CONCLUSIONS

The kidney isolated *in situ* shows a good metabolizing power evaluated by studying the glucuronide conjugation of oxazepam and the demethylation and acetylation of aminopyrine, both in the dog and the monkey. The tested substances and their metabolites were present in the excreted urine and in the renal tissues. During the extracorporeal renal perfusion (from 1 to 2 hr.) no significant differences were observed between the initial and final values of plasma electrolytes concentrations and urine pH, flow, and electrolytes excretion.

In comparison with the metabolizing power of other organs isolated *in situ* the kidney shows a lower activity than the liver (1) and a higher activity than the brain (2).

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Keyphrases

Renal metabolism—*in situ* determination
Kidney, perfused—dog, monkey
Oxazepam—renal metabolism
Aminopyrine—renal metabolism

Influence of Calcium and Antiarrhythmic Drugs on Palmitate Uptake by Rabbit Heart Slices

By C. M. MOKLER* and P. P. MATHUR

Nonesterified fatty acids diffuse into myocardial cells at a rate which is proportional to the concentration of unbound acid, and are used in intracellular oxidative and synthetic reactions. Quinidine is known to inhibit cellular oxidative processes. The effects of quinidine, disopyramide, and pronethalol on oxygen consumption and palmitate uptake by rabbit ventricle slices were determined by standard Warburg manometric techniques. In the absence of Ca^{++}, all three drugs in concentrations of 10^{-5}–10^{-4} moles/l. induced marked increases in the rate of palmitate uptake from its albumin-bound form in incubation media without significant changes in tissue Q_{O_2}. This effect was not seen at drug concentrations of 10^{-6} or 10^{-3} moles/l., or when 2 m*M* Ca^{++} was present. A relationship is suggested between palmitate, Ca^{++}, antiarrhythmic drugs, and extracellular protein, which may involve the binding of fatty acid to the protein moiety.

PLASMA NONESTERIFIED FATTY ACIDS (NEFA) serve an important function in mammalian myocardial nutrition (1). They apparently diffuse across cell membranes and become available to cytoplasmic and mitochondrial enzyme systems in quantities which are related to the molar ratio of NEFA–plasma albumin (2) because of the reversible manner in which NEFA are bound to plasma albumin. Quinidine is also bound to plasma albumin (3), as is ionic calcium (Ca^{++}) (4), which is known to play an important role in cardiac function (5). Nayler (6) has shown an additional relationship between Ca^{++}, quinidine, and cell lipids. Quinidine will inhibit the movement of Ca^{++} from an aqueous phase into a chloroform phase which contains lipids extracted from myocardial membranes and

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microsomes. A similar effect was demonstrated with pronethalol and propranolol (7) which also possess antiarrhythmic properties. These findings suggest that the antiarrhythmic drugs may modify the affinity of membrane lipids for Ca^{++}, or that they may compete with these lipids for Ca^{++}.

The work described here will show a further interaction of quinidine, pronethalol, and disopyramide with myocardial cells which results in modified uptake of palmitate by these cells, and a reversal of the effect with extracellular Ca^{++}.

METHODS

Standard Warburg manometric techniques were employed. Healthy, male, nonfasting animals were used in this study. New Zealand white rabbits were stunned by blows on the head and hearts were removed and placed in chilled buffer without substrate. Thin longitudinal slices (0.5 mm.) were taken from ventricles with a Stadie-Riggs slicer. Slices were halved and distributed equally between control and drug-containing vessels. Each Warburg reaction vessel contained 150–200 mg. of tissue in buffer containing the drug but no substrate. Each experiment included a thermobarometric control flask (no tissue) and at least four control flasks which contained tissue and buffer but no drug. Calcium levels were the same in all flasks of a given experiment. The low calcium buffers contained 88 m*M* NaCl, 5 m*M* KCl, 2 m*M* $MgCl_2$, 40 m*M* Na_2HPO_4, and either no calcium or 0.2 m*M* $CaCl_2$. The high mmoles calcium buffer differed only by having 2 m*M* Na_2HPO_4, 2 m*M* $CaCl_2$, and 124 m*M* NaCl to keep ionic strength constant. The pH of each solution was adjusted with 1 *N* HCl to 7.4 before use. The substrate solution was prepared by adding warm sodium palmitate solution dropwise with stirring to a solution of bovine albumin (Cohn's fraction V[1]), to give 3 mg. palmitate per ml. of 16% albumin solution. This mixture was refrigerated overnight and filtered at least once before use. When added from the side arm of the Warburg flask, 0.5 ml. gave flask concentrations of 5–8 μmoles palmitate and 80 mg. albumin per 3 ml. of medium, or a molar ratio of about 5:1.

The reaction vessels were gassed for 5 min. with 100% oxygen, equilibrated for 5 min. at 37 ± 0.1° and, after initial manometer readings, substrate was added from the side arm. Manometer readings were made every 15 min. for 2 hr, but calculation of oxygen consumption was based on readings from the first hour.

At the end of the incubation period, flasks were immediately chilled in crushed ice. A 2.0-ml. aliquot of medium was quickly removed from each flask, the fatty acids were extracted, and the concentration of fatty acids in each extract was determined by the microtitration method of Dole and Meinertz (8). To calculate tissue uptake of palmitate, the quantity remaining in the medium of each control and experimental flask was compared with that remaining in the thermobarometric control flask.

Tissue slices were blotted, weighed, and dried overnight at 104° before the final weighing. Oxygen consumption was expressed as Q_{O_2}, or microliters of oxygen used per milligram dry weight of tissue per hour.

Drugs used were quinidine sulfate (USP crystals, Mallinckrodt), crystalline disopyramide,[2] and pronethalol.[3] Drug concentrations ranged from 10^{-6} *M* to 10^{-3} *M*.

The data from drug-treated heart slices were compared with those from untreated control slices by Fisher's *t* test, using the 5% significance level.

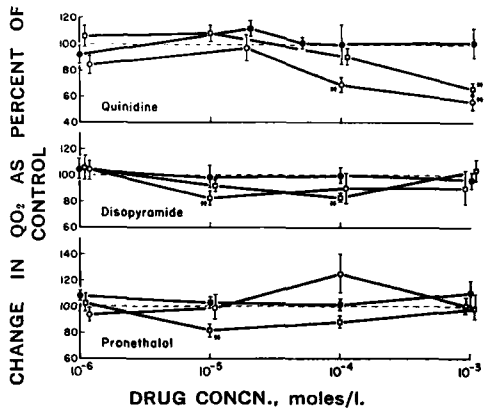

Fig. 1—Comparison of drug-treated heart slices with untreated control slices with respect to the oxygen consumption at varying concentrations of drugs and calcium ions. Asterisk (*) indicates significant difference from controls (p < .05). The vertical bar in each figure indicates the standard error of the change from control found with each treatment. Key: ●, No Ca; □, 0.2 mM Ca; ○, 02.0 mM Ca.

TABLE I—RESPONSE OF HEART SLICES TO CHANGES OF CALCIUM CONCENTRATION IN THE INCUBATION MEDIUM

Ca^{++} (m*M*)	$Q_{O_2} \pm S/E$	*p*	Palmitate Uptake, (μmol per g./hr.) ● S/E	*p*
0	5.6 ± 0.54(8)[a]		0.82 ± 0.20(8)	
0.2	4.0 ± 0.55(6)	>.05	0.75 ± 0.26(6)	>0.5
1.0	3.3 ± 0.29(6)	<.005[b]	0.66 ± 0.18(6)	>0.5
2.0	3.2 ± 0.42(6)	<.005[b]	0.60 ± 0.12(6)	>0.2

[a] Number of flasks. [b] Significant difference from control (0 calcium).

RESULTS

Figure 1 summarizes the effects of varying drug concentrations and Ca^{++} levels on the rate of oxygen consumption by rabbit ventricle slices. Significant changes in Q_{O_2} were found only with 2 m*M* Ca^{++} at quinidine concentrations of 10^{-6} to 10^{-3} *M*, and with 0.2 m*M* Ca^{++} only at 10^{-3} *M* quinidine. That this effect may be due to Ca^{++} is illustrated by Table I, which indicates a significant reduction in Q_{O_2} in untreated heart slices when incubated with 2 m*M* Ca^{++}. This reduction of Q_{O_2} was in general

[1] Palmitate and albumin from Nutritional Biochemicals Corporation.

[2] Kindly supplied by Dr. Donald L. Cook, G. D. Searle & Co., Chicago, Ill.

[3] Kindly supplied by Dr. Alex Sahagian-Edwards, Ayerst Laboratories, New York, N. Y.

not seen when disopyramide or pronethalol was present.

Figures 2–4 show how these factors altered the rate of disappearance of palmitate from the medium of Warburg flasks. The rate of palmitate uptake appears to be inversely related to the Ca^{++} concentration at the intermediate drug levels in each case, but is less dependent on Ca^{++} concentration at higher and lower concentrations of drugs. In the absence of drugs, palmitate uptake was not depressed by increasing Ca^{++} concentrations (Table I).

While reasonably small differences in Q_{O_2} and palmitate uptake were observed between control slices from any one heart, considerable variation between hearts was observed. Thus, the treated slices from any heart were compared only with untreated control slices from the same heart, and results are expressed as the observed change from control values.

Control studies revealed no significant differences between vessels containing 2 m*M* phosphate and those containing 40 m*M* phosphate with respect to either Q_{O_2} or palmitate uptake from the medium (Table II).

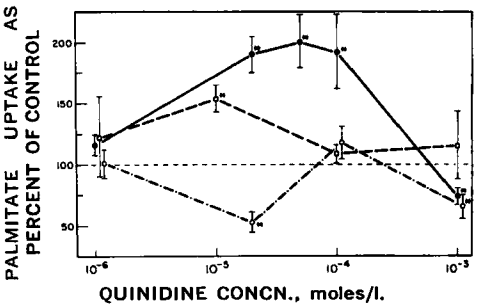

Fig. 2—Changes in palmitate uptake by heart slices as induced by quinidine at various levels of calcium. Changes in Q_{O_2} of these slices are shown in Fig. 1. Asterisk (*) indicates significant difference from controls ($p < .05$). *Key: ●, No Ca; □, 0.2 mM Ca; ○, 2.0 mM Ca.*

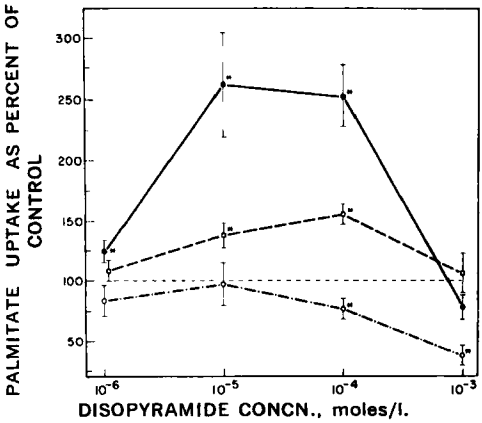

Fig. 3—Changes in palmitate uptake by heart slices as induced by disopyramide at various levels of calcium. Changes in QO_2 of these slices are shown in Fig. 1. Asterisk (*) indicates significant difference from controls ($p < .05$). *Key: ●, No Ca; □, 0.2 mM Ca; ○, 2.0 mM Ca.*

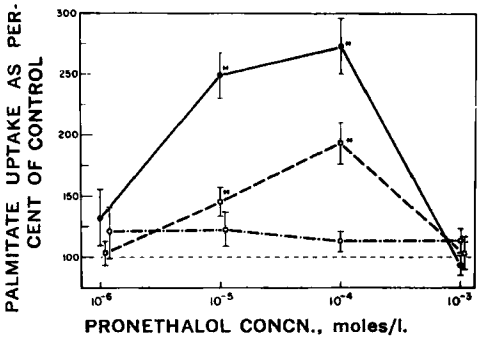

Fig. 4—Changes in palmitate uptake by heart slices as induced by pronethalol at various levels of calcium. Changes in Q_{O_2} of these slices are shown in Fig. 1. Asterisk (*) indicates significant difference from controls ($p < .05$). *Key: ●, No Ca; □, 0.2mM Ca; ○, 2.0 mM Ca.*

TABLE II—EFFECT OF PHOSPHATE CONCENTRATION ON Q_{O_2} AND PALMITATE UPTAKE BY RABBIT VENTRICLE SLICES (No Ca^{++})

Phosphate Concn.	*N*	Q_{O_2}	Palmitate Uptake (μmoles/g. tissue/hr.)
2 m*M*	6	8.2 ± 0.4[a]	2.90 ± 0.25
40 m*M*	6	8.4 ± 0.7	2.44 ± 0.30

[a] Mean ± *SE*.

DISCUSSION

As Evans (2) has indicated, the movement of fatty acids across cell membranes is probably a physical process since the rates of its loss from the medium (9) and its oxidation to CO_2 (10) appear to be dependent on the quantity of unbound or loosely bound NEFA in the medium. The experiments described here show that quinidine, disopyramide, and pronethalol will, at certain concentrations, enhance the rate of disappearance of palmitate from the medium in which rabbit ventricle slices are incubated. The effect is not present when calcium is added in concentrations (2 mmoles/l.) which approach the normal concentrations found in plasma. The increased rate of palmitate uptake is not accompanied by a parallel change in the rate of oxygen consumption by the ventricle tissue. Thus, although additional palmitate appears to be moving into the cells under these conditions, it must be used in nonoxidative or synthetic reactions of a nature which is not revealed by these experiments.

Several possible explanations of this phenomenon exist. One such explanation is related to the binding of fatty acids to plasma albumin. Goodman (11) has shown that palmitate is bound to plasma albumin in molar ratios of up to 27:1, with approximately 20 moles of palmitate per mole of protein having a relatively low association constant. If the antiarrhythmic drugs alter this binding to lower the association constant or to displace fatty acid from this binding, more NEFA will be available to be taken up by intacellular proteins (2). That quinidine is also bound to albumin (3) increases the plausibility of such an explanation, although there is no *a priori* reason to believe that albumin could not bind both fatty acid and quinidine independently.

At the higher drug concentrations where palmitate uptake is reduced to control values or below, explanations for the observed data become more speculative. It is possible that, even though palmitate is made more available for diffusion into the cells, less is taken up because of drug inhibition of intracellular enzyme systems which would normally utilize the fatty acid as a substrate in oxidative or synthetic reactions. The concentration gradient for diffusion of fatty acids into the cells would not be as great as normal under these circumstances. There is evidence that palmitate oxidation is inhibited (12) by quinidine in concentrations (greater than 10^{-4} *M*) at which the rate of uptake (Fig. 2) begins to diminish. Other workers have demonstrated inhibited oxidation of citric acid cycle intermediates (13, 14) in the presence of high concentrations of quinidine.

However, the rate of oxygen utilization (Fig. 1) did not diminish with increased quinidine concentration in those slices (no Ca^{++}) which showed the greatest change in fatty acid uptake. Nor was the Q_{O_2} much altered by disopyramide or pronethalol, both of which induced similar changes in palmitate uptake. These observations could mean that the rate of incorporation of the fatty acid into neutral lipid molecules is diminished by the higher concentrations of the drugs, but additional experiments are needed to support this theory. A more plausible explanation for the quinidine data is that quinidine inhibits endogenous substrate oxidation, but that the absence of calcium permits easier entry of palmitate into the cell where it is available for oxidation and prevents a fall in the oxygen consumption. This possibility also requires additional experimentation for support.

The role of Ca^{++} in cancelling the drug effect is also obscure. Ca^{++} itself is bound to plasma protein, to quinidine (15), and to fatty acids, and thus could significantly affect acid release from albumin in several ways.

A third possible explanation for the phenomenon may be seen in the alteration of cell-membrane permeability by Ca^{++} and the antiarrhythmic drugs. It is known that Ca^{++} and quinidine have definite effects on membrane electrical characteristics which are, in turn, dependent on the relative movements of ions across the membrane. It is possible that Ca^{++} and the antiarrhythmic drugs could alter the permeability of myocardial cell membranes to palmitate and exert their individual effects on NEFA uptake in this manner.

The alteration of palmitate uptake by heart slices which is caused by antiarrhythmic drugs in the absence of Ca^{++} ions reveals a relationship between the three factors which may have some bearing on the action of this class of drugs. The intracellular presence of quinidine and fatty acid is measurable and relatively constant during therapy, and sarcoplasmic Ca^{++} concentrations vary considerably during the cardiac cycle. If fatty acid binding to intracellular proteins varies inversely with the drug concentration, the presence of these drugs may alter the availability of the fatty acids for enzymatic processes, and thus affect the economy of the cell.

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Keyphrases

Palmitate uptake—rabbit heart slices
O_2 consumption—ventricle slices
Drug effect—O_2 consumption
Calcium effect—drug-altered palmitate uptake, O_2 consumption