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Enhanced myocardial preservation by nicotinic acid, an antilipolytic compound: mechanism of action*)

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Summary: The cardioprotective effects of an antilipolytic compound, nicotinic acid, on arrested-reperfused myocardium were investigated in the isolated *in situ* pig heart preparation. Hearts were preperfused for 15 min in the presence of (5-^{3}H)-glucose and (U-^{14}C)-palmitic acid. Half of the hearts were then perfused with 0.08 mM nicotinic acid for an additional 15-min period, while the remaining control hearts received unmodified perfusion. Arrest was then induced in all animals for 2 h using hypothermic K^+ cardioplegia, followed by 60 min of normothermic reperfusion. In control hearts, there were significantly greater levels of long-chain acyl Co-A and acyl carnitine and lower levels of membrane phospholipids than in the nicotinic acid group. While nicotinic acid inhibited β-oxidation during pre-ischemia and reperfusion, it also prevented the degradation of membrane phospholipids. The net result was a reduction of free fatty acid accumulation during arrest and reperfusion in the nicotinic acid group. Glycolysis, as reflected in $^{3}H_2O$ production, was significantly increased by nicotinic acid administration. In the control heart as compared to the nicotinic acid group, the incorporation of ^{14}C-label from palmitate into triglyceride and cholesterol during arrest was enhanced, while incorporation into phospholipids was depressed. The cardioprotective effects of nicotinic acid were demonstrated by decreased release of creatine kinase and improved coronary blood flow, and cardiac contractility in the reperfused myocardium supplemented with nicotinic acid compared to the control group. These results suggest that nicotinic acid significantly protects the arrested-reperfused myocardium by a) preventing elevation of myocardial fatty acid levels, b) stimulating glycolysis by limiting fatty acid oxidation, c) inhibiting degradation of membrane phospholipids, and d) preventing accumulation of fatty acid metabolites with harmful detergent properties.

Key words: heart; myocardial preservation; nicotinic acid; ischemia; reperfusion

Introduction

Myocardial ischemia results in the accumulation of free fatty acids from the inhibition of β-oxidation (10), degradation of membrane phospholipids (7, 20), and enhanced endogenous lipolysis (17). Free fatty acids and their long-chain esters are potentially injurious to myocardium because of their enzyme-inhibitory and detergent properties (13, 31).

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Mammalian heart can utilize both fatty acids and glucose as energy substrates. During aerobic metabolism, fatty acid is the preferred substrate for heart muscle, while during ischemia, fatty acid utilization is rapidly depressed because of the inhibition of β-oxidation. It has been suggested that conversion of myocardial metabolism from predominantly lipid to predominantly carbohydrate would be of considerable benefit in preserving myocardial viability during ischemia (18). Theoretically, any stimulation of carbohydrate metabolism relative to fatty acid utilization should provide "oxygen saving" for any given hemodynamic state. Using this concept, we evaluated an antilipolytic drug, nicotinic acid, to study its cardioprotective effect during cardioplegic arrest and reperfusion.

Materials and methods

Twenty-four Yorkshire pigs of either sex, weighing between 18 and 25 kg, were tranquilized with ketamine (Ketaject 50 mg/kg intramuscularly) and anesthetized with intravenous pentobarbital (Nembutal 25 mg/kg). Each animal was placed on controlled respiration in room air, and the chest was opened through a median sternotomy. After sodium heparin (500 units/kg) was administered, the animals were placed on cardio-pulmonary bypass with a bubble oxygenator (Bentley BOS-5), and the heart was isolated from the systemic circulation by cross-clamping the aorta as described previously (21, 24).

The isolated heart was perfused via the clamped aortic root by its own bubble oxygenator. Systemic perfusion was discontinued, and the circulating blood volume was drained into the oxygenator. Superior and inferior vena cavae, as well as the axygos and hemiazygos veins were ligated, and the coronary sinus return coming from the pulmonary artery was recirculated via the pump oxygenator, thereby maintaining a closed circuit with the isolated, perfused heart.

The perfusate was temperature controlled (at 37 °C for control and reperfusion periods and at 4–8 °C for hypothermic cardioplegia). Perfusate hematocrit during reperfusion varied from 20–25 %. The isolated heart was maintained in a constant temperature bath enclosed by the suspended pericardial well, and myocardial temperature was thereby maintained at normothermia during reperfusion and hypothermia (8–10 °C) during cardioplegic arrest. Half of the animals were utilized for metabolic studies and half for tissue biopsy measurements. In the metabolic group, after 15 min of stabilization, tracer amounts of radiolabeled (U-^{14}C)-palmitic acid (sp. activity 550 μCi/mmol, New England Nuclear, Massachusetts) (50 μCi complexed to 2 % bovine serum albumin to yield a final concentration of 0.05 nM) and (5-^{3}H)-glucose (sp. activity 22 Ci/mmol, New England Nuclear) (100 μCi to yield a final concentration of 0.5 nM) were introduced into the perfusion circuit. These radioactive tracers were never removed from the perfusion circuit, and they were present throughout the experiment. The heart was perfused in the presence of these isotopic compounds for 15 min. In half of the animals (six metabolic, six biopsy studies), 0.08 mM nicotinic acid was added to the perfusion medium, and the heart was perfused for an additional 15 min-period. Control experiments were performed in the remaining 12 animals in the absence of nicotinic acid.

Global hypothermic cardioplegic arrest was then induced using potassium crystaloid cardioplegia (KCC) for 2 h with topical hypothermia at 10 °C. An aliquot of 50 ml of KCC was introduced every 15 min into the aortic root during the arrest period. Following arrest, the heart was reperfused for another 60 min at normothermia. The myocardial perfusate and coronary sinus effluents were sampled prior to the addition of nicotinic acid, prior to arrest, and 15, 30, 45, and 60 min following the onset of reperfusion. Biopsies of left ventricular myocardium were taken from one-half of the nicotinic acid and control groups, and the other half of each group was used for the metabolic experiments. Myocardial biopsies were taken prior to the addition of nicotinic acid, prior to arrest, immediately after arrest, and after 60 min reperfusion.

Measurement of glucose oxidation

The glycolytic flux was measured by estimating the rate of $^{3}H_2O$ production from (5-^{3}H) glucose as described previously (8). Coronary effluent samples collected at various times prior to and following arrest were subjected to column chromatography using a 0.5 × 2 cm column of Dowex CG1×8 (formate form) to separate ^{3}H-glucose from lactate. Lactate was eluted with 0.4 M Na-formate (23). Separation

of ^3H_2O from (5-^{3}H) glucose was achieved by chromatography on Dowex CG1×8 (formate) as described previously (23). The radiolabeled glucose was retained by the column. An aliquot of the ^3H_2O washed from the column was counted for radioactivity using a liquid scintillation counter (Packard Tri-Carb, IL), equipped with automatic quenching correction to correct any error due to quenching.

Production of ^3H_2O was used as an index for glucose utilization through the glycolytic pathway. After the isolated heart had been perfused for 15 min in the presence of ^{3}H-glucose, the amount of ^3H_2O present in the perfusate was taken as 100%. The determination of ^3H_2O production in the coronary effluent was used to determine the percent change in glucose utilization.

Measurement of palmitate oxidation

Fatty acid oxidation was analyzed by monitoring $^{14}CO_2$ production in the perfusate. The sample used to estimate $^{14}CO_2$ from (U-^{14}C) palmitate was removed directly from the closed system with a syringe and placed in a stoppered tube containing hyamine hydroxide (1 M) in a center well as described previously (8, 15). The perfusate sample was acidified with 1 ml of 9 N H_2SO_4, and the flask was shaken for 3 h. The hyamine trap containing $^{14}CO_2$ was placed in scintillation cocktail and counted for radioactivity. Rates of fatty acid oxidation were measured as $^{14}CO_2$ production from (U-^{14}C) palmitate and are expressed as μmoles of palmitate oxidized per gm dry weight per minute. This was calculated as follows: μmoles of palmitate oxidized/gm/min = cpm of $^{14}CO_2$/ml perfusate × coronary flow (ml/min) sp activity perfusate palmitate (cpm/μmol) × gm of dry weight.

Assay for CoA, acetyl CoA, carnitine, acetyl carnitine, long-chain acyl CoA, long-chain acyl carnitine, NAD and NADH

Myocardial biopsies were immediately frozen under liquid nitrogen and divided into sections. One part was immediately weighed to determine the wet weight of the tissue, and it was then dried to a constant weight to measure the dry weight. Another part was used to extract fatty acids and lipids, while adenine nucleotides were measured in yet a third section. The remaining biopsy was used to determine the tissue levels of CoA, acetyl CoA, carnitine, etc. according to Das et al. (6), as modified by Lopaschuk et al. (15).

Extraction and separation of lipids

Lipids were extracted according to Folch et al. (11), as modified by Das et al. (7), to eliminate phospholipid loss during extraction. The extracted lipids were separated into neutral lipids and phospholipids by both silicic acid column chromatography and thin-layer chromatography (11). The respective lipids were scraped off silica gel plates into scintillation vials in order to estimate the incorporation of labels from (U-^{14}C)-palmitate. Phospholipid contents were quantified by estimating phosphate according to Bartlett (1).

Estimation of free fatty acids

The neutral lipid extract was used for determination of free fatty acids (FFA). Samples were added to SEP-PAK silica cartridge (Waters Associates, Milford, Massachusetts) for separation of neutral lipids and FFA as described by Hamilton and Comai (12). Phenacyl esters of FFA derivatized from the pooled eluents were analyzed by high performance liquid chromatography (HPLC) using a modification of the method of Wood and Lee (32).

Estimation of CK release from heart, myocardial contractility, and coronary blood flow

CK was assayed in the perfusate plasma by enzymatic assay method as described elsewhere (7). Maximum rate of rise of left ventricular pressure (LV max dp/dt) and coronary blood flow were measured according to previously described methods (20).

Statistical analysis

All measurements are expressed as the mean values plus or minus the standard error of the mean. Student's paired *t*-test or two-sample *t*-test was used for statistical analysis. Results were considered significant when $p < 0.05$.

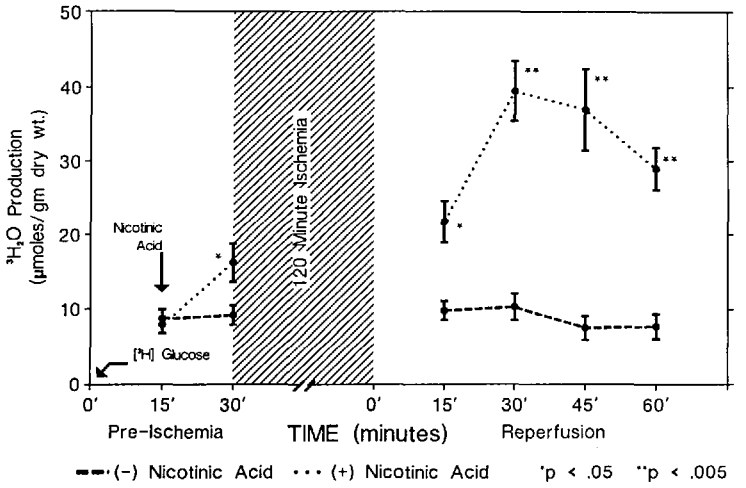

Fig. 1. ^3H_2O production as a manifestation of glycolysis during pre-ischemia and reperfusion, comparing animals receiving nicotinic acid pretreatment with control.

Results

Glucose utilization

The nicotinic acid-treated heart immediately showed a highly significant 16 % stimulation in glucose oxidation compared to the untreated control (Fig. 1). Following 120 min of cardioplegic arrest, the amount of ^3H_2O production rose in the NA group during 15 and 30 min of reperfusion, but not in control. Radioactive water production then fell slightly in both groups, such that after 60 min of reperfusion the untreated group returned to the control level of glucose utilization. The treated group continued to show high glucose utilization (80 % greater than pre-ischemia) even after 60 min of reperfusion.

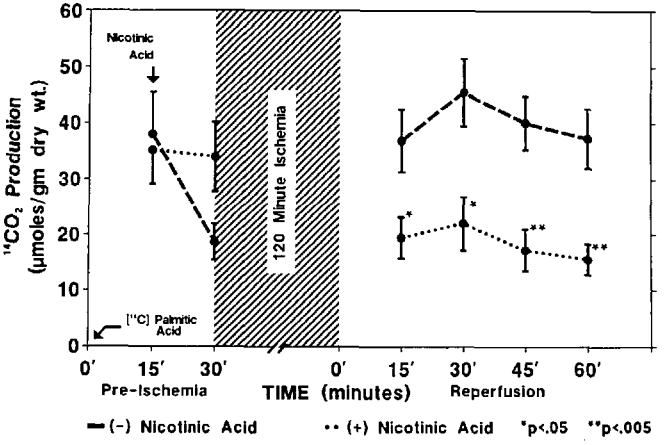

Fig. 2. $^{14}CO_2$ production, as a manifestation of β-oxidation, prior to ischemia and during reperfusion in animals receiving nicotinic acid pretreatment compared to control.

Table 1. Effect of nicotinic acid on CoA, acetyl CoA, carnitine, acetyl carnitine, long-chain acyl CoA, long-chain acyl carnitine, and reducing equivalents in heart during ischemia and reperfusion.

(μmol/gm d.w.)	Treatment	Control	Pre-ischemia	120 min ischemia	15 min reperfusion	60 min reperfusion
CoA	−NA	0.38 ± 0.01	0.36 ± 0.01	0.22 ± 0.02*	0.22 ± 0.02*	0.28 ± 0.01*
	+NA	0.38 ± 0.01	0.36 ± 0.01	0.24 ± 0.03*	0.27 ± 0.03*	0.33 ± 0.03
Acetyl CoA	−NA	0.15 ± 0.02	0.18 ± 0.02	0.10 ± 0.01	0.13 ± 0.01	0.14 ± 0.01
	+NA	0.18 ± 0.01	0.20 ± 0.02	0.13 ± 0.01*	0.14 ± 0.02	0.16 ± 0.01
Carnitine	−NA	3.76 ± 0.05	3.74 ± 0.11	2.10 ± 0.18*	2.28 ± 0.16*	2.68 ± 0.16*
	+NA	3.72 ± 0.11	3.89 ± 0.11	2.27 ± 0.25*	2.50 ± 0.26*	2.98 ± 0.24*
Acetyl carnitine	−NA	0.26 ± 0.01	0.24 ± 0.01	0.19 ± 0.03*	0.19 ± 0.02*	0.20 ± 0.01*
	+NA	0.26 ± 0.01	0.25 ± 0.02	0.21 ± 0.02*	0.22 ± 0.02	0.21 ± 0.01*
Long-chain acyl CoA	−NA	0.16 ± 0.01	0.16 ± 0.00	0.28 ± 0.01*	0.25 ± 0.01*	0.20 ± 0.01*
	+NA	0.16 ± 0.00	0.16 ± 0.00	0.24 ± 0.01*	0.21 ± 0.01*	0.18 ± 0.01*
Long-chain acyl carnitine	−NA	1.85 ± 0.07	1.85 ± 0.07	3.78 ± 0.14*	3.40 ± 0.14*	2.63 ± 0.15*
	+NA	1.85 ± 0.06	1.85 ± 0.07	3.30 ± 0.15*Δ	2.95 ± 0.14*Δ	2.11 ± 0.06*Δ
$\frac{NADH}{NAD}$	−NA	2.48 ± 0.05	2.42 ± 0.08	14.71 ± 0.84*	11.22 ± 0.84*	6.22 ± 0.51*
	+NA	2.48 ± 0.07	2.49 ± 0.07	14.54 ± 1.16*	10.90 ± 0.76*	5.46 ± 0.51*

* $p < 0.05$, compared to pre-ischemia
Δ $p < 0.05$, −NA vs +NA

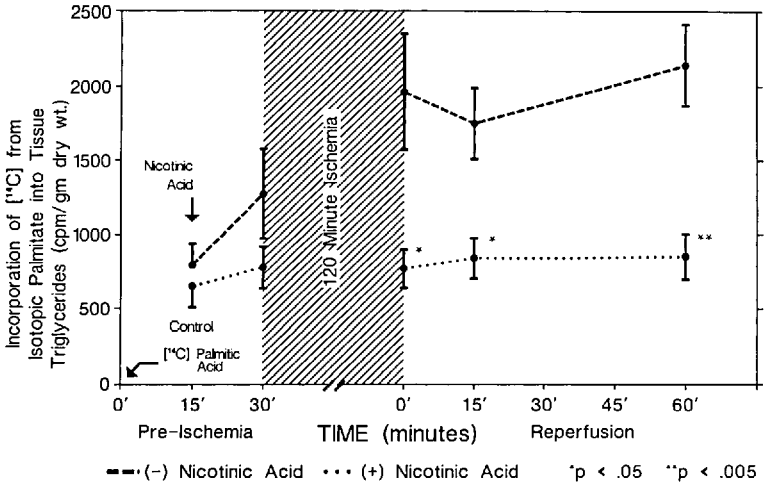

Fig. 3. The incorporation of radioactive carbon from palmitate into tissue triglycerides during pre-ischemia and reperfusion in animals receiving nicotinic acid compared to control.

Fatty acid utilization

$^{14}CO_2$ production was lowered by nicotinic acid 45 % compared to the untreated controls after just 15 min of preperfusion prior to arrest (Fig. 2). A comparable pattern of CO_2 production (fatty acid utilization or β-oxidation) persisted during reperfusion, such that even after 60 min of reperfusion $^{14}CO_2$ production in the nicotinic acid-treated group was 58 % lower compared to the untreated group. This clearly shows that nicotinic acid significantly lowers fatty acid oxidation which persists during reperfusion of ischemic myocardium.

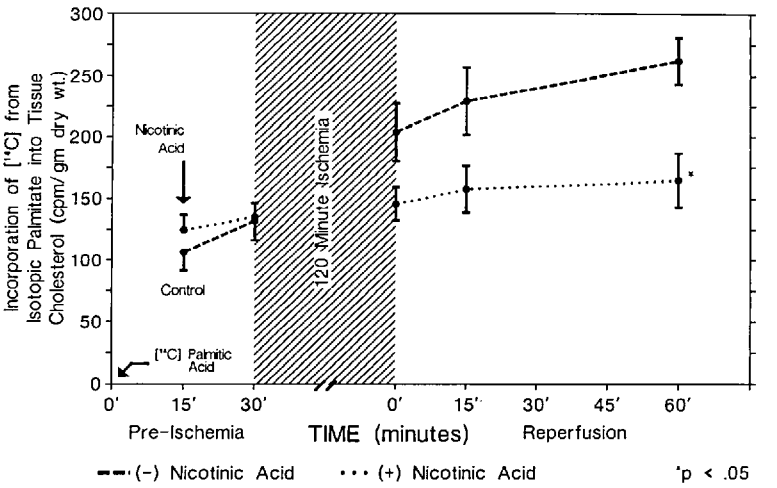

Fig. 4. ^{14}C-incorporation into cholesterol from radiol-labeled palmitate during pre-ischemia and reperfusion, comparing nicotinic acid-pretreated animals with control.

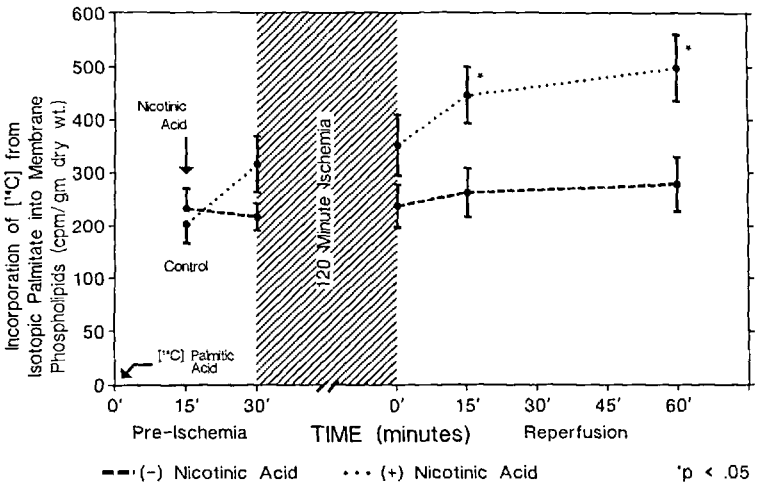

Fig. 5. ^{14}C-incorporation into membrane phospholipids from radioactive palmitate during pre-ischemia and reperfusion in animals treated with nicotinic acid compared to control.

The metabolites of the β-oxidation of fatty acids were measured from the treated and untreated groups. The addition of nicotinic acid had no effect on any of these metabolites prior to ischemia (Table 1). However, at the end of arrest and during reperfusion, nicotinic acid significantly lowered the levels of long-chain acyl CoA and long-chain acyl carnitine relative to the control group. The acetyl carnitine level was slightly increased during reperfusion as a result of nicotinic acid treatment, but the difference was not significant.

Recovery of (^{14}C)-label into various lipid fractions

Incorporation of (^{14}C)-label into the triglyceride pool showed a 65 % increase in the untreated group compared to the nicotinic acid-treated heart during the first 15 min of preperfusion prior to ischemia (Fig. 3). After 2 h of cardioplegic arrest incorporation of ^{14}C-label into triglyceride was increased to 156 % and remained elevated at 152 % following 60 min of reperfusion in the control compared to the NA group. Incorporation of ^{14}C-activity into triglyceride was significantly inhibited in the nicotinic acid group and remained unaltered after ischemic arrest and during reperfusion.

(^{14}C)-incorporation into cholesterol increased signficantly during ischemia and reperfusion in the absence of nicotinic acid (Fig. 4). Nicotinic acid treatment significantly reduced isotopic incorporation, such that no increase was noticed during ischemia and reperfusion.

(^{14}C)-incorporation into membrane phospholipids in the untreated animal did not increase after 120 min of arrest, but it gradually increased (n.s.) after 60 min of reperfusion (Fig. 5). In the nicotinic acid group, (^{14}C)-incorporation was enhanced by 37 % before and 49 % after ischemia, 80 % after 15 min of reperfusion, and 99 % after 60 min of reperfusion. There was a significant difference between the two groups.

Tissue content of free fatty acids and membrane phospholipids

In the untreated group, total FFA increased after cardioplegic arrest and during 60 min of reperfusion (Table 2), and nicotinic acid prevented these increases. Among the individual fatty acids, only $C_{20:4}$ (arachidonic acid) was increased significantly in both groups after 60

Table 2. Effect of nicotinic acid on the myocardial fatty acid compositions during ischemia and reperfusion.

Fatty acid (nmol/gm d.w.)	Treatment	Pre-ischemia	15 min preperfusion	120 min ischemia	15 min reperfusion	60 min reperfusion
C_{16}	−NA	405 ± 40	327 ± 34*	553 ± 105	528 ± 84	558 ± 89
Palmitic acid	+NA	412 ± 67	426 ± 90	355 ± 52	366 ± 56	325 ± 70
C_{18}	−NA	444 ± 42	441 ± 59	541 ± 50	613 ± 129	671 ± 117
Stearic acid	+NA	534 ± 79	434 ± 47	432 ± 75	403 ± 69	412 ± 64
C_{18}: 1	−NA	210 ± 21	151 ± 11	253 ± 63	257 ± 55	140 ± 24
Oleic acid	+NA	146 ± 24	203 ± 38	161 ± 26	195 ± 20	102 ± 25
C_{18}: 2	−NA	124 ± 17	110 ± 15	166 ± 40	142 ± 15	287 ± 97
Linoleic acid	+NA	86 ± 17	92 ± 13	101 ± 18	109 ± 20	187 ± 37
C_{20}: 4	−NA	118 ± 16	115 ± 12	129 ± 14	162 ± 21	240 ± 24*
Arachidonic acid	+NA	100 ± 20	108 ± 17	112 ± 19	150 ± 20	183 ± 49*
Total FFA	−NA	1410 ± 105	1238 ± 120	1741 ± 256	1792 ± 280	2003 ± 303
	+NA	1365 ± 152	1359 ± 184	1228 ± 139	1256 ± 195	1242 ± 195

* $p < 0.05$, compared to pre-ischemia

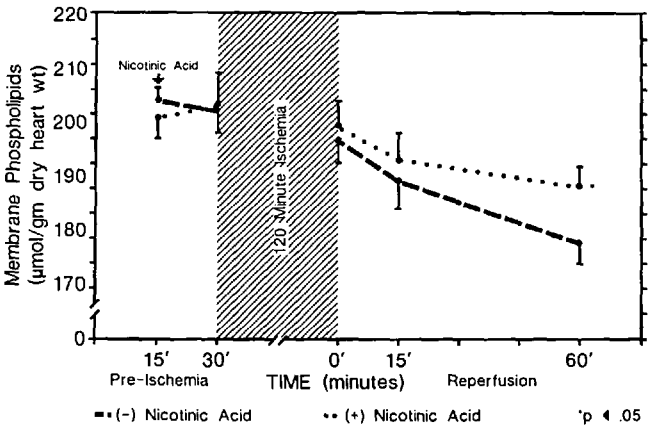

Fig. 6. The level of membrane phospholipids measured per gram dry weight in myocardial tissue prior to ischemia at the end of 2 h of ischemia and during reperfusion; comparison of animals pretreated with nicotinic acid with a control group.

min of reperfusion. While nicotinic acid lowered this response, the nicotinic acid-mediated inhibition of arachidonic acid production was not statistically significant.

In both groups, very little loss ($p > 0.1$) of membrane phospholipids was noticed immediately after 2 h of arrest (Fig. 6). However, after 15 min of reperfusion, phospholipid content was decreased by 7 % in control; after 60 min of reperfusion, it was reduced by 15 % ($p < 0.05$). In the nicotinic acid-treated group, phospholipid loss after 120 min of ischemia and 15 min of reperfusion was negligible, and it was only 7 % after 60 min of reperfusion (significantly less than the untreated group).

Myocardial recovery during ischemia and reperfusion

We monitored CK release, an indicator of tissue necrosis, and the cardiac contractility as well as coronary flow to judge the myocardial performance during nicotinic acid treatment. These measurements were not significantly altered during pretreatment with nicotinic acid before HCA (Table 3). Plasma CK levels were increased after reperfusion in nontreated and nicotinic acid-treated animals, respectively. There was an increase in CK release during the rest of the reperfusion period in nontreated animals, which was inhibited in hearts treated with nicotinic acid. Coronary flow decreased during reperfusion in nontreated animals, whereas in nicotinic acid-treated animals flow was maintained near the baseline level. There was a 40 % decrease in LV max dp/dt from baseline level 15 min after reperfusion in nontreated hearts. Treatment with nicotinic acid prevented this decline in LV max dp/dt significantly, compared to nontreated animals throughout the reperfusion period.

Discussion

Current knowledge suggests at least one feature common to the pathogenesis of both ischemic and reperfusion injury. Accumulation of fatty acids and their esters has been observed during ischemia (3, 29) as well as during reperfusion (4). These accumulated fatty acids and their esters have been shown to be a major cause of myocardial dysfunction (5, 13, 14). There are three known sources of free fatty acids in the ischemic-reperfused myocar-

Table 3. Effect of nicotinic acid on CK release from heart, myocardial contractility, and coronary blood flow during ischemia and reperfusion.

	Treatment	Control	Pre-ischemia	15 min reperfusion	30 min reperfusion	60 min reperfusion
CK release	(−) NA	203 ± 27	215 ± 20	223 ± 22	270 ± 35	269 ± 37
(IU/L)	(+) NA	187 ± 19	195 ± 18	191 ± 15	195 ± 7	215 ± 10
LV max dp/dt	(−) NA	1990 ± 145	1982 ± 38	1147 ± 37	1210 ± 27	1077 ± 37
(mm Hg/sec)	(+) NA	2015 ± 37	2038 ± 41	1730 ± 42*	1870 ± 29**	1800 ± 29*
Coronary flow	(−) NA	6.96 ± 0.45	6.95 ± 0.76	5.86 ± 1.06	5.20 ± 1.12	5.02 ± 1.25
(ml/min/100 gm)	(+) NA	8.13 ± 0.46	8.06 ± 0.83	8.16 ± 1.64	7.53 ± 0.72	8.31 ± 1.01

* $p < 0.05$
** $p < 0.005$

dium: 1) from inhibition of fatty acid oxidation; 2) from lipolysis of endogenous triglycerides; and 3) from the breakdown of membrane phospholipids. The present study was primarily designed to reduce ischemic-reperfusion injury by inhibiting endogenous lipolysis with a well-known antilipolytic drug, nicotinic acid. Another objective of this study was to use this antilipolytic drug to enhance the utilization of glucose relative to free fatty acid during cardioplegic arrest. The study was designed to preserve ischemic myocardium in the clinical setting of crystalloid cardioplegia, and in this study nicotinic acid successfully reduced free fatty acid as expected and protected the myocardium during both ischemic and reperfusion phases.

The concept of protecting an ischemic heart with nicotinic acid is not new. During past years, this antilipolytic agent was used to inhibit myocardial infarction. With nicotinic acid, Vik-Mo (30) observed improved coronary blood flow after ischemia, and Russell and Oliver (26) noticed transient reduction of ST segment elevation during ischemia in man. Rowe et al. used an analogue of nicotinic acid to control ventricular arrhythmias during myocardial infarction (25). Nicotinic acid is readily absorbed from all portions of the intestinal tract and then distributed to all tissues. Nicotinic acid is known to inhibit lipolysis in adipose tissue, decrease esterification of triglycerides in the liver, and increase the activity of lipoprotein lipase. The primary site of action may be at the fatty acid activation step or at the acyl carnitine transferase reaction. The use of systemic nicotinic acid for myocardial preservation has not been popular because of undesirable side-effects such as flushing, nausea, or vomiting at doses necessary to ensure continued control of fatty acids. The use of nicotinic acid as an agent during open heart surgery, however, seems to be highly promising, since it can be administered in high concentrations to the isolated coronary circulation.

During cardioplegic arrest both β-oxidation and anaerobic glycolysis are inhibited (17, 18). Accumulated fatty acids are known to increase myocardial oxygen demand (16). Theoretically, oxygen saving should be enhanced if myocardial glucose utilization can be stimulated relative to fatty acid oxidation. Using this concept, glucose-insulin-potassium (GIK) therapy was introduced initially by Sodi-Pallares (28), and was thereafter adapted by other investigators (19). This GIK therapy has never become popular because of the adverse effects of this therapy, particularly associated with hyperosmolarity and the risks of potassium manipulation (19). In the present study, nicotinic acid has been found to stimulate anaerobic glycolysis, although the mechanism behind it is not completely clear. The method used to measure glucose utilization was to monitor the rate of production of ^3H_2O during perfusion with (5-^{3}H) glucose. It has been shown that the theoretical and measured values of glucose utilization based on recovery of isotope from either (5-^{3}H) or (U-^{14}C) glucose are similar (8). We have used only a tracer amount of labeled glucose to monitor ^3H_2O formation. At this low concentration, the administered glucose does not have any effect on either glycolysis or fatty acid metabolism. In the untreated group, glucose utilization was restored within 15 min of reperfusion and remained unchanged during the 60 min reperfusion period. In the nicotinic acid group, however, the appearance of ^3H_2O increased two-fold within 15 min of preperfusion (Fig. 1). ^3H_2O concentration continued to increase up to 30 min of reperfusion following 2 h of arrest. Glucose utilization remained three-fold higher compared to the untreated control after 60 min of reperfusion.

Membrane-derived nonesterified fatty acids (NEFA) appear from the breakdown of phospholipids during ischemia and reperfusion. The recent observation by Chien et al. (3) and by van der Vusse et al. (29) of arachidonic acid accumulation in ischemic myocardium has been confirmed by our group (4, 9). The breakdown of membrane phospholipids becomes more apparent during early reperfusion of ischemic myocardium, resulting in significant amounts of NEFA accumulation (7, 20, 22). This degradation of membrane

phospholipids was accompanied by the accumulation of free fatty acids, especially arachidonic acid. However, the accumulated fatty acid could account for only a small fraction of the fatty acids derived from the breakdown of membrane phospholipids. At least three possibilities can be speculated for these discrepancies. First, the arachidonate is a well known substrate for the cyclooxygenase pathway. Significant amounts of prostaglandin and thromboxane production have been noticed during reperfusion of ischemic myocardium (22). Secondly, a portion of this fatty acid may be reincorporated into the tissue triglyceride as indicated from the present study. Finally, a significant amount of FFA, including arachidonate, may be released from the heart into the perfusate as metabolized or nonmetabolized fatty acids. Our results here have indicated that nicotinic acid significantly prevented degradation of membrane phospholipids during reperfusion. Nicotinic acid also prevented the arachidonate acid accumulation to some extent. However, the decrease in accumulation did not reach statistical significance, probably because of the large degree of variance. Nicotinic acid also enhanced the incorporation of (^{14}C)-label from isotopic palmitic acid into membrane phospholipids. Although the mechanism behind these interesting observations is not clear, some possibilities may be speculated. Nicotinic acid could enhance (^{14}C)-incorporation by stimulating the fatty acid activation step and removing the block in the reacylation of lysophosphoglycerides which normally occur during ischemia and reperfusion (7, 20, 22). It is also possible that nicotinic acid inhibits phospholipase by some unknown mechanism.

The second route through which fatty acid accumulates intracellularly is from the inhibition of fatty acid oxidation. The major regulatory factor for β-oxidation of fatty acids appears to be the concentration of FFA in the tissue. During ischemia, tissue levels of CoA, carnitine, acetyl CoA, and acetyl carnitine are lowered, with the concomitant increase in long-chain acyl CoA and long-chain acyl carnitine, suggesting that β-oxidation becomes rate-limiting for fatty acid oxidation (31). In the present study, nicotinic acid slightly increased acetyl carnitine but reduced the levels of long-chain acyl CoA and acyl carnitine significantly, even though net fatty acid oxidation was not enhanced. This would tend to suggest that acetyl CoA may be derived from enhanced glycolysis, and the primary site of action of nicotinic acid would be at the fatty acid activation step or at the acyl carnitine transferase reaction. A decrease in long chain esters of CoA and carnitine may at least partially explain improved myocardial function observed after ischemia and reperfusion during nicotinic acid therapy.

An alternative route of fatty acid accumulation in ischemic myocardium is via the stimulation of lipolysis of endogenous triglycerides releasing glycerol and free fatty acids within the ischemic myocardium (2). The immediate fate of released fatty acids is re-esterification to triglycerides, which is increased considerably during ischemia (27). Thus, it appears that the breakdown and resynthesis of triglycerides occur simultaneously in the ischemic myocardium. Our results demonstrated significant enhancement of (^{14}C) incorporation into the triglyceride pool in the untreated group. Nicotinic acid effectively inhibited the isotopic incorporation into tissue triglycerides by its antilipolytic effect and thus would be expected to block fatty acid accumulation.

Nicotinic acid provided protection to the ischemic-reperfused myocardium as shown by improved cardiac contractility. However, in this study, when there was no systemic circulation, uncontrolled activation of various reflexes triggered by end-organ system death may secondarily alter myocardial metabolism. Nevertheless, our study conclusively indicates myocardial preservation during reperfusion of ischemic myocardium. Among the many beneficial effects observed, the inhibition of endogenous lipolysis in conjunction with enhanced anaerobic glycolysis should warrant nicotinic acid being a promising agent for the protection of ischemic myocardium during open-heart surgery.

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