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NALOXONE ATTENUATES AUGMENTATION OF cAMP LEVELS AND ARRHYTHMIAS FOLLOWING MYOCARDIAL ISCHAEMIA AND REPERFUSION IN THE ISOLATED PERFUSED RAT HEART

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SUMMARY

1. The effects of myocardial ischaemia and reperfusion on arrhythmias and cAMP levels were studied using the Langendoff isolated perfused rat heart preparation.

2. Myocardial ischaemia and reperfusion caused arrhythmias and augmentation of cAMP levels concurrently, supporting the suggestion that myocardial cAMP is related to arrhythmias.

3. Pretreatment with naloxone attenuated both arrhythmias and augmentation of cAMP levels to a similar extent. The results suggest that the anti-arrhythmic effect of naloxone may involve myocardial cAMP.

Key words: arrythmias, cAMP, isolated rat heart, myocardial ischaemia, naloxone, reperfusion.

INTRODUCTION

Naloxone has been shown to block arrhythmias following myocardial ischaemia and reperfusion in the conscious and anaesthetized rat (Fagbemi *et al.* 1982), in the isolated perfused rat heart (Zhan *et al.* 1985) and in the anaesthetized dog (Huang *et al.* 1986). Since it has been suggested that cardiac arrhythmias, induced by myocardial ischaemia and reperfusion, are associated with elevated levels of adenosine 3′, 5′-cyclic monophosphate (cAMP) (Opie 1982), it is of interest to know whether or not the anti-arrhythmic effect of naloxone involves myocardial cAMP activity. In this investigation naloxone was administered before ischaemia and the changes in cardiac rhythm and cAMP levels following reperfusion were studied in an isolated rat heart preparation.

METHODS

The Langendoff isolated perfused rat heart preparation described previously (Zhan *et al.* 1985) was employed. Female Sprague-Dawley rats (210–230 g) were used. Electrocardiogram (ECG)

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was monitored continuously (Zhan *et al.* 1985); recordings were made at 0–3, 9–12 and 19–22 min following reperfusion.

All hearts were mounted for perfusion for 10 min and any heart showing functional instability in this period was discarded. Perfusion was then stopped to produce myocardial ischaemia for 20 min. Naloxone (DuPont) was injected 2 min before the start of ischaemia. A dose of 200 μg per heart was used. To test the dose-related effect of naloxone on suppressing augmented cAMP levels, an additional dose of 66.7 μg per heart was used for the group of hearts frozen at 10 min following reperfusion. At 10 min the difference in cAMP levels, in the ischaemic groups, between these hearts with or without naloxone (200 μg per heart) was most marked (see result). The perfused hearts were frozen by two copper blocks previously chilled in dry ice at 1, 10 and 20 min following reperfusion for the ischaemic group or at 21, 30 and 40 min after continuous perfusion for the non-ischaemic control. All hearts were then stored at $-70°C$ until extraction and assay.

The method of Gilman (1970) was adopted for extraction and assay of cAMP. It was assayed in duplicate, using a commercial cAMP assay kit (Amersham TRK 432). The recovery was found to be 83%. Due to the limitation that there were at maximum only 28–32 tubes for each assay, the control was always assayed at the same time as the corresponding experimental samples to avoid interassay variation.

Comparisons were made between the control and ischaemic groups as well as between the ischaemic groups with or without naloxone treatment. Chi-squared test (combining 2×2 tables) and analysis of variance for split-plot design were used to test the difference in the incidence of hearts exhibiting ventricular arrhythmia and myocardial cAMP levels, respectively. Student's *t*-test was used to analyse the difference in cAMP levels of hearts frozen 10 min following reperfusion and between the ischaemic groups with or without receiving naloxone (66.7 μg per heart).

RESULTS

Figure 1 shows the effects of naloxone on both arrhythmias and augmentation of cAMP levels induced by myocardial ischaemia and reperfusion in the isolated perfused rat heart preparation. In agreement with the previous findings (Penny & Sheridan 1983; Zhan *et al.* 1985), myocardial ischaemia and reperfusion invariably caused cardiac arrhythmias. The incidence and severity of arrhythmias reached their peak after 9–12 min reperfusion. The arrhythmias were accompanied by a corresponding augmentation of myocardial cAMP levels. The increase was statistically significant. Pretreatment with naloxone (200 μg per heart) significantly attenuated both the incidence of arrhythmias and augmentation of cAMP levels to similar extents. A lower dose of naloxone (66.7 μg per heart) also attenuated significantly the augmentation of myocardial cAMP contents in hearts frozen 10 min following reperfusion, but to a lesser extent as compared with the higher dose of naloxone. The effect of naloxone in suppressing the ischaemia-reperfusion-induced elevated cAMP levels seemed to be dose-related.

DISCUSSION

Ventricular arrhythmias induced by myocardial ischaemia with or without reperfusion has been shown to be preceded by or associated with an increase in myocardial cAMP levels in the cat (Corr *et al.* 1978), in the dog (Krause *et al.* 1978), in the pig (Podzuweit & Lubbe 1977; Podzuweit *et al.* 1981); and in the isolated working rat heart (Crome *et al.* 1983), suggesting that the eleva-

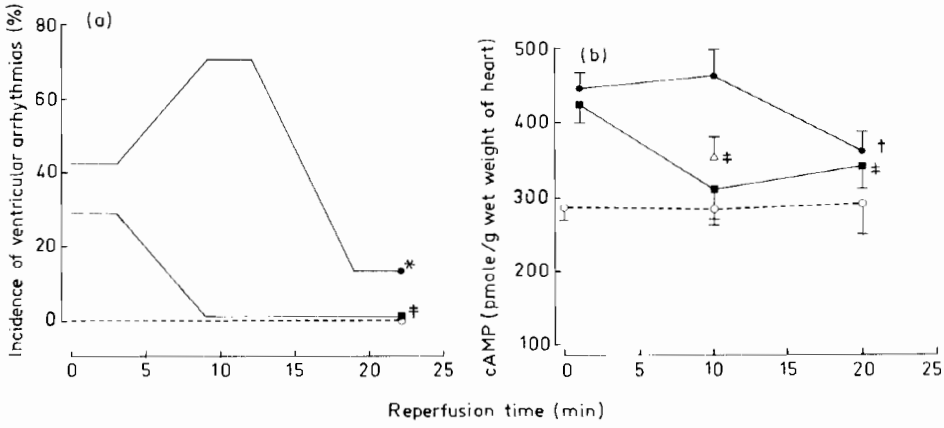

Fig. 1. Effects of ischaemia and reperfusion, either alone (●) or after, with or without pretreatment with 200 μg per heart (■) or 66.7 μg per heart (△) naloxone on (a) the incidence of cardiac arrhythmias and (b) myocardial cAMP levels in the isolated perfused rat heart (O control). Values are mean and s.e.m. of seven hearts for all groups, and of six hearts for the ischaemic group receiving 66.7 μg naloxone. Significant difference from the corresponding control groups: * $P<0.01$, † $P<0.001$, respectively. ‡ Significant difference from the corresponding group without naloxone pretreatment: $P<0.05$.

tion of cAMP may predispose to ventricular arrhythmias. However, Kane *et al.* (1985) could not find a similar relationship between the cardiac arrhythmias and cAMP levels in the anaesthetized rat. In this study, myocardial ischaemia and reperfusion induced ventricular arrhythmias which were accompanied by corresponding increases in cAMP levels. Attenuation of these arrhythmias by naloxone was accompanied by a corresponding reduction in cAMP levels. These results support the notion that cAMP may be related to ventricular arrhythmias.

This study has not only confirmed the anti-arrhythmic effect of naloxone in the same preparation (Zhan *et al.* 1985), but more importantly it has shown that attenuation of arrhythmias and reduction of cAMP by naloxone occur in parallel, suggesting that the anti-arrhythmic action of naloxone may involve cAMP. If the anti-arrhythmic effect of naloxone is an indication of the involvement of endogenous opioid peptides in arrhythmogenesis, the results of this study may mean that such peptides are released during myocardial ischaemia and reperfusion and increase cAMP levels, thus causing ventricular arrhythmias. However, further studies are needed to verify this.

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