

Naloxone Reduces Release of Creatine Kinase in the Isolated Ischemic Rat Heart (42089)

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Abstract. The effects of naloxone, propranolol, or both on the release of creatine kinase (CK) from the isolated ischemic rat heart were studied. Naloxone at concentrations of 1.1 and 3.6 mmole liter⁻¹ in the perfusate at a rate of 1-2 ml min⁻¹ reduced the release of CK from the isolated ischemic rat heart during myocardial ischemia in a dose-dependent manner. Propranolol at a concentration of 7 μ mole liter⁻¹ in the perfusate also reduced the release of CK. Addition of naloxone (1.1 mmole liter⁻¹) to propranolol further reduced the release of CK. The effect of the joint administration of the two drugs seemed to be additive. © 1985 Society for Experimental Biology and Medicine.

Naloxone, an opiate antagonist, markedly reduced both the incidence and severity of β -endorphin-induced cardiac arrhythmias in the isolated perfused rat heart (1). Naloxone has also been shown to attenuate the cardiac arrhythmias following myocardial ischemia in anesthetized or conscious rats (2) and in the isolated perfused rat heart (3). As the incidence and severity of cardiac arrhythmias due to myocardial ischemia are known to be related to the extent of myocardial infarction (4), it is likely that the antiarrhythmic action of naloxone may lead to protection from myocardial infarction. In this study we made use of the activity of creatine kinase (CK) as an index of the extent of myocardial tissue damage (5) in the isolated rat heart preparation. Naloxone was found to have dose-dependent effects on myocardial CK release. When naloxone was used in conjunction with propranolol, another drug which reduces CK release (6), the effects of the two drugs appeared to be additive.

Materials and Methods. *Perfusion of isolated heart.* Female Sprague-Dawley rats of 210-230 g were killed by decapitation. The heart was rapidly excised and mounted within 1 min for perfusion by the Langendorff technique. The hearts were perfused retrogradely through the aorta at a constant perfusion pressure of about 100 mm Hg at a flow rate of 8-10 ml min⁻¹ with Krebs' Ringer solution (pH 7.4) containing (mmol liter⁻¹) NaCl 118, KCl 4.7, MgSO₄ 1.2, KH₂PO₄ 1.1, NaHCO₃

24, CaCl₂ 2.5, and glucose 10, held at 37°C, and equilibrated with 95% O₂:5%CO₂ mixture. A water jacket was also provided for the entire system, maintaining the whole isolated perfused heart at 32°C.

Drugs and treatments. The isolated rat heart model of global (low flow) ischemia of Manning and co-workers (6) was adopted with slight modification. The isolated perfused rat heart was allowed to equilibrate for 30 min. The amount of CK released during this period was determined. Any heart showing functional instability or releasing significant amount of CK in this period was discarded. At the start of the experiment naloxone (DuPont Pharmaceutical Co.) and where indicated propranolol (Imperial Chemical Industries) were added to the perfusion fluids. The heart was then made ischemic by reducing the perfusion rate to 1 to 2 ml min⁻¹ using a two-way flow stopper.

Assessment of tissue injury. Ischemic injury was assessed by the leakage of creatine kinase to the perfusate. Perfusate was collected every 30 min throughout the entire ischemic period. Two samples from each hour were pooled together for the determination. CK was measured by the method of Oliver (7) and was assayed in an Abbott biochromatic analyzer (Abbott Laboratories) using a standard uv test kit (A-Gent CK-NAC kit, Abbott Laboratories).

Statistical analysis. Analysis of variance for split plot design was used to test differences between groups, and Tukey's test was used for pairwise comparison between means.

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Results. Figure 1 shows the effect of administration of naloxone on the release of CK from the isolated perfused rat heart during myocardial ischemia. In the control group, the amount of CK released from the heart increased gradually with time, indicating an increase in the extent of myocardial tissue damage. Naloxone at concentrations of 1.1 and 3.6 mmole liter⁻¹ reduced the leakage of CK in a dose-dependent manner. In the group treated with 1.1 mmole liter⁻¹ of naloxone the leakage of CK during the first 3 hr was the same as that in the control group. However, from the fourth hour onward, the CK released was significantly lower compared with the control. In the other group treated with a higher dose of naloxone the amount of CK released was significantly lower from the first hour of myocardial ischemia.

Figure 2 shows the effect of propranolol alone or with naloxone on the leakage of CK from the isolated rat heart during myocardial ischemia. Propranolol at the concentration

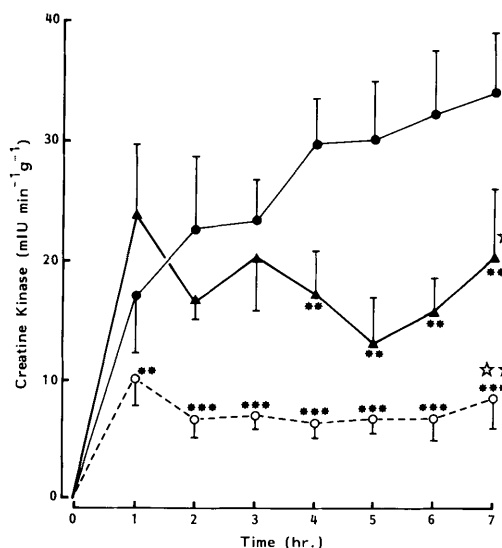


FIG. 1. Effects of naloxone on the release of creatine kinase from the isolated heart during myocardial ischemia. Values represent the mean $\pm$ SEM of seven hearts in each group. ☆ and ☆☆, Statistical difference to the control group at the levels $P < 0.05$ and $P < 0.01$, respectively. ☆☆☆ and ☆☆☆☆, Statistical difference to the corresponding means of the control group at the levels $P < 0.01$ and $P < 0.001$, respectively. ●, Control; ▲, naloxone at a concentration of 1.1 mmole liter⁻¹; ○, naloxone at a concentration of 3.6 mmole liter⁻¹.

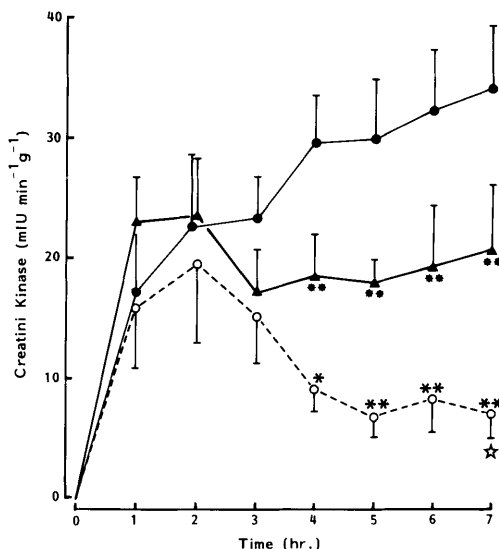


FIG. 2. Effects of propranolol alone or with naloxone on the release of creatine kinase from the isolated rat heart during myocardial ischemia. Values represent the mean $\pm$ SEM of seven hearts in each group. ☆ and ☆☆, As given under Fig. 1. * and **, Statistical difference to the corresponding means of the group treated with propranolol alone at the levels $P < 0.05$ and $P < 0.01$, respectively. ●, Control; ▲, propranolol at a concentration of 7 μ mole liter⁻¹; ○, propranolol at a concentration of 7 μ mole liter⁻¹ and naloxone at the concentration 1.1 mmole liter⁻¹.

of 7 μ mole liter⁻¹ produced an effect similar to that of naloxone in that compared with the control there was no difference in the release of CK in the first 3 hr, but from the fourth hour on the release was significantly lowered. Addition of naloxone further reduced the release of CK.

Discussion. We found that naloxone reduced the leakage of CK from the ischemic heart, indicating that, like propranolol, naloxone possesses a protective effect against myocardial ischemia. While the effect of naloxone suggests that endogenous opioid peptides may be released during myocardial ischemia, it is also possible that naloxone acts via mechanisms unrelated to opiate antagonism. For example, naloxone has been shown to inhibit proteolysis and stabilize lysosomal membrane in hemorrhagic shock in the cat (8). It is not unlikely that naloxone also produces similar effects in the isolated ischemic heart, thus preventing development of

serious myocardial injury. The exact mechanism of naloxone in its protective action awaits further study. Joint administration of both naloxone and propranolol further reduced the leakage of CK and the effect seemed to be additive. The results suggest that different mechanisms may be employed by these two drugs in producing this protective action.

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