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Effects of dynorphin$_{1-13}$ on cardiac rhythm and cyclic adenosine monophosphate (cAMP) levels in the isolated perfused rat heart

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We have studied the effects of dynorphin$_{1-13}$ on cardiac rhythm and cAMP levels in the isolated perfused rat heart. The standard Langendorff isolated heart preparation was used. The myocardial cAMP levels and the incidence of cardiac arrhythmias were determined after injection of dynorphin$_{1-13}$. Dynorphin$_{1-13}$ caused simultaneously cardiac arrhythmias and an increase in myocardial cAMP levels in a dose-dependent manner, and both effects were antagonized by naloxone. Further studies are needed to determine whether myocardial cAMP mediates the dynorphin-induced cardiac arrhythmias.

There are several lines of evidence supporting the hypothesis that endogenous opioid peptides (EOPs) are involved in cardiac arrhythmogenesis. Firstly, β-endorphin [7] and dynorphin$_{1-13}$ [11] cause naloxone-reversible arrhythmia in the isolated perfused rat heart. Secondly, naloxone is antiarrhythmic in vivo [1, 3, 6, 10] and in vitro [12]. Thirdly, only those opiate antagonists with specific antagonistic properties are antiarrhythmic whereas their isomers with no opiate antagonistic properties are not [9]. Fourthly, when tolerance to dynorphin has developed after chronic morphine treatment, rats are protected to a significant extent from arrhythmias induced by myocardial ischaemia and reperfusion [12]. The most important questions outstanding, therefore, are the role and mode of action of EOPs in cardiac arrhythmogenesis.

It is believed that cyclic adenosine 3′,5′-monophosphate (cAMP) is a mediator of cardiac arrhythmias as elevation of cAMP levels have been shown to occur in parallel with cardiac arrhythmias [8]. Since both β-endorphin [7] and dynorphin [12] cause cardiac arrhythmias in the isolated perfused rat heart and another group of EOPs, the enkephalins, increase cAMP levels in the cultured chick embryo heart cells [4], it is possible that EOPs induce cardiac arrhythmias and increase cAMP levels in par-

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allel in the isolated perfused rat heart and that they are linked together functionally. In this study, we administered dynorphin$_{1-13}$ to the isolated perfused rat heart in addition to the perfusion fluid and determined the cAMP levels and monitored the electrocardiogram (ECG).

Female Sprague-Dawley rats were used, from which Langendorff isolated heart preparations were made as previously described [7]. The heart was perfused through the aorta with a Krebs Ringer solution at a pressure of 120 mmHg and a flow rate of 8–10 ml/min. The pH was maintained at 7.4 with a 95% O_2–5% CO_2 mixture. The temperature of the heart was kept at 31–32°C by means of a water jacket. The first 10 min after mounting was used to allow the heart to stabilize and any heart exhibiting cardiac arrythmias during this period was discarded. Dynorphin$_{1-13}$ (Peninsula Labs.) dissolved in Krebs–Ringer solution to a total of 6.4 and 20 μg/heart (or Krebs–Ringer solution alone as control) was then added to the perfusion fluid. The volume and time of injection was 20 μl and 1 min, respectively. To study the antagonistic effect of naloxone against dynorphin, naloxone (DuPont) dissolved in Krebs–Ringer solution was administered 2 min prior to the dynorphin. The ECG was continuously monitored with a positive electrode hooked into the apex of the heart and a negative electrode at the aorta. Recordings were made 8–10 min after injection. At the end of the 10 min the heart was frozen by two copper blocks previously chilled in dry-ice and stored at −75°C until extraction and assay for cAMP levels. A period of 10 min was chosen as, in preliminary experiments on the effects of dynorphin on the time course of changes in cardiac rhythm and cAMP levels, the drug caused maximal cardiac arrhythmias and a simultaneous increase in myocardial cAMP levels at this time interval. For determination of cAMP levels, the method of Gilman [2] for extraction and assay was adopted. A commercial cAMP assay kit (Amersham TRK 432) was used: the recovery was 83%.

The χ^2-test was employed to test the difference in incidence of cardiac arrhythmias between the control and dynorphin-injected groups and between dynorphin-injected groups with or without pretreatment with naloxone at 10 min after injection of dynorphin. Analysis of variance (one-way ANOVA) was used to analyze the effects of dynorphin on myocardial cAMP levels at 10 min after injection. Student's t-test was used to test the difference in myocardial cAMP levels between the dynorphin-injected groups with or without pretreatment with naloxone at 10 min after injection of dynorphin.

Fig. 1 shows the arrhythmogenic effect of dynorphin at 8–10 min after its injection. The effect of dynorphin was statistically significant and dose-related. Moreover, the myocardial cAMP levels were significantly elevated in a dose-related manner following administration of dynorphin, as shown in Fig. 2. Naloxone, which itself had no effect on either cardiac rhythm or cAMP levels, reversed the effects of dynorphin significantly (Figs. 1 and 2).

It has been reported that cardiac arrhythmias are preceded by, or associated with, an elevation of myocardial cAMP levels [5, 8], suggesting a possible mediating role of cAMP in induction of cardiac arrhythmias. In this study we have also shown that cardiac arrhythmias and an elevation of myocardial cAMP levels occurred concur-

Fig. 1.

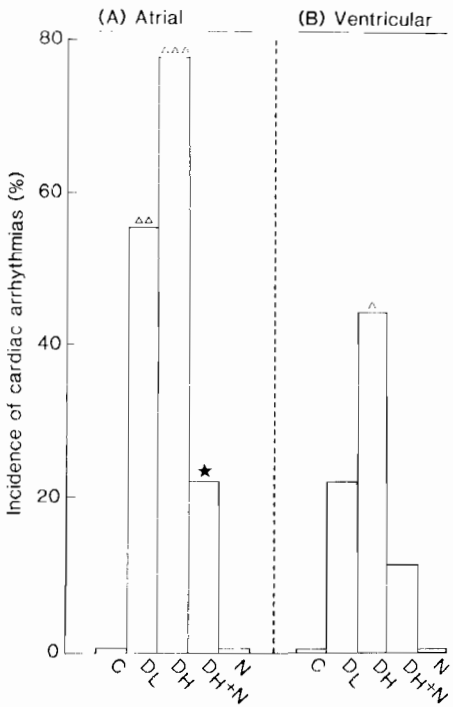

Fig. 2.

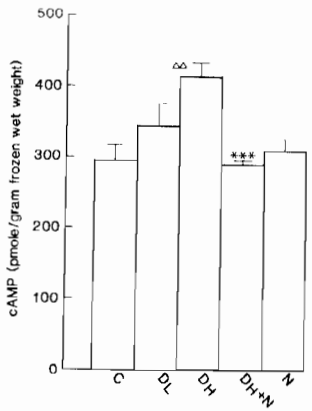

Fig. 1. Effects of dynorphin$_{1-13}$ on the cardiac rhythm of the isolated perfused rat heart at 8–10 min after its injection. C, control, D_L, dynorphin$_{1-13}$ at a dose of 6.4 μg/heart; D_H, dynorphin$_{1-13}$ at a dose of 20 μg/heart; D_{H+N}, dynorphin$_{1-13}$ at a dose of 20 μg heart, pretreated with naloxone at a dose of 200 μg/heart; N, naloxone at a dose of 200 μg/heart. Number of hearts is 9 in all groups. Atrial arrhythmias include premature atrial contraction, paroxysmal atrial tachycardia, atrial fibrillation and A–V block. Ventricular arrhythmias include premature ventricular contraction, ventricular tachycardia and ventricular fibrillation. △, △△, △△△ Indicate statistically significant difference to the corresponding control groups by χ^2-test at the levels $P<0.05$, $P<0.01$ and $P<0.001$, respectively. ★ Indicates statistically significant difference to the group receiving dynorphin$_{1-13}$ (20 μg/heart) only.

Fig. 2. Effects of dynorphin$_{1-13}$ on the cAMP levels in the isolated perfused rat heart at 10 min after its injection. Values are mean ± S.E.M. of 9 samples (one sample from one heart). For abbreviations see Fig. 1. △△ Indicates a statistically significant effect of dynorphin$_{1-13}$ by analysis of variance (one-way ANOVA) at the level $P<0.01$. ***Indicate a statistically significant difference to the dynorphin-treated group by Student's t-test at the level $P<0.001$.

rently following administration of dynorphin$_{1-13}$ and that naloxone reversed the effects of dynorphin$_{1-13}$ on both.

The results are particularly interesting in view of our previous findings that myocardial ischaemia and reperfusion lead to the simultaneous occurrence of cardiac arrhythmias and an elevation in myocardial cAMP, both of which are prevented by naloxone [5]. An obvious speculation is that myocardial cAMP mediates the cardiac arrhythmias induced by dynorphin or myocardial ischaemia and reperfusion, with the further possibility that the latter procedure acts via the release of EOPs [11]. It

would be of interest firstly to determine whether ischaemia and reperfusion cause release of EOPs and secondly to find out whether arrhythmias are still produced by ischaemia and reperfusion or by EOPs if the increase in cAMP is prevented.

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