival. In this investigation we have studied the effects of treatment of naloxone, propranolol or both on cardiac arrhythmias, durations from respiratory arrest to ventricular asystole and cardiac standstill in the unanaesthetized young rats induced to show ventricular fibrillation by a modified chloroform hypoxia technique (Erker & Baker 1980; Baker & Erker 1980).

## METHODS

### *Modified chloroform hypoxia technique*

Sprague Dawley rats of either sex weighing between 70–100 g were used. Ventricular fibrillation (VF) was induced using the chloroform hypoxia technique described by Erker and Baker (1980). Theophylline (Sigma) dissolved in 0.9% NaCl solution at a concentration of 0.4% was injected intramuscularly into the rat at a dose of 20 mg/kg. Thirty min after injection, the rat was placed into a 1 l beaker with 50 ml of chloroform absorbed by cotton wool on the bottom of the beaker. Excessive liquid chloroform was maintained to provide a relatively constant chloroform vapour pressure. The rat was placed on wire gauze on an iron stand inside the beaker so that it did not touch chloroform on the bottom of the beaker. At respiratory arrest which usually occurred within 2 min the rat was removed from the beaker.

### *Measurement of arrhythmias*

Standard lead II electrocardiogram (ECG) were monitored with a Heart Monitor System Model No. 633 BM (Fukuda, Japan) continuously and recorded every 5 min, with a positive electrode connected to the left leg, a negative electrode to the right arm and a ground electrode to the left arm. The durations from the time of removal from chloroform to the times of appearance of ventricular asystole and cardiac standstill were recorded. The heart was considered to exhibit ventricular asystole if the ECG showed only minute fibrillary twitchings with no ventricular deflection as shown in Fig. 1. Cardiac standstill was determined by the appearance of a straight line in the ECG tracing for over 5 min, indicating a complete absence of electrical activity of the heart (Fig. 1). The appearance of VF was determined. It was considered to have cardiac antiarrhythmic protection (CAP) if VF did not appear at all or disappeared within 30 min of respiratory arrest. The CAP (%) of ten rats in every group was taken as an index in assessing the antiarrhythmic effect of the drugs.

### *Drugs and treatment*

Naloxone from Dupont Pharmaceuticals and propranolol from Imperial Chemical Industries were dissolved in 0.9% NaCl solution. It was injected intraperitoneally 5 min before the rat was subjected to chloroform hypoxia. The doses of naloxone were 1, 2.5, 5, 10 and 20 mg/100 g body weight and those of propranolol 6, 25, 100, 460 and 1850 μg/100 g. In the experiments involving joint administration of propranolol and naloxone, a fixed dose of naloxone of 5 mg/100 g was used while the doses for propranolol were the same as those when propranolol alone was ad-

ministered. The volume of injection was 2.0 ml/100 g because of the low solubility of propranolol in 0.9% NaCl solution. In the control group, 0.9% NaCl solution of the same volume was injected.

*Statistical analyses*

Analysis of variance (one way Anova or two way Anova) was used to test for a difference between the control and treated groups and between the treated groups, respectively. Chi square test was employed to test the difference in cardiac rhythm between the groups treated with propranolol alone or with naloxone.

## RESULTS

Figure 1 shows the time course changes of cardiac rhythm after respiratory arrest induced by administration of theophylline and inhalation of chloroform in rats pretreated with naloxone. In the saline injected rats, nine out of ten rats showed VF immediately following respiratory arrest. In only one out of the nine rats showing VF was there a transient disappearance of VF for 5 min. They showed ventricular asystole at about 15 min and died at about 26 min after respiratory arrest. Intraperitoneal injection of naloxone 5 min before the rats were subjected to chloroform decreased the incidence of VF in a dose dependent manner. The cardiac antiarrhythmic protection (CAP) therefore increased dose dependently and maximum CAP (100%) was reached in the group receiving 20 mg/100 g of naloxone (Fig. 2). The times from respiratory arrest to the appearance of ventricular asystole and cardiac standstill were also prolonged in a dose-dependent manner (Fig. 3).

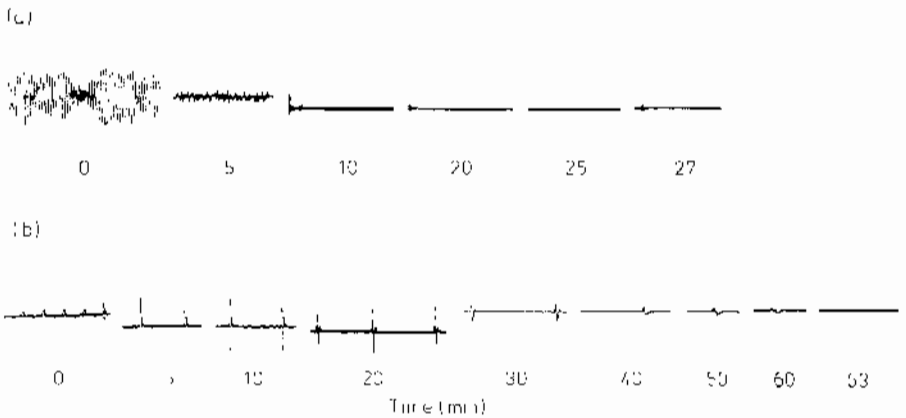


**Fig. 1.** Time course changes of cardiac rhythm after respiratory arrest induced by administration of theophylline and inhalation of chloroform in rats. (a) The electrocardiogram of a rat which exhibits no cardiac antiarrhythmic protection. There is ventricular fibrillation immediately following respiratory arrest. Ventricular asystoles and cardiac standstill occurred 20 and 27 min, respectively after respiratory arrest. (b) The electrocardiogram of rat which exhibits cardiac antiarrhythmic protection. There is no ventricular fibrillation immediately following respiratory arrest. Ventricular asystoles and cardiac standstill occurred 40 and 63 min, respectively after respiratory arrest.

Figure 4 shows the relationship between the cardiac rhythm of propranolol with or without naloxone. As expected, propranolol increased CAP dose-dependently. When administered together with 5 mg/100 g of naloxone, the dose-response curve was shifted to the left. At the doses 6, 25 and 100 μg/100 g of propranolol the CAP was significantly increased with the addition of naloxone at the dose of 5 mg/100 g, indicating that the shift was significant. The $ED_{50}$ were 64.45 μg/100 g with a 95% confidence interval of 48.39–85.86 μg/100 g and 5.23 μg/100 with a 95% confidence interval of 3.68–7.44 μg/100 g for the groups treated with propranolol alone and both propranolol and naloxone, respectively. The $ED_{50}$ of propranolol was reduced 12 times after addition of naloxone.

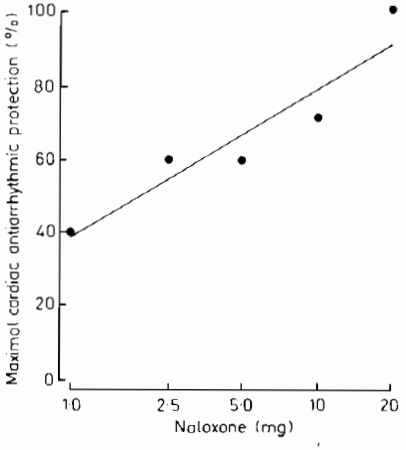


**Fig. 2.** The maximum cardiac antiarrhythmic regression of naloxone ($r = 0.94$, $y = 40 \log x + 38.5$).

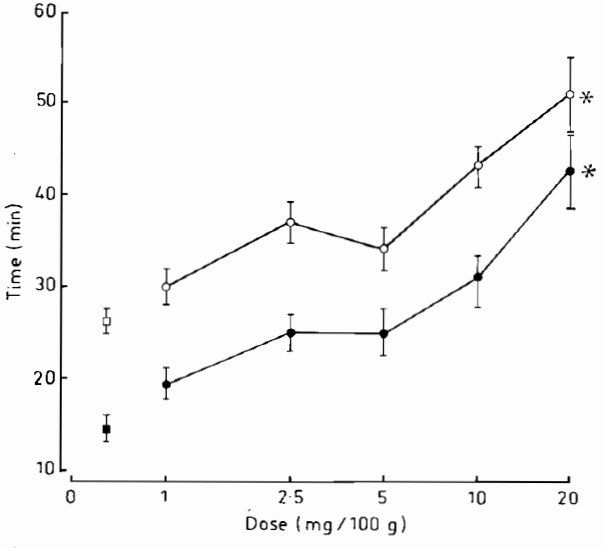


**Fig. 3.** Durations from respiratory arrest to ventricular asystole (●) and cardiac standstill (○)—Effect of naloxone. Values are mean and s.e.m. of ten animals. ■ and □ are durations, from respiratory arrest to ventricular asystole and cardiac standstill in the control group, respectively. * Indicates a significant effect of naloxone by analysis of variance (one way Anova) at the level $P < 0.01$.

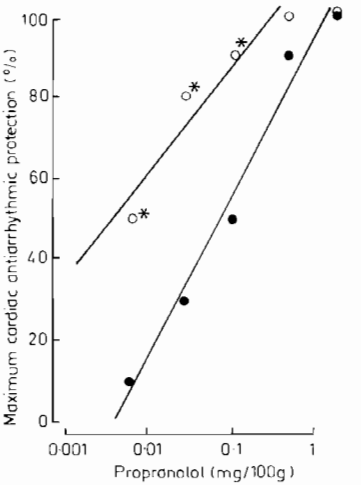


**Fig. 4.** The maximum cardiac antiarrhythmic protection regression of propranolol alone (●, $y = 25.6 \log x + 10.7$, $r = 0.95$) or propranolol with naloxone at the dose 5 mg/100 g (○, $y = 38.5 \log x - 60.4$, $r - 0.99$). * Indicates significant difference to the corresponding control groups receiving propranolol only to the level $P < 0.01$ by Chi square test.

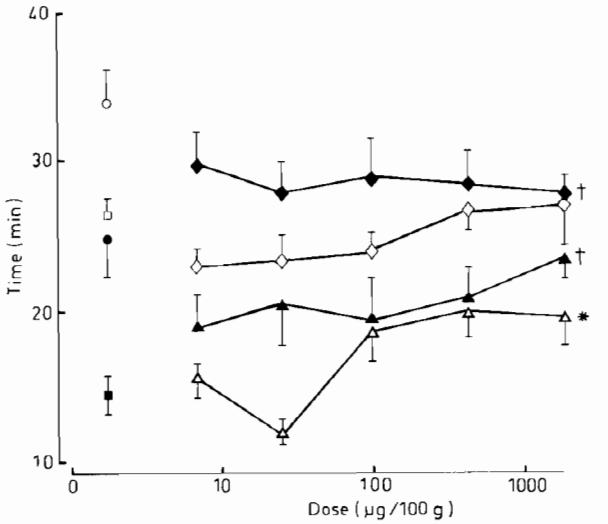


**Fig. 5.** Durations from respiratory arrest to ventricular asystole and cardiac standstill – Effect of propranolol, alone or with naloxone. Values are mean and s.e.m. of ten animals. * Indicates a significant effect of propranolol by analysis of variance (one way Anova) at the level $P < 0.05$. † Indicates a significant difference to the corresponding group receiving propranolol alone by analysis of variance (two way Anova) at the level $P < 0.05$.

Duration from respiratory arrest to ventricular asystole: ■ control; ● naloxone (5 mg/100 g); △ propranolol; ▲ propranolol + naloxone (5 mg/100 g). Duration from respiratory arrest to cardiac standstill: □ control; ○ naloxone (5 mg/100 g); ◇ propranolol; ◆ propranolol + naloxone (5 mg/100 g).

Figure 5 summarizes the effects of propranolol alone or with naloxone on the duration from respiratory arrest to ventricular asystole and cardiac standstill. Propranolol prolonged the duration from respiratory arrest to ventricular asystole significantly, but had no effect on the duration from respiratory arrest to cardiac standstill. Addition of naloxone (5 mg/100 g) significantly shifted the dose-response curve up, indicating an augmentatory effect. However, this is misleading. When compared with the group that received naloxone (5 mg/100 g) alone the durations from respiratory arrest to ventricular asystole and cardiac standstill in groups receiving both propranolol and naloxone were even shorter although there was no statistical difference.

## DISCUSSION

In this study naloxone reduced the incidence of ventricular fibrillation in unanaesthetized young rats pretreated with theophylline and chloroform (Fig. 2), indicating that naloxone produced an antiarrhythmic effect. The result is in agreement with the previous finding in the rat (Fagbemi *et al.* 1982). It is, however, incompatible with the finding in the pigs (Bergey & Beil 1983). Since the experimental designs of these two studies were basically the same and the doses of naloxone used were of similar order of magnitude, the discrepancy may be due to species differences. In agreement with the well known antiarhythmic property of the drug, propranolol also reduced the incidence of ventricular fibrillation (Fig. 3). The efficiency of propranolol found in this study was however greater than that reported by Baker & Erker (1980). The difference is obviously due to the fact that we studied the electrocardiogram whereas they observed the pattern of contraction of the heart. Addition of 5 mg/100 g of naloxone shifted the dose-response curve to the left significantly (Fig. 3), indicating an additive effect of the two drugs on their antiarrhythmic activity. This simple additive action of the two drugs suggests that naloxone and propranolol may produce this antiarrhythmic effect via different mechanisms.

Naloxone also prolonged both the durations from respiratory arrest to ventricular asystole and to cardiac standstill (Fig. 4), indicating that naloxone prolonged the survival time in addition to its beneficial effects on the electrical activities of the heart. The results are again compatible with the previous finding in the rat (Fagbemi *et al.* 1982) but not with that in the pig (Bergey & Beil 1983). The ability of naloxone to prolong survival time suggests that naloxone probably antagonizes the inhibitory actions of endogenous opioid peptides on respiratory arrests.

Propranolol at doses that produced antiarrhythmic effects, prolonged the time from respiratory arrest to ventricular asystole but not that from respiratory arrest to cardiac standstill (Fig. 5). The ability of propranolol to delay the appearance of ventricular asystole is probably related to its antiarrhythmic property. The fact that propranolol, at the doses that produced antiarrhythmic effects, did not prolong the survival time indicates that the beneficial effects on the electrical activities of the heart does not necessarily lead to prolongation of the survival time and suggests that different mechanisms may be involved in these two events.

Although both naloxone and propranolol prolonged the duration from respiratory arrest to ventricular asystole, joint administration of them did not further prolong this duration (Fig. 5). Joint administration of these drugs did not further prolong the survival time either (Fig. 5). In contrast, the durations from respiratory arrest to ventricular asystole and cardiac standstill were even shorter in groups receiving both drugs than in the group receiving naloxone only, although the difference was not statistically significant. Does it imply an attenuating action of propranolol on these beneficial effects of naloxone?

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