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Chronic morphine treatment reduces the incidence of ventricular arrhythmias in the isolated rat heart induced by dynorphin$_{1-13}$ or myocardial ischemia and reperfusion

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We have studied the effects of a daily morphine injection for 2 weeks on induction of naloxone-reversible arrhythmias in the Langendorff isolated rat heart preparation caused by dynorphin$_{1-13}$ or myocardial ischemia and reperfusion. Chronic morphine treatment significantly reduced the incidence and severity of arrhythmias and increased recovery following both administration of dynorphin and myocardial ischemia and reperfusion. The results support the notion that myocardial ischemia and reperfusion induce cardiac arrhythmias via endogenous opioid peptides.

It has been shown that β-endorphin induces naloxone-reversible cardiac arrhythmias in the isolated perfused rat heart [3]. Myocardial ischemia and reperfusion-induced arrhythmias have also been shown to be attenuated by naloxone [1, 2, 6]. The results suggest that endogenous opioid peptides (EOP) may be involved in cardiac arrhythmogenesis. It is well known that animals may develop tolerance to morphine or cross-tolerance to other opiates in opiate receptor-mediated effects after morphine treatment. It is therefore reasoned that if myocardial ischemia and reperfusion do induce cardiac arrhythmias via EOP, animals receiving morphine treatment may develop tolerance to myocardial ischemia and reperfusion in a similar way as to opiates. In this investigation, we injected morphine into rats for 2 weeks and studied the incidence of cardiac arrhythmias induced by myocardial ischemia and reperfusion or dynorphin$_{1-13}$ in the isolated heart preparation described previously [6]. We used dynorphin$_{1-13}$ in this study because a preliminary study (personal observation) showed that it causes ventricular arrhythmias which also result from myocardial ischemia and reperfusion and it has been shown to be present in the heart by immunological techniques [5].

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Female Sprague Dawley rats of 150–190 g were used. They received a daily i.p. injection of morphine sulfate (MacFarlan Smith) in 0.9% NaCl solution or 0.9% NaCl solution only as controls at 9.30 and 21.30 h for 2 weeks. The dosage (mg/kg body wt.) was 10, 20, 30, 50, 60, 80, 100, 110, 120, 130, 150, 160, 180 and 200 from day 1 to day 14. On the 15th day rats were killed and hearts removed for the experiments.

The Langendorff isolated rat perfused heart preparation [6] was used. The time between decapitation of the rat and mounting of the heart was 4 min. The hearts were perfused retrogradely through the aorta at a constant pressure of 120 mmHg and a flow rate of 8–10 ml/min with Krebs–Ringer solution containing (mmol/l) NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.1, $NaHCO_3$ 24, $CaCl_2$ 2.5, glucose 10, and equilibrated with a 95% O_2 5% CO_2 gas mixture at pH 7.4. A water jacket surrounding the isolated heart was used to maintain the temperature at 32°C during perfusion and 31°C during ischemia. The electrocardiogram (ECG) was determined with a positive electrode hooked at the apex of the heart and a negative electrode at the aorta.

Two series of experiments were performed. The procedures were previously described [6]. In the first series, hearts were mounted and allowed to stabilize for 10 min. Any heart showing functional instability such as cardiac arrhythmias was discarded. Dynorphin$_{1-13}$ (Peninsula Labs.), at a dose of 4 nmol/heart was given and an observation was made for 80 min. In the second series of experiments, immediately after the heart was mounted, ischemia took place by stoppage of perfusion for 3 or 5 min followed by reperfusion for 35 min. Preliminary experiments showed that if there is recovery to sinus rhythm, it usually takes place within 35 min. The ECG was always normal within the first minute after ischemia and this one minute served as the control period. In both series of experiments the ECG was monitored throughout.

χ^2-Tests and Student's t-tests were used to analyze the difference in incidence and duration of cardiac arrhythmias in the different groups.

TABLE I

EFFECTS OF DYNORPHIN (4 nmol/heart) ON INDUCTION OF VENTRICULAR ARRHYTHMIAS IN THE ISOLATED PERFUSED HEARTS OF RATS RECEIVING A MORPHINE INJECTION FOR TWO WEEKS

Values are mean ± S.E.M.; n and no. are number of hearts. C, control group; M, morphine-injected group; PVC, premature ventricular contraction; VT, ventricular tachycardia; VF, ventricular fibrillation. *Significant difference to the corresponding control groups at the level $P < 0.05$ using χ^2-test.

	n	PVC	VT	VF	Total	Recovery to sinus rhythm	
						no.	%
C	10	7	5	3	8	1	12.5
M	10	2*	1*	1	4	2	50

TABLE II

INCIDENCE OF VENTRICULAR ARRHYTHMIAS DURING MYOCARDIAL ISCHEMIA (A, 3 min; B, 5 min) AND REPERFUSION (35 min) IN THE ISOLATED PERFUSED HEART OF RATS RECEIVING A MORPHINE INJECTION FOR TWO WEEKS

Values are mean ± S.E.M.; *n* and no. are number of hearts. For abbreviations, see Table I. *,** Significant difference to the corresponding control groups at the levels $P < 0.05$ and $P < 0.01$, respectively using χ^2-test.

	n	Ischemia		Reperfusion						Recovery	
		PVC (no.)	VT (no.)	PVC (no.)	VT		VF			no.	%
					no.	Duration (min)	no.	Interval from reperfusion to VF (min)	Duration (min)		
A											
C	11	11	3	11	8	2.31 ± 1.21	7	1.66 ± 0.91	14.5 ± 5.5	8	72
M	11	4**	0	11	8	2.31 ± 0.55	2*	3.75 ± 1.41	2.75 ± 1.25	11	100
B											
C	12	11	1	12	12	1.43 ± 0.44	12	2.43 ± 0.64	13.5 ± 2.12	8	67
M	12	8	0	12	11	1.38 ± 0.51	11	3.36 ± 0.96	11.0 ± 2.33	12*	100

Table I shows the effects of dynorphin$_{1-13}$ on cardiac rhythm. Dynorphin induced ventricular arrhythmias in 8 of 10 hearts in the control group. Only one heart recovered to normal sinus rhythm. In the morphine-treated group, only 4 out of 10 hearts exhibited ventricular arrhythmias and two of them recovered. In addition, the number of hearts exhibiting premature ventricular contraction (PVC) and ventricular tachycardia (VT) was significantly reduced in the morphine-treated group, indicating a reduction in severity of arrhythmias. The results showed that the hearts from morphine-treated rats were less vulnerable to dynorphin in induction of ventricular arrhythmias. The arrhythmogenic effects of dynorphin were attenuated by pretreatment with naloxone (0.83 nmol/heart) in both control and morphine-treated groups (results not shown).

In Table II, the effects of myocardial ischemia and reperfusion on cardiac rhythm are presented. In agreement with our previous findings [6] myocardial ischemia and reperfusion invariably led to cardiac arrhythmias. In the morphine-treated group subjected to myocardial ischemia for 3 min, the number of hearts exhibiting PVC during ischemia and ventricular fibrillation (VF) during reperfusion was significantly lower than in the corresponding group. The interval from reperfusion to VF was prolonged, the duration of VF shorter and recovery to sinus rhythm greater in the morphine-treated group although the difference was not statistically significant. In the morphine-treated group subjected to myocardial ischemia for 5 min the incidence and severity of cardiac arrhythmias were less than the corresponding control group, but the difference was not statistically significant. Recovery to sinus rhythm was, however, significantly increased in this group. The results show that the arrhythmogenic effects of myocardial ischemia and reperfusion were attenuated after chronic morphine treatment in a similar way to that of dynorphin. Treatment of naloxone significantly antagonized the arrhythmogenic effects of myocardial ischemia and reperfusion in a similar way as it did for dynorphin effects (results not shown).

In the present study we have demonstrated that after 2 weeks of morphine injection, rats have already developed tolerance to the arrhythmogenic effect of opiates as shown by the fact that cross-tolerance to dynorphin has occurred.

We have further shown that the isolated hearts from morphine-treated rats have developed tolerance to myocardial ischemia and reperfusion in a similar way to the effects of dynorphin. The results support the concept that myocardial ischemia and reperfusion induce arrhythmias via EOP. We have shown that both β-endorphin [6] and dynorphin are arrhythmogenic in the isolated rat heart. This is the first piece of evidence suggesting that EOP may be involved in cardiac arrhythmogenesis, but it should be considered with reservation as the doses used were pharmacological. The second piece of evidence in support of this suggestion is the demonstration of an antiarrhythmic effect of naloxone, an opiate antagonist in vivo [1, 2] and in vitro [6] preparations. However, the possibility of naloxone exerting its antiarrhythmic effect via its membrane stabilizing action rather than by opiate antagonism cannot be excluded. The finding in the present study that tolerance to the arrhythmogenic effect of myocardial ischemia and reperfusion after morphine treatment occurs in a similar way to the tolerance to the dynorphin effect is more compelling evidence that EOP

are indeed involved in cardiac arrhythmogenesis. This is in agreement with the recent finding by Parratt and Sitsapesan [4], who found that two opiate antagonists, (−)-Mr 1452 and (−)-WIN 44,441-3 are antiarrhythmic while their isomers without opiate antagonistic properties are not, indicating that the opiate antagonism is responsible for their antiarrhythmic effect. Further studies are needed to define the extent of involvement of EOP in arrhythmogenesis, mechanisms of action of EOP and the receptor types involved in cardiac arrhythmogenesis.

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