Experimental Studies

Effects of Procainamide and Disopyramide on Long Chain Acyl Carnitine and Long Chain Acyl CoA Concentrations in the Ischemic Heart

Akira KOBAYASHI, M.D., Hiroshi WATANABE, M.D., *and* Noboru YAMAZAKI, M.D.

SUMMARY

Though the efficacies of procainamide and disopyramide in treating arrhythmias are well established, their precise mechanisms of antiarrhythmic action remain unclear. Arrhythmias which occur during acute myocardial ischemia can be explained partly on a metabolic basis. The accumulation of intermediates subsequent to impaired β-oxidation of free fatty acids has been suggested as a cause of serious arrhythmias. The purpose of this study was to investigate changes in free carnitine, long chain acyl carnitine and long chain acyl CoA concentrations in the ischemic canine heart following the administration of procainamide and disopyramide. The coronary artery was occluded for 40 min and myocardial samples were prepared from both nonischemic and ischemic areas. Procainamide and disopyramide prevented the accumulation of long chain acyl carnitine and long chain acyl CoA in the ischemic myocardium. The results showed that procainamide and disopyramide had beneficial effects on fatty acid metabolism. It was suggested that one of the antiarrhythmic mechanisms of these drugs might be the prevention of the accumulation of fatty acyl derivatives in the ischemic myocardium.

Additional Indexing Words:

Free fatty acids Carnitine Ventricular arrhythmias Antiarrhythmic drugs Antiarrhythmic mechanism Cyclic AMP

VENTRICULAR arrhythmias have been reported to account for many sudden deaths in patients with myocardial infarction. The efficacy of antiarrhythmic drugs in treating ventricular arrhythmias is well established. However, their precise mechanisms of antiarrhythmic action remain unclear. Recently, increasing emphasis has been placed on biochemical factors in the genesis of arrhythmias, and some arrhythmias occurring during acute myocardial ischemia can be explained on a metabolic basis. Much attention has

From the Third Department of Internal Medicine, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan.

Address for reprints: Akira Kobayashi, M.D., Third Department of Internal Medicine, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu, Shizuoka 431-31, Japan.

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been focused on the arrhythmogenicity of free fatty acids (FFA) under conditions of ischemia from many clinical and experimental observations. An increased concentration of circulating FFA has been reported in patients with acute myocardial infarction, and elevated concentrations of FFA have been correlated with the appearance of ventricular arrhythmias.[1] In the ischemic heart, elevated FFA leads to the accumulation of fatty acyl derivatives, such as long chain acyl carnitine and long chain acyl CoA as a result of the inhibition of β-oxidation of FFA.[2] Accumulation of FFA and its intermediates has been advocated as a cause of serious cardiac arrhythmias because of their direct toxic effects on the cell membrane, perhaps with structural changes in the ionic channels.[3] Sodium-2-[5-(4-chlorophenyl)-pentyl]-oxirane-2-carboxylate (POCA) is an inhibitor of carnitine acyltransferase I and prevents the increase in long chain acyl carnitine in hypoxic cells.[4] Pretreatment with POCA markedly attenuated the electrophysiologic derangements induced by hypoxia as assessed by intracellular transmembrane action potential recordings.[4] Thus, it is likely that the inhibition of the accumulation of intermediates of FFA might have a beneficial effect on the prevention of arrhythmias associated with myocardial ischemia. Cyclic AMP accelerates lipolysis both in adipose tissue and in the heart. Propranolol decreases the infarct size and improves cardiac metabolism in acute ischemia, and one identified mechanism of its protective effects is the inhibition of lipolysis and the reduction of circulating FFA concentrations through alteration of the cyclic AMP system.[5] Quinidine, procainamide and disopyramide have been reported to reduce atrial cyclic AMP concentrations in guinea pigs and to inhibit hormone-stimulated lipolysis in isolated fat cells.[6],[7] Although local anesthetics presumably act to stabilize the cell membrane and prevent arrhythmias,[8] they may also block the effect of cyclic AMP on lipid metabolism. However, there is no report available regarding the effects of antiarrhythmic drugs on the intermediates of FFA in the ischemic heart. Therefore, an investigation was undertaken to study the changes in the myocardial concentrations of long chain acyl carnitine and long chain acyl CoA following the administration of procainamide and disopyramide.

Materials and Methods

Twenty-four mongrel dogs of both sexes, weighing from 10 to 15 kg, were used. All dogs were anesthetized with a bolus injection of sodium pentobarbital (30 mg/kg i.v.). The trachea was intubated and respired with room air using a Harvard respirator. Thoracotomy was performed and the heart suspended in a pericardial cradle. The left anterior descending coro-

nary artery was dissected free from surrounding tissue just distal to the origin of the first diagonal branch and a 1–0 silk thread was placed around the left anterior descending coronary artery. The coronary artery was occluded with an intracranial arterial clamp for 40 min. Soon after the onset of coronary artery occlusion, the ischemic area was clearly apparent as a sharply delineated cyanotic area. Animals which did not exhibit epicardial cyanosis over the left ventricle were excluded from this study. Lead II of the standard electrocardiogram was recorded continuously during the experiment. Twenty-four dogs were divided into 3 groups as follows. The control group (n=8) received isotonic saline solution. The procainamide-treated group (n=8) was intravenously injected at a dose of 20 mg/kg 30 min prior to coronary occlusion followed by an infusion of 0.1 mg/kg/min to the end of the experiment. The disopyramide-treated group (n=8) was intravenously injected at a dose of 5 mg/kg 30 min prior to coronary occlusion followed by an infusion of 0.1 mg/kg/min to the end of the experiment. The doses of both agents were based on previous reports.[9),10)] After 40 min of coronary artery occlusion, the beating hearts were immediately removed and transmural samples representing the ischemic area (supplied by the occluded left coronary artery) and nonischemic area (supplied by the circumflex coronary artery) were rapidly excised. Tissue samples were immediately frozen with a Wollenberger clamp which was cooled to the temperature of liquid nitrogen and then stored at −80°C. The animals which developed ventricular fibrillation were excluded from the analysis of metabolism because of different durations of ischemia. The concentration of free carnitine was measured by coupled enzymatic assays following the method of Marquis and Fritz.[11)] The concentration of long chain acyl CoA was assayed as free CoA after alkaline hydrolysis at pH 11.5 to 12.0 in the presence of dithiothreitol and measured by the enzymatic cycling method of Veloso and Veech.[12)] We have reported the assay methods for free carnitine, long chain acyl carnitine and long chain acyl CoA in another report.[13)] In order to analyze their frequency and severity, ventricular arrhythmias were quantified by an arbitrary scoring system that was devised based on a modification of Lown and Wolf's ventricular arrhythmia grading system[14)] (Table I). Maximum scores were recorded in individual dogs every 5 min and added in each group. Values of the metabolites were expressed as mean±SD and statistical analysis was done using Student's t-test. Statistical analysis of ventricular arrhythmias was done by the Chi-square method and the Wilcoxon signed rank test. P values of less than 0.05 were considered significant.

Table I. Scoring System for Ventricular Arrhythmias

Grade	Arrhythmias
0	No VPB
1	Isolated uniform VPBs<5/min
2	Isolated uniform VPBs>5/min
3	Multiform VPBs
4	Couplets or salvos VPBs
5	Ventricular tachycardia (VT*)
6	Ventricular fibrillation (VF)

VPB=ventricular premature beat.
* More than 5 consecutive VPBs were considered VT.

Results

1. Effects on ventricular arrhythmias

In the control group, 1 dog had ventricular fibrillation and 2 dogs had ventricular tachycardia during coronary artery occlusion. On the other hand, no dog showed ventricular fibrillation or ventricular tachycardia in the procainamide-treated and disopyramide-treated groups. Figure 1 shows the changes in the mean score of ventricular arrhythmias. The mean scores at 30, 35 and 40 min after coronary occlusion in the procainamide-treated and disopyramide-treated groups were significantly lower than those in the control group.

2. Myocardial concentrations of free carnitine

Figure 2 shows the myocardial concentrations of free carnitine in the ischemic and nonischemic areas in each group. In the control group, mean free carnitine concentrations in the ischemic and nonischemic areas were 632 ± 223 nmol/g wet tissue and 1072 ± 350 nmol/g wet tissue, respectively. In the procainamide-treated group, mean free carnitine concentrations in the ischemic and nonischemic areas were 671 ± 129 nmol/g wet tissue and 1025 ± 103 nmol/g wet tissue, respectively. In the disopyramide-treated group, mean free carnitine concentrations in the ischemic and nonischemic areas were 840 ± 46 nmol/g wet tissue and 994 ± 105 nmol/g wet tissue, respectively. The concentration of free carnitine in ischemic areas decreased significantly compared with that in nonischemic areas in all groups. In ischemic areas, the concentration of free carnitine in the disopyramide-treated group was kept at a higher level than that in the control group ($p<0.05$), but there was no significant difference between procainamide-treated and control groups.

3. Myocardial concentrations of long chain acyl carnitine

Figure 3 shows the myocardial concentrations of long chain acyl car-

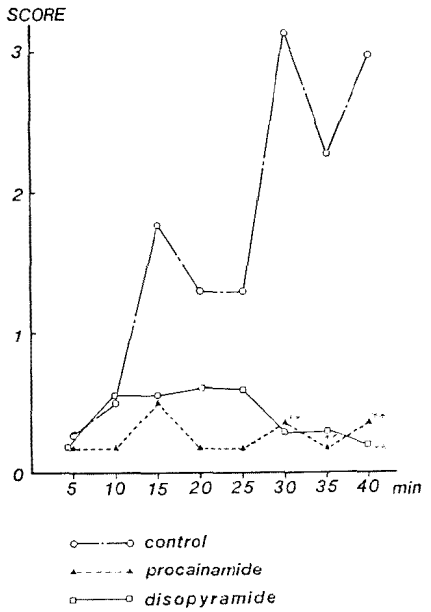

Fig. 1. Changes in the mean scores for ventricular arrhythmias during myocardial ischemia. Asterisks represent the significance of changes between the control group and the treated group. ** $p<0.01$.

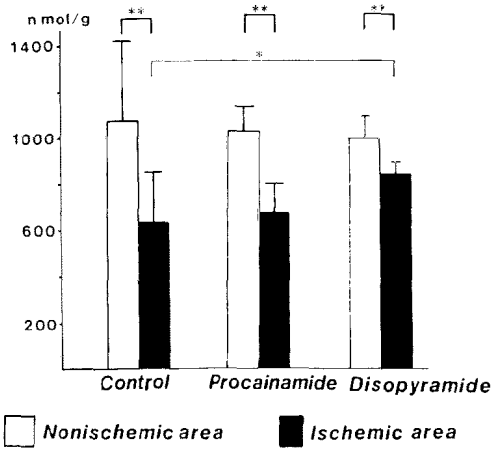

Fig. 2. Effects of procainamide and disopyramide on tissue levels of free carnitine. The significance of the changes is represented as follows: non-ischemic area vs ischemic area (paired t-test), the control group vs treated group (nonpaired t-test). * $p<0.05$, ** $p<0.01$.

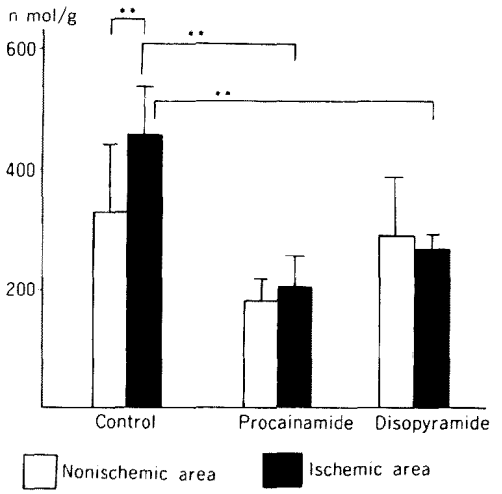

Fig. 3. Effects of procainamide and disopyramide on tissue levels of long chain acyl carnitine. The significance of the changes is represented as follows: nonischemic area vs ischemic area (paired t-test), the control group vs treated group (nonpaired t-test). ** $p<0.01$.

nitine in the ischemic and nonischemic areas in each group. In the control group, mean long chain acyl carnitine concentrations in the ischemic and nonischemic areas were 458 ± 80 nmol/g wet tissue and 331 ± 102 nmol/g wet tissue, respectively. In the procainamide-treated group, mean long chain acyl carnitine concentrations in the ischemic and nonischemic areas were 203 ± 51 nmol/g wet tissue and 186 ± 23 nmol/g wet tissue, respectively. In the disopyramide-treated group, mean long chain acyl carnitine concentrations in the ischemic and nonischemic areas were 268 ± 62 nmol/g wet tissue and 291 ± 96 nmol/g wet tissue, respectively. The concentration of long chain acyl carnitine in the control group was significantly higher in the ischemic area than in the nonischemic area ($p<0.01$). On the other hand, there was no significant difference between the ischemic and nonischemic areas in the procainamide-treated and disopyramide-treated groups. In ischemic areas, the concentrations of long chain acyl carnitine in the procainamide-treated and disopyramide-treated groups were significantly lower than those in the control group ($p<0.01$, $p<0.01$). This shows that procainamide and disopyramide prevented the accumulation of long chain acyl carnitine in ischemic myocardium.

4. Myocardial concentrations of long chain acyl CoA

Figure 4 shows the myocardial concentrations of long chain acyl CoA in the ischemic and nonischemic areas in each group. In the control group, mean long chain acyl CoA concentrations in the ischemic and nonischemic

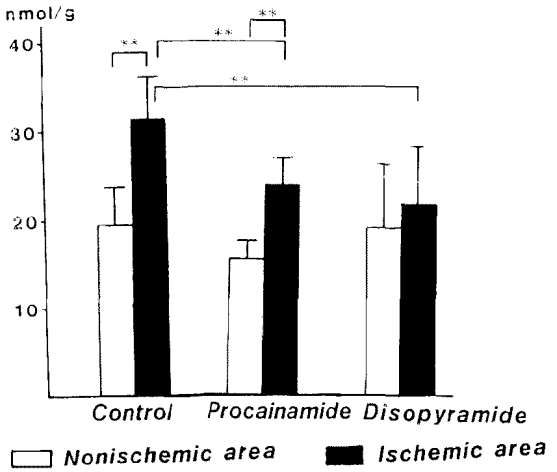

Fig. 4. Effects of procainamide and disopyramide on tissue levels of long chain acyl CoA. The significance of the changes is represented as follows: nonischemic area vs ischemic area (paired t-test), the control group vs treated group (nonpaired t-test). ** $p<0.01$.

areas were 31.2 ± 4.9 nmol/g wet tissue and 19.4 ± 4.4 nmol/g wet tissue, respectively. In the procainamide-treated group, mean long chain acyl CoA concentrations in the ischemic and nonischemic areas were 23.7 ± 2.7 nmol/g wet tissue and 15.7 ± 2.1 nmol/g wet tissue, respectively. In the disopyramide-treated group, mean long chain acyl CoA concentrations in the ischemic and nonischemic areas were 21.9 ± 7.4 nmol/g wet tissue and 19.5 ± 7.0 nmol/g wet tissue, respectively. The concentrations of long chain acyl CoA in the control and procainamide-treated groups were significantly higher in the ischemic areas than in the nonischemic areas ($p<0.01$, $p<0.01$). However, the concentration of long chain acyl CoA in the procainamide-treated group was significantly lower than that in the control in the ischemic area ($p<0.01$). On the other hand, there was no significant difference in the concentration of long chain acyl CoA between ischemic and nonischemic areas of the disopyramide-treated group. The concentration of long chain acyl CoA in the disopyramide-treated group was significantly lower than that in the control group in the ischemic area ($p<0.01$). This shows that disopyramide and procainamide prevented the accumulation of long chain acyl CoA in the ischemic myocardium.

Discussion

The efficacies of procainamide and disopyramide in treating ventricular

arrhythmias are well established. Both agents are thought to be capable of suppressing automatic and reentrant arrhythmias, since they modify refractoriness and slow conduction, and suppress automaticity in Purkinje fibers and ventricular muscle cells.[15] However, their precise mechanisms of antiarrhythmic action remain unclear. Arrhythmias which occur during acute myocardial hypoxia can be partly explained on a metabolic basis. Increased levels of circulating FFA have been reported in patients with acute myocardial infarction,[1] and elevated levels of FFA have been correlated with the appearance of ventricular arrhythmias.[2] An increased release of catecholamine is prominent in the ischemic heart and can lead to an increase in lipolysis and to an enhancement of the uptake of FFA into the myocyte.[3] Since β-oxidation of FFA is inhibited by the limited supply of oxygen to the ischemic heart, fatty acid derivatives, such as long chain acyl carnitine and long chain acyl CoA, increase in the myocyte.[2] Therefore, elevated FFA leads to the accumulation of long chain acyl carnitine and long chain acyl CoA in the presence of myocardial ischemia. The accumulation has been suggested as a cause of cellular damage and ventricular arrhythmias in the ischemic heart.[3] The administration of exogenous palmitoyl carnitine, which is a long chain acyl carnitine, decreases maximum diastolic potential, amplitude, V_{max} and action potential duration in canine Purkinje fibers.[16] POCA, an inhibitor of carnitine acyltransferase, prevents the accumulation of long chain acyl carnitine in hypoxic myocytes and markedly attenuates the electrophysiologic derangements induced by hypoxia assessed by intracellular transmembrane action potential recordings.[5] Carnitine is essential for the transport of fatty acyl derivatives to the site of β-oxidation in the mitochondria, and itself improves mechanical and electrophysiological function in the ischemic heart.[17] These experimental results indicate that it may be beneficial to reduce long chain acyl carnitine and long chain acyl CoA in the ischemic myocardium. In this study, procainamide and disopyramide prevented the accumulation of long chain acyl carnitine and long chain acyl CoA in the ischemic myocardium. Pande et al reported that quinidine, disopyramide and lidocaine prevented the increase in plasma FFA concentrations in the cat.[9] Siddle et al reported that local anesthetics, such as lignocaine and dibucaine, inhibited hormone-stimulated lipolysis in adipose tissue.[7] Mirro reported that quinidine, procainamide and disopyramide reduced the cyclic AMP content of guinea pig atria.[6] Quinidine, procainamide and disopyramide produced concentration-dependent reductions in atrial rate which were paralleled by reductions in atrial cyclic AMP concentration. A number of investigators have proposed that myocardial infarction induces the release of catecholamines from both adrenal glands and sympathetic nerve terminals, and hence stimulates both

peripheral and intramyocardial lipolysis which is mediated by cyclic AMP. The precise mechanisms by which procainamide and disopyramide prevent the accumulation of fatty acyl derivatives in the ischemic myocardium are unknown. It was suggested that the mechanisms which prevented the accumulation of fatty acyl derivatives might be the inhibition of lipolysis and the reduction of uptake into the myocyte as a result of decreased circulating FFA concentration. The inhibition of lipolysis may be influenced, in part, through alterations in the adenylate cyclase-cyclic AMP system. The results showed that procainamide and disopyramide have beneficial effects on FFA metabolism and it was suggested that one of the antiarrhythmic mechanisms of these drugs might be the prevention of the accumulation of FFA derivatives in the ischemic myocardium. The present study points out that knowledge of the biochemical effects of antiarrhythmic drugs may provide further insight into the mechanisms of action of these drugs.

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