Naloxone Reversal of Ischemic Arrhythmia Is Stereospecific and Suggests Role of Endogenous Opioid Peptides in Ischemic Heart Disease (43464)

M. N. KAN, Y. T. CHEN, AND A. Y. S. LEE[1]

Heart Research Laboratory, Departments of Cardiology and Medical Research, Taichung Veterans General Hospital, Taichung, Taiwan, Republic of China

Abstract. **The effects of the stereoisomers of naloxone during myocardial ischemia were studied. (−)-Naloxone (but not the (+)-isomer naloxone) attenuated the ischemia-induced cardiac arrhythmias, hypotension, and bradycardia that result from coronary artery occlusion in anesthetized rats. From these findings, it may be inferred that endogenous opioid peptides may play a role in the pathophysiology of myocardial ischemia. It is also suggested that naloxone may have therapeutic value in the prevention and treatment of ischemic heart disease.** [P S E B M 1992 Vol 200]

The endogenous opioid system includes three major families of peptides: dynorphins (derived from pre-proenkephalin B), endorphins (derived from pre-proopiomelanocortin), and enkephalins (derived from pre-proenkephalin A). Multiple forms of opioid peptides are derived fro these major precursors and many of them possess potent cardiovascular properties.

Endogenous opioid peptides (EOP) and opioid receptors are widely distributed throughout the body, including the heart (1, 2). Lang *et al.* (3) have demonstrated the presence of enkephalins in the guinea pig heart. Immunoreactive dynorphin (4) and prodynorphin-derived opioid peptides (5) have also been shown to be present in the hearts of rats and guinea pigs, respectively. The existence of opioid receptors in the heart has been demonstrated by the blocking action of the opioid antagonist naloxone against cardiac effects of opioids, as well as by the receptor binding studies (6). Because EOP are secreted by the pituitary gland in response to stress (7), it seems likely that EOP may also be released from the heart during various cardiovascular stress situations, such as hypovolemic shock (8), heart failure (9), and myocardial ischemia (10), which might contribute to their respective detrimental effects. We have previously shown that naloxone reversed the cardiac arrhythmias induced by myocardial ischemia in the rat (11–13) and dog (14). To determine whether the protection observed is related to antagonism to opioid receptors and prevention of putative disturbing effects of EOP, or whether it is a result of nonopiate effects of the drug, the effects of the stereoisomers of naloxone were examined. (−)-Naloxone (but not the (+)-isomer naloxone, which has no opioid antagonistic activity) attenuated the ischemia-induced cardiac arrhythmias, hypotension, and bradycardia that result from coronary artery occlusion in anesthetized rats. The results strongly suggest that EOP may have a role in the pathophysiology of myocardial therapeutic value in the prevention and treatment of ischemic heart disease.

Materials and Methods

Sprague-Dawley rats of either sex weighing between 350 and 400 g were used. They were anesthetized with pentobarbitone sodium (60 mg/kg) intraperitoneally, tracheotomized, intubated, and artificially ventilated. The respiratory rate was similar to that of the rat (60–80 strokes/min, 1 ml/100 g). The left femoral artery and vein were cannulated for the measurement of blood pressure (BP) and heart rate (HR) by a Statham

[1] To whom requests for reprints should be addressed at Heart Research Laboratory, Departments of Cardiology and Medical Research, Taichung Veterans General Hospital, Taichung 40705, Taiwan, Republic of China.

Received September 3, 1991 [P.S.E.B.M. 1992, Vol 200]
Accepted March 3, 1992.

0037-9727/92/2004-0518$3.00/0

pressure transducer and a Biotechnometer (Gould), respectively, and for the administration of drugs. Electrocardiograms were recorded from lead II limb leads using the Lifepak ECG Monitor (Physio-Control Corp.).

Left thoracotomy in the fifth intercostal space was then performed, the heart was exposed and a ligature (6-0 silk suture) was placed around the left coronary artery. The rat was then allowed to equilibrate for 15 min. Afterward, (−)-naloxone (Sigma at the doses of 0.92 and 2.75 μmol/kg, (+)-naloxone at the dose of 2.75 μmol/kg, or saline as control were administered intravenously 10 min before the ligature was tied. BP, HR, and the electrocardiogram were continuously monitored before and throughout the 30-min postligation period.

To enable a quantitative comparison, an arrhythmia scoring system modified from that of Curtis and Walker (15) was used. Each rat was given one score representing the most severe type of arrhythmia observed anytime during the entire postligation period of 30 min. The arrhythmias were scored as follows: 0, no arrhythmia; 1, occasional ventricular premature contraction (VPC); 2, frequent VPC when there were three or more VPC occurring within 1 min; 3, ventricular tachycardia (VT; one to two episodes); 4, VT (three to five episodes); 5, VT (more than five episodes); 7, VF (three to five episodes); and 8, VF (more than five episodes).

The chi-square test was used to analyze the difference in the number of rats with VT or VF present versus the number with no VT or VF between control and treated groups, and between the groups receiving 2.75 μmol/kg of (+)- and (−)-naloxone. Student's t test was used to test the difference in arrhythmia score and the difference in the onset of arrhythmias between control and treated groups and between the groups receiving 2.75 μmol/kg of (+)- and (−)-naloxone, respectively. Repeated-measures analysis of variance, followed by multiple comparisons, was used to compare the difference in time course changes in mean arterial pressure and heart rate between control and treated groups, and between the groups receiving (+)- and (−)-naloxone. A 0.05 significance level was used for the analysis of variance and a 90% overall confidence level was used for the multiple comparisons.

Results

Table I summarizes the effects of (−)- and (+)-stereoisomers of naloxone on cardiac rhythm after coronary artery occlusion. Myocardial ischemia invariably caused ventricular arrhythmias, including VPC, VT, and VF. Of the eight rats in the control group, eight showed VPC, eight showed VT, and four showed VF, with onset of arrhythmias at 3.18, 13.88, and 20 min, respectively. The overall arrhythmia score was 4.88. Pretreatment with 0.92 μmol/kg of (−)-naloxone before coronary artery occlusion significantly reduced the incidence and severity of ischemia-induced arrhythmias. Of the eight rats, eight showed VPC, but only five showed VT and two showed VF, with onset of arrhythmias at 9.63 min, 11 min, and 8 min, respectively. The overall arrhythmia score was 3, in which was significantly lower than the score for the control group ($P < 0.05$). At a higher dose, 2.75 μmol/kg, (−)-naloxone further reduced the incidence and severity of ischemia-induced arrhythmias. All rats had VPC, but only six and two out of eight rats exhibited VT and VF, with onset of arrhythmias at 11.63 min, 11.75 min, and 8 min, respectively. The overall arrhythmia score was 2.5, which was significantly lower than that for the control group ($P < 0.01$). The antiarrhythmic effect of (−)-naloxone was dose related. Pretreatment with (+)-naloxone (2.75 μmol/kg) before coronary artery occlusion, however, was not effective in reversing the ischemia-induced arrhythmias. Of eight rats, eight showed VPC, eight showed VT, and five showed VF, with onset of arrhythmias at 6, 8.38, and 10.4 min, respectively. The overall arrhythmia score was 5.38, which was statistically not different from that of the control group but significantly higher than the group receiving (−)-naloxone ($P < 0.01$).

Figures 1 and 2 show the effects of (−)- and (+)-stereoisomers of naloxone on the changes in BP and HR after coronary artery occlusion. Myocardial ischemia invariably caused a marked decrease in both BP and HR. Pretreatment with (−)-naloxone at the dose of 2.75 μmol/kg before coronary artery occlusion significantly prevented the reduction in both BP ($P < 0.01$) and HR ($P < 0.05$). Pretreatment with (+)-naloxone (2.75 μmol/kg) before coronary artery occlusion, however, was not effective in reversing the ischemia-induced hypotension and bradycardia, which were statistically not different from the control group but significantly lower than the group receiving (−)-naloxone.

Discussion

It is well known that coronary artery occlusion leads to cardiogenic shock, bradycardia, and ischemia-induced arrhythmias, all of which may become fatal complications secondary to acute myocardial infarction. Similar effects were shown in the present study in the rat, in which coronary artery ligation soon led to marked reduction in the arterial blood pressure, bradycardia, malignant arrhythmias, and even death.

The findings of this study clearly demonstrate that naloxone blocks the arrhythmias, hypotension, and bradycardia secondary to myocardial ischemia. Such findings are in agreement with our previous experiments on the ischemic, isolated rat heart, in which naloxone markedly attenuated the cardiac arrhythmias and abolished the reduction in left ventricular pressures

Table I. Effects of (−)- and (+)-Stereoisomers of Naloxone on the Cardiac Rhythm after Coronary Artery Occlusion in the Rat[a]

	n	Arrhythmia score	VPC		VT		VF		Survival
			n	Onset (min)	n	Onset (min)	n	Onset (min)	
Control	8	4.88 ± 0.55	8	3.18 ± 1.27	8	13.88 ± 3.33	4	20 ± 0.35	7
(−)-Naloxone (0.92 μmol/kg)	8	3 ± 0.57[b]	8	9.63 ± 2.49[b]	5	11 ± 2.45	2	8 ± 2	8
(−)-Naloxone (2.75 μmol/kg)	8	2.5 ± 0.65[c]	8	11.63 ± 3.02[b]	4[d]	11.75 ± 4.77	1	8	8
(+)-Naloxone (2.75 μmol/kg)	8	5.38 ± 0.53[e]	8	6 ± 0.63	8[f]	8.38 ± 1.9	5[f]	10.4 ± 3.12	5

[a] Values are mean ± SEM; *n*, number of rats.
[b] Statistical difference to the corresponding control group at the level of $P < 0.05$ by Student's *t* test.
[c] Statistical difference to the corresponding control group at the level of $P < 0.01$ by Student's *t* test.
[d] Statistical difference to the corresponding control group at the level of $P < 0.05$ by chi-square test.
[e] Statistical difference to the group receiving 2.75 μmol/kg of (−)-naloxone at the level of $P < 0.01$ by Student's *t* test.
[f] Statistical difference to the group receiving 2.75 μmol/kg of (−)-naloxone at the level of $P < 0.05$ by chi-square test.

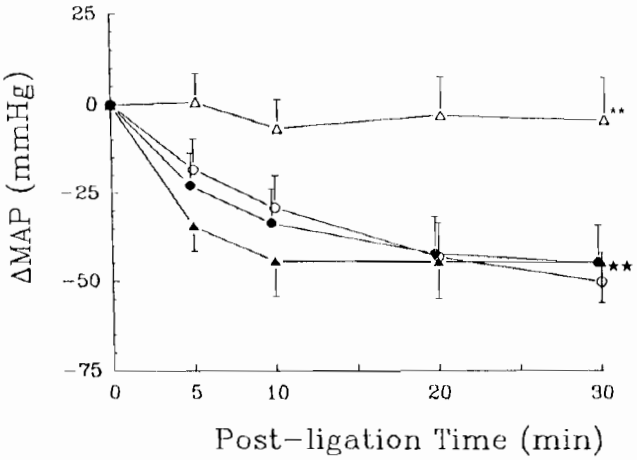

Figure 1. Effects of (−)- and (+)-stereoisomers of naloxone on the change in mean arterial blood pressure (ΔMAP), expressed in mm-Hg, after coronary artery occlusion in the rat. Values are mean and SE (vertical bars) of eight animals. ○, Saline; ●, (−)-naloxone (0.92 μmol/kg); △, (−)-naloxone (2.75 μmol/kg); ▲, (+)-naloxone (2.75 μmol/kg). Asterisks signify statistical difference to the corresponding saline-injected control group by repeated-measures analysis of variance ($df = 1,14$; $F = 9.82$; $P = 0.008$). Stars signify statistical difference to the group receiving 2.75 μmol/kg of (−)-naloxone by repeated-measures analysis of variance ($df = 1,14$; $F = 10.32$; $P = 0.006$).

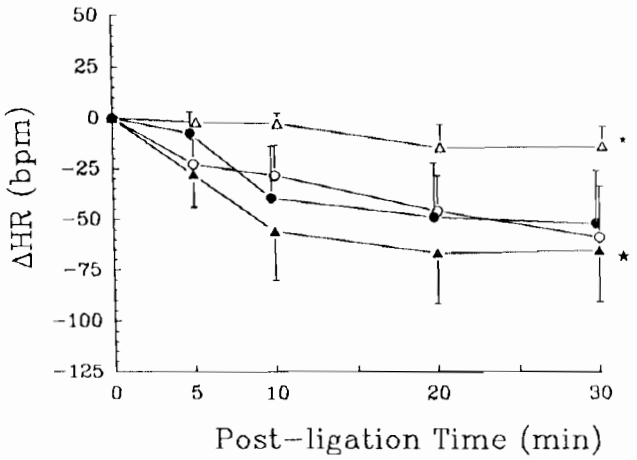

Figure 2. Effects of (−)-l and (+)-stereoisomers of naloxone on the change in heart rate (ΔHR), expressed in beats per minute, after coronary artery occlusion in the rat. Asterisk signifies statistical difference to the corresponding saline-injected control group by repeated-measures analysis of variance ($df = 1,14$; $F = 6.45$; $P = 0.024$). Star signifies statistical difference to the group receiving 2.75 μmol/kg of (−)-naloxone by repeated-measures analysis of variance ($df = 1,14$; $F = 7.80$; $P = 0.014$). For details see Figure 1.

resulting from myocardial ischemia and reperfusion (11). These findings are also compatible with those of Fagbemi *et al.* (16), in which naloxone reduced the incidence and severity of arrhythmias resulting from coronary artery ligation in the rat. Moreover and most importantly, the above effects of naloxone were stereospecific, the above effects of naloxone were stereospecific, because naloxone's (+)-isomer (without opiate antagonistic property) was not effective in reversing the ischemia-induced arrhythmias, hypotension, and bradycardia. Thus, the present study, using the two stereoisomers of naloxone, indicates that the effects of naloxone against myocardial ischemia are mediated by opiate receptors.

The efficacy of the opiate antagonist naloxone as a modifier of physiologic events has been used as a tool to infer EOP in various systems (17). From that perspective, the effects of the two stereoisomers of naloxone in the present study strongly implicate EOP as a factor in the pathophysiology of myocardial ischemia. Our results are also consistent with the hypothesis that EOP may be released from the heart upon myocardial ischemia, thereby causing arrhythmias, hypotension, and bradycardia. Naloxone, by virtue of its antagonistic action against opiates, rectifies these fatal complications secondary to myocardial ischemia, which suggests an

important role for EOP in ischemic heart disease. The beneficial effects of opiate antagonism certainly have great clinical implications in the prevention and treatment of ischemic heart disease. More studies are needed to define the therapeutic values of opiate antagonists.

This study was supported by the National Science Council, Republic of China. (+)-Naloxone was kindly supplied by the National Institute on Drug Abuse.

1. Hughes J, Kosterlitz HW, Smith TW. The distribution of methionine-enkephalin and leucine-enkephalin in the brain and peripheral tissues. Br J Pharmacol **61**:639–647, 1977.
2. Krumins SA, Faden AL, Fuerstein G. Opiate binding in rat hearts: Modulation of binding after hemorrhagic shock. Biochem Biophys Res Commun **127**:120–128, 1985.
3. Lang RE, Hermann K, Dietz R, Gaida W, Ganten D, Kraft K, Unger Th. Evidence for the presence of enkephalins in the heart. Life Sci **32**:399–406, 1983.
4. Spampinato S, Goldstein A. Immunoreactive dynorphin in rat tissue and plasma. Neuropept **3**:193–212, 1983.
5. Weihe E, McKnight AT, Corbett AD, Kosterlitz HW. Proenkephalin- and prodynophin-derived peptides in guinea pig heart. Neuropeptides **5**:453–456, 1985.
6. Lord JAH, Waterfield AA, Hughes J, Kosterlitz HW. Endogenous opioid peptides: Multiple agonists and receptors. Nature **267**:495–499, 1977.
7. Guillemin R, Vargo T, Rossier J, Minick S, Ling N, Rivier C, Vale W, Bloom F. Beta-endorphin and adrenocorticotrophin are secreted concomitantly by the pituitary gland. Science **197**:1367–1369, 1977.
8. Holaday JW. Cardiovascular consequences of endogenous opiate antagonism. Biochem Pharmacol **32**:573–583, 1983.
9. Liang CS, Imai N, Stone CK, Woolf PD, Kawashima S, Tuttle RR. The role of endogenous opioids in congestive heart failure: Effects of nalmefene on systemic and regional hemodynamics in dogs. Circulation **75**:443–451, 1987.
10. Lee AYS. Endogenous opioid peptides and cardiac arrhythmias. Int J Cardiol **27**:145–151, 1990.
11. Zhan CY, Lee AYS, Wong TM. Naloxone blocks the cardiac effects of ischemia and reperfusion in the isolated rat heart. Clin Exp Pharmacol Physiol **12**:373–378, 1985.
12. Lee AYS, Wong TM. Naloxone attenuates augmentation of cAMP levels and arrhythmias following myocardial ischemia and reperfusion in the isolated perfused rat heart. Clin Exp Pharmacol Physiol **13**:707–710, 1986.
13. Lee AYS, Wong TM. Antiarrhythmic potency of naloxone determined by a screening test using the isolated ischemic perfused rat heart preparation. Arch Int Pharmacol Pharmacodyn **286**:212–215, 1987.
14. Huang XD, Lee AYS, Wong TM, Zhan CY, Zhao YY. Naloxone blocks cardiac arrhythmias due to myocardial ischemia and reperfusion in the anesthetized dog. Br J Pharmacol **87**:475–477, 1988.
15. Curtis MJ, Walker MJA. Quantification of arrhythmias using scoring systems: An examination of seven scores in an in vivo model of regional myocardial ischemia. Cardiovasc Res **22**:656–665, 1988.
16. Fagbemi O, Lepran I, Parratt JR, Szekeres L. Naloxone inhibits early arrhythmias resulting from acute coronary ligation. Br J Pharmacol **76**:504–506, 1982.
17. Holaday JW, Faden AI. Naloxone reversal of endotoxin hypotension suggests role of endorphins in shock. Nature **275**:450–451, 1978.