The Effect of Hypoxia on Thallium Kinetics in Cultured Chick Myocardial Cells

Bruce J. Friedman, Robert Beihn, and Joan P. Friedman

Division of Cardiology, and Radiation Medicine, University of Kentucky Medical Center, Lexington, Kentucky

To assess the effect of hypoxia on cellular thallium-201 (^{201}Tl) uptake and washout independent of coronary flow, we studied thallium kinetics during normoxia and hypoxia in cultured chick ventricular cells. Monolayers of contracting ventricular cells grown on coverslips were placed in a chamber and perfused to asymptote with media containing ^{201}Tl. Perfusates were equilibrated with 5% CO_2-95% air or 5% CO_2-95% nitrogen for normoxia and hypoxia, respectively. Washout thallium kinetics were then observed during perfusion with unlabeled media. Twenty paired experiments were performed, randomly alternating the sequence of normoxia and hypoxia. Pharmacokinetics for thallium were determined by computer using standard formulae. Thallium uptake and washout were best described by assuming that intracellular thallium was contained within a single compartment. Cellular thallium uptake, as well as transfer rate constants for thallium uptake and for thallium washout during normoxia and hypoxia, were compared using paired t-tests. During normoxia and hypoxia, respectively, thallium uptake was 22 ± 7% and 19 ± 7% of asymptote ($p < 0.01$); the compartmental rate constant for uptake by the cell was 0.16 ± 0.07 min^{-1} and 0.15 ± 0.06 min^{-1} (N.S.); and the transfer rate constant for washout from the cell was 0.26 ± 0.06 min^{-1} and 0.23 ± 0.05 min^{-1} ($p < 0.01$). We conclude that there was a small (14%) decrease in thallium uptake during hypoxia. The rate of thallium uptake and washout was slightly less during hypoxia, although only the rate of washout was significantly less. These data show that cellular accumulation of thallium and the rate of washout of thallium were minimally decreased by hypoxia independent of blood flow.

J Nucl Med 28:1453–1460, 1987

Despite the widespread use of thallium-201 (^{201}Tl) as a myocardial perfusion imaging agent, the effects of hypoxia on cellular uptake and washout of thallium have not been well established. Removal of oxygen results in prompt alterations of cardiac electrolytes (*1–3*), including a fall in intracellular potassium levels within seconds of hypoxia (*4*). Like potassium, thallium is a monovalent cation that is, primarily, intracellularly localized (*5*). Both cations have similar ionic radii (*5*), and many physiological similarities have been demonstrated (*6–8*).

Previous attempts to characterize cellular uptake of thallium during hypoxia have all been limited by the presence of diffusion barriers and multiple extracellular compartments (*9–11*). These barriers complicate the determination of intracellular-extracellular ion exchanges in intact cardiac muscle. Many of these models are further complicated by increases or decreases in coronary flow (*12*), and by an inability to distinguish the effects of these changes in flow from changes in the intrinsic cellular ability to extract thallium. Thus, thallium kinetics during normoxia and hypoxia were studied in a monolayer of cultured chick ventricular cells to assess the effect of hypoxia on cellular thallium uptake and washout, independent of coronary flow and of diffusional limitations resulting from intervascular and interstitial compartments.

METHODS

Tissue Culture

Monolayer cultures of contracting chick embryo myocardial cells were prepared using the method of DeHann (*13*) with modifications as described by Barry et al. (*14*). Eight- to 10-day-old chick embryo hearts were removed under sterile conditions in a laminar flow hood. The ventricles were cut

Received Aug. 11, 1986; revision accepted Mar. 12, 1987.

For reprints contact: Bruce J. Friedman, MD, University of Kentucky Medical Center, Div. of Cardiology, Rm. MN-670, Lexington, KY 40536-0084.

into 0.5-mm fragments and placed in calcium- (Ca) and magnesium- (Mg) free Hanks' solution.* The ventricular fragments were gently agitated in 10 ml of 0.025% (wt/vol) trypsin in Ca- and Mg-free Hanks' solution, at 37°C for four 7-min cycles. The supernatent suspension containing the cells, decanted during each cycle, were then placed in 20 ml of cold trypsin inhibitor medium (50% heat inactivated fetal calf serum and 50% Ca- and Mg-free Hanks'). This suspension was centrifuged at 1,600 rpm for 10 min, the supernatent was discarded, and the cells were resuspended in culture medium consisting of 6% heat inactivated fetal calf serum, 40% medium 199*, 0.1% penicillin-streptomycin antibiotic solution, and 54% low-potassium salt solution containing: NaCl, 116 mmol; NaH_2PO_4, 1.0 mmol; $MgSO_4$, 0.8 mmol; KCl 1.8 mmol; $NaHCO_3$, 26.2 mmol; $CaCl_2$, 0.87 mmol; and glucose, 5.5 mmol. The cell suspension was diluted to 400,000 cells/ml and placed in sterile plastic culture dishes containing 25-mm circular glass coverslips. Cultures were incubated in a humidified 5% CO_2-95% air atmosphere at 37°C. Within 2–3 days there were confluent monolayers of cells attached to the coverslips. Seventy to 90% of the cells contracted synchronously.

Experimental Protocol

Two circular glass coverslips with monolayers of contracting cells were used to form two surfaces of a chamber.† The coverslips were separated by a 2.5-mm-thick silicone gasket that also maintained an internal seal. The coverslips and gasket were held together by an aluminum ring which had multiple ports, allowing for entry and exit of the perfusate. The chamber volume was 0.75 ml. A sodium iodide detector (3 in. × 3 in.), appropriately shielded with lead, monitored gamma and x-ray emission from the chamber; while a single channel spectrometer and scaler timer with automated printing were used to sequentially record the counts each minute.

The chamber and perfusion media were enclosed in a Lucite box with controlled temperature (37°C). The inlet to the perfusion chamber was connected to a syringe pump by high pressure tubing surrounded by polyethylene tubing. Media was infused at a constant rate of 0.76 ml/min. This media was composed of 94% balanced salt solution (see "Tissue Culture") and 6% fetal calf serum with no added glucose. In order to produce the normoxic and hypoxic conditions the perfusate, prior to infusion, was gassed for 1 hr with either 5% CO_2-95% air, or 5% CO_2-95% nitrogen, respectively. Prolonged gassing of these solutions with 5% CO_2 resulted in a pH of ~7.35. Similarly the space surrounding the high pressure and polyethylene tubing was also gassed with the appropriate mixture for normoxia or hypoxia.

After 1 hr of gassing, the perfusion medium was aspirated into a 50-ml glass syringe and infused into the chamber. The oxygen concentration of the effluent was monitored with a pH/gas analyzer model 213.‡ The desired oxygen concentration for hypoxia was <12 mmHg and for normoxia < 120 mmHg. Equilibration with hypoxic media for 30 to 40 min was required before the chamber effluent oxygen reached the desired range. After obtaining the desired oxygen concentration, infusion with thallium-201 (^{201}Tl)§ (0.5–1 Ci/ml) labeled media was begun. The thallium-201 count reached an asymptote within 30 min of infusion. Perfusion was then continued with unlabeled media, and the washout of thallium counts was monitored for 60 min. The same oxygen concentration was maintained during both infusion with the ^{201}Tl-labeled media, and washout with the unlabeled media, respectively.

To determine the characteristics of the perfusion chamber without myocardial cells, a set of ten experiments was performed. Infusion and washout were performed during normoxia (number = 5) and hypoxia (number = 5) using coverslips without myocardial cells. Twenty experiments were performed in a paired (normoxic and hypoxic) manner, with random alternation of the sequence of normoxia and hypoxia. To help ensure the responsiveness of the system to metabolic interventions, a series of experiments was performed with ouabain, a Na-K ATPase inhibitor. Paired experiments (n = 4) were done during normoxic conditions with and without ouabain in concentrations from 10^{-5} to $10^{-3}M$. The cells were observed at the end of each experiment to ensure that they remained adherent to the coverslips and continued to contract. If the cells were detached from the coverslip, the experiment was repeated.

After noting the small magnitude of change in thallium uptake (see "Results") during hypoxia, an attempt was made to more closely simulate the clinical situation during hypoxia by also allowing the pH to fall. Paired experiments (n = 4) were performed comparing normoxia ($pO_2 > 120$ mmHg, pH = 7.35) versus hypoxia and mild acidosis ($pO_2 < 12$ mmHg, pH = 7.10). The mild acidosis was created by addition of 1N HCl to the perfusion media. During exposure of cells to severe acidosis (pH = 6.5) cells ceased to contract.

Data Analysis

Thallium activity (counts) was plotted versus time (min) for the 60-min washout period (Figs. 1 and 2). Using manual sequential curve stripping (*15*) the experimentally obtained curves were easily resolved into multiple exponentials (Figs. 1 and 2). These findings were confirmed using the CSTRIP program (*16*) that also provided initial estimates of the elimination rate constant (λ_i) of the ith exponential term (min^{-1}), and of the coefficient (C_i) of the ith exponential term that represents counts of the ith exponential at time 0. Using these estimates, counts (C) at time (t) is described by:

$$C = \sum_{i=1}^{n} C_i e^{-\lambda_i t} \qquad \lambda_i \gg \lambda_{i+1}$$

At the start of washout (time = 0) C was set equal to the asymptote. The initial estimates were then used in the NONLIN program (*17*) for derivation of final estimates by nonlinear iterative least-squares curve fitting. For these estimates, thallium activity (counts) was weighted by the inverse of the square of the observed counts because of the wide range (two log cycles) of counts (*18,19*). Goodness of fit was assessed by visual inspection of residuals, by comparison of the experimental and calculated curves, as well as by examination of R^2, and the percent of the derived elimination rate constants (λ_1) and coefficients (C_i) (*19*).

The experimentally obtained curves were accurately described by two or three exponentials depending on the presence or absence of cells. Compartmental analysis theory (*20*) has shown that a system composed of at least a corresponding number of compartments may be used to model the thallium kinetics.

In accordance with the experimental design, elimination occurs from the central compartment (Compartment 1) that represents media in the chamber. The model assumes that the

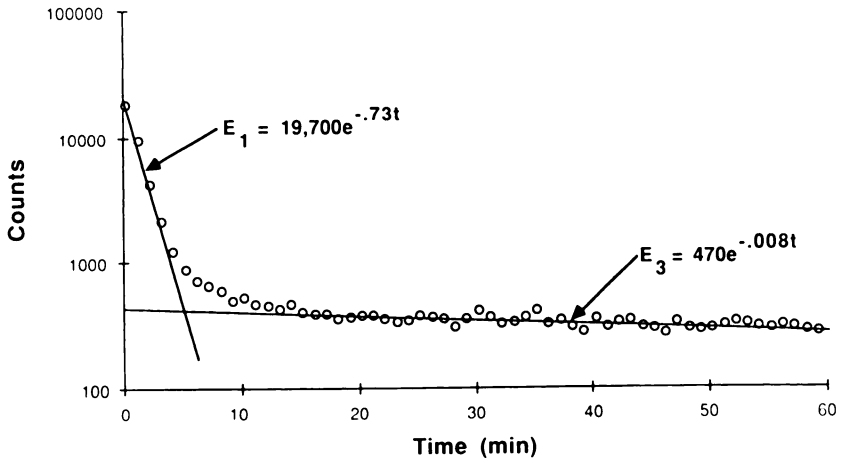

FIGURE 1
Sample plot showing washout of thallium (counts) from the chamber without cells. The two exponentials (E_1 and E_3) derived by the NONLIN program to describe the experimental data are shown. E_1, E_3 = exponentials describing thallium washout from Compartment 1 (media) and Compartment 3 (nonspecific binding to glass and gasket), respectively; t = time.

central compartment is also reversibly connected to both "shallow" or cellular, and "deep" or noncellular (glass and silicone gasket) peripheral compartments, numbers 2 and 3, respectively (Fig. 3). Once C_1 and λ_1 are known, the individual intercompartmental transfer rate constants (k_{12}, k_{21}, k_{31}, and k_{13}) may be calculated by standard formula (*21*). Each rate constant (k_{ij}) represents the fraction of activity exchanged from Compartment i to Compartment j per unit time.

Cellular thallium uptake during normoxia and hypoxia was compared during each paired experiment as a fraction of asymptote. This was calculated as C_2 divided by C. Comparing the cellular thallium uptake in this manner enabled the comparison of relative cellular thallium uptake during normoxia and hypoxia without correction for ^{201}Tl decay (70-hr half-life), differences in thallium activity, or for variations in the number of myocardial cells on each set of coverslips. Uptake by the "deep" peripheral compartment was calculated in a similar manner, dividing C_3 by C. The decrease in cellular thallium uptake for each intervention (hypoxia, ouabain, hypoxia, and mild acidosis) was calculated as the difference between control C_2/C and intervention C_2/C divided by control C_2/C.

Statistical Analysis

Two-way analysis of variance for four groups was used to compare uptake and pharmacokinetic parameters of the chamber both with and without cells, during normoxia and hypoxia. The Student's t-test for paired data (normoxia versus hypoxia) was used to test for significant differences in cellular thallium uptake (C_2/C), the cellular elimination constant (λ_2), and the rate constants (k_{12}, k_{21}) for transfer between the cellular and central compartments.

RESULTS

Typical plots of thallium washout from the chamber without cells (Fig. 1) and with cells (Fig. 2) are illustrated on a semi-log scale. Using manual sequential curve stripping (*15*) and CSTRIP (*16*), the washout curve for the chamber with cells was easily resolved into three exponential curves; while the washout curve for the chamber without cells was easily resolved into two exponential curves. Shown in Figures 1 and 2 are the computer derived exponential curves E_1, E_2, and E_3 ($E_i = C_i e^{-\lambda_i t}$) representing the central (chamber volume), cellular, and noncellular (chamber walls) compartments, respectively. Also illustrated in Figure 2 is the close agreement between both the observed and calculated data points.

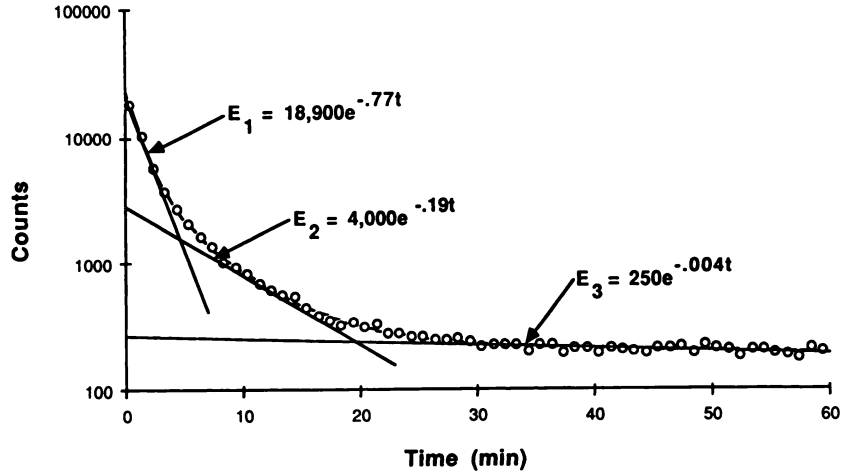

FIGURE 2
Sample plot showing washout of thallium (counts) from the chamber with cells. The three exponentials (E_1, E_2, and E_3) derived by the NONLIN program to describe the experimental data are shown. The broken line connecting the circles represents the thallium counts (C) as calculated by the model, where $C = E_1 + E_2 + E_3$. E_1, E_3, and t as in Figure 1. E_2 = exponential describing cellular thallium washout.

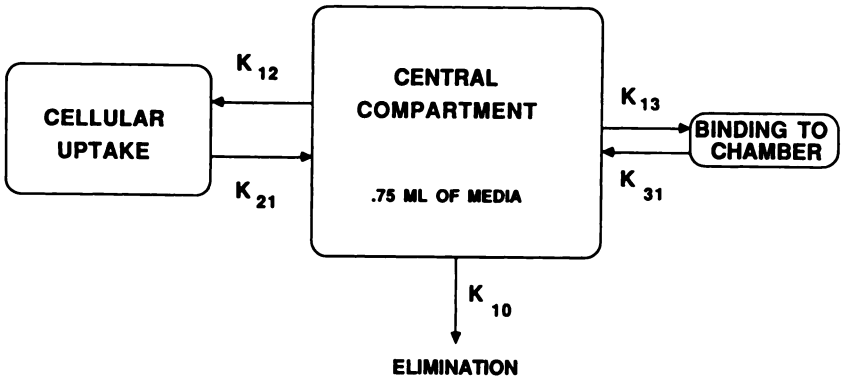

FIGURE 3
This three-compartment model accurately described thallium distribution. The central compartment represents media (0.75 ml) within the chamber. The peripheral cellular compartment represents cellular uptake. The peripheral noncellular chamber represents nonspecific binding to the glass and silicone gasket. In accordance with the experimental design, elimination occurs from the central compartment. Intercompartmental transfer rate constants are symbolized by k_{ij}.

Cells were no longer attached to the coverslips and noncontractile in only three experiments that were repeated. Cells were also noted to be noncontractile during hypoxia and severe acidosis (pH = 6.5). Observed data from the experiments that were successfully completed with contractile cells correlated very closely, 1.0 ± 0.0004 with the calculated data. Visual examination of these data showed a random distribution of weighted residuals.

Comparison of Chamber With and Without Cells

The fractional uptake by each peripheral compartment, and the intercompartmental transfer rate constants for experiments with and without cells are shown in Table 1. Note, there was no significant difference in the transport rate constant from the central compartment k_{10}, regardless of either the presence or absence of cells. This rate constant was also unaffected by the oxygen concentration. Relative uptake (C_3/C) and transport rate constants both into (k_{13}) and out of ($k/_{31}$) the noncellular compartment were also unaffected by either the oxygen concentration, or the presence or absence of cells. The peripheral compartment, represented by the second exponential (E_2), was only observed when cells were present. It followed that the presence of this additional exponential reflected a cellular effect.

Differences in Cellular Thallium Distribution During Normoxia and Hypoxia

The measured oxygen concentration during hypoxia was 9 ± 3 mmHg (mean ± s.d.). The cellular elimination rate constants (λ_2) for normoxia and hypoxia were 0.16 ± 0.02 min^{-1} and 0.14 ± 0.02 min^{-1} ($p < 0.01$), respectively. The relative uptake and transport rate constants for the "shallow" or cellular compartment are shown in Table 1. Thallium uptake C_2/C during normoxia and hypoxia was 0.22 ± 0.07 and 0.19 ± 0.07 of asymptote ($p < 0.01$), respectively (Fig. 4). The rate constants for cellular uptake (k_{12}) of thallium during normoxia and hypoxia were 0.16 ± 0.07 min^{-1} and 0.15 ± 0.06 min^{-1} (N.S.), respectively (Fig. 5). There was a small but significant difference ($p < 0.01$) in the rate constants for cellular thallium washout (k_{21}) during normoxia and hypoxia 0.26 ± 0.06 min^{-1} and 0.23 ± 0.05 min^{-1}, respectively (Fig. 6).

The addition of ouabain resulted on a dose dependent decrease in cellular thallium uptake. The decrease in cellular thallium uptake compared with control was 31 ± 9%, 42 ± 5% and 61 ± 2% at $10^{-5}M$, $10^{-4}M$, and $10^{-3}M$ ouabain, respectively. Thallium uptake C_2/C during normoxia and hypoxia along with mild acidosis was 0.23 ± 0.06 and 0.20 ± 0.07, respectively. This 13% decrease in cellular thallium uptake during hy-

TABLE 1
Values of Transport Rate Constants k_{ij} (min^{-1}) and Relative Compartmental Sizes (C_i/C)

	Without cells		With cells		Analysis of variance
	Normoxia	Hypoxia	Normoxia	Hypoxia	
k_{10}	0.17 ± 0.05	0.16 ± 0.06	0.22 ± 0.08	0.19 ± 0.05	$p > 0.1$ (N.S.)
k_{13}	0.21 ± 0.08	0.20 ± 0.07	0.26 ± 0.08	0.27 ± 0.07	$p > 0.1$ (N.S.)
k_{31}	0.016 ± 0.007	0.014 ± 0.005	0.020 ± 0.005	0.018 ± 0.005	$p > 0.1$ (N.S.)
C_3/C	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.01	$p > 0.1$ (N.S.)
					Paired t-test
k_{12}			0.16 ± 0.07	0.15 ± 0.06	N.S.
k_{21}			0.26 ± 0.06	0.23 ± 0.05	$p < 0.01$
C_2/C			0.22 ± 0.07	0.19 ± 0.07	$p < 0.01$

1 = Central compartment; 2 = Cellular compartment; 3 = Nonspecific binding.

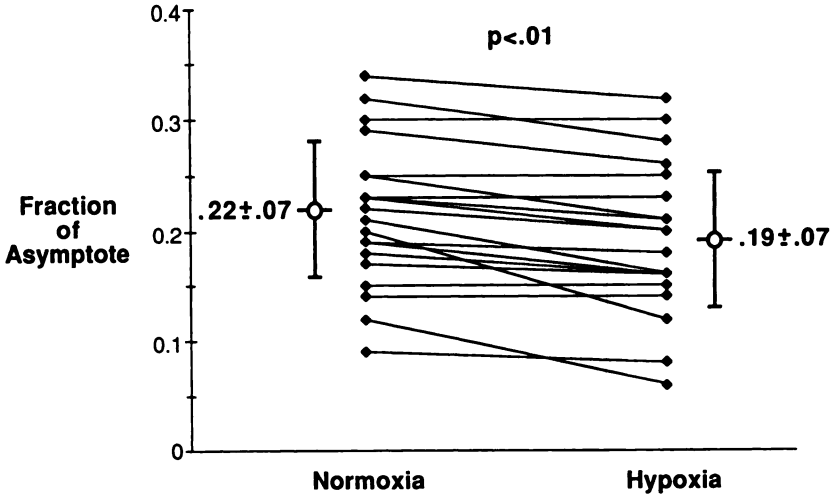

FIGURE 4
Cellular thallium uptake expressed as a fraction of asymptote during normoxia and hypoxia.

poxia and mild acidosis is nearly identical to the 14% decrease during hypoxia alone.

DISCUSSION

This study using monolayers of cultured chick myocardial cells, suggests that intracellular thallium is contained within a single compartment. A decrease in oxygen concentration was shown to decrease the intracellular thallium content by a small but statistically significant percentage. Rates of uptake and washout of thallium were slightly less during chronic hypoxia, although only the rate of washout was significantly less. Thus, hypoxia alone has a surprisingly small effect on thallium transport. This study is the first, to our knowledge, to demonstrate an effect of hypoxia albeit small on cellular thallium uptake independent of flow and of intervascular or interstitial diffusional limitations.

Cellular Compartmentalization

McCall et al. (*22*) using cultured rat myocardial cells also reported monoexponential uptake and washout of thallium most consistent with localization of intracellular thallium in a single compartment during normoxia. They reported the elimination rate constant (λ_2) of thallium to be 0.14 ± 0.01 min^{-1} versus 0.16 ± 0.02 min^{-1} in this study. Goldhaber et al. (*11*) reported biexponential tissue washout due to an extracellular and cellular related compartment. Llaurado et al. (*9*) studied thallium kinetics in isolated rat myocardial tissue and proposed an extracellular, main intracellular, and subcellular compartment for thallium. They reported enlargment of the subcellular space and increased thallium influx into this space after exercise. Similarly, Krivokapich et al. (*10*) in the isolated rabbit ventricular septum found rapidly and slowly exchanging cellular compartments. These discrepancies may be

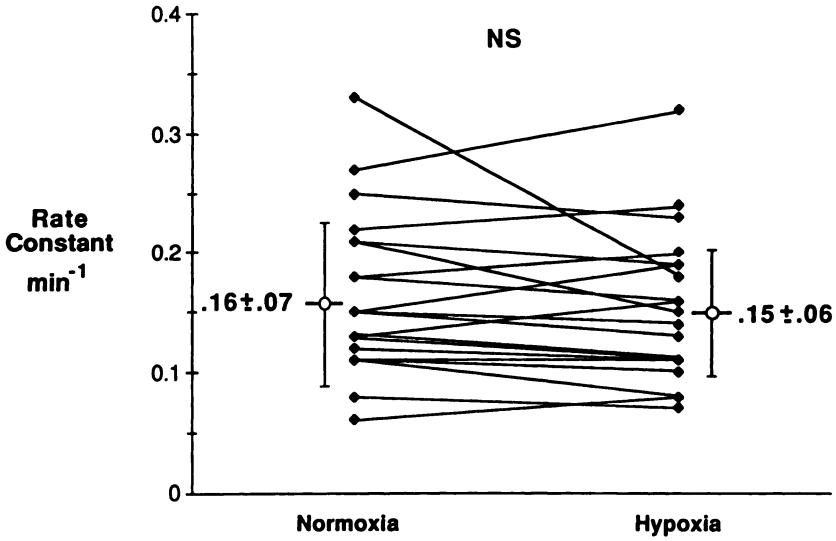

FIGURE 5
Transport rate constant (k_{12}) expressed as min^{-1} for cellular thallium uptake during normoxia and hypoxia.

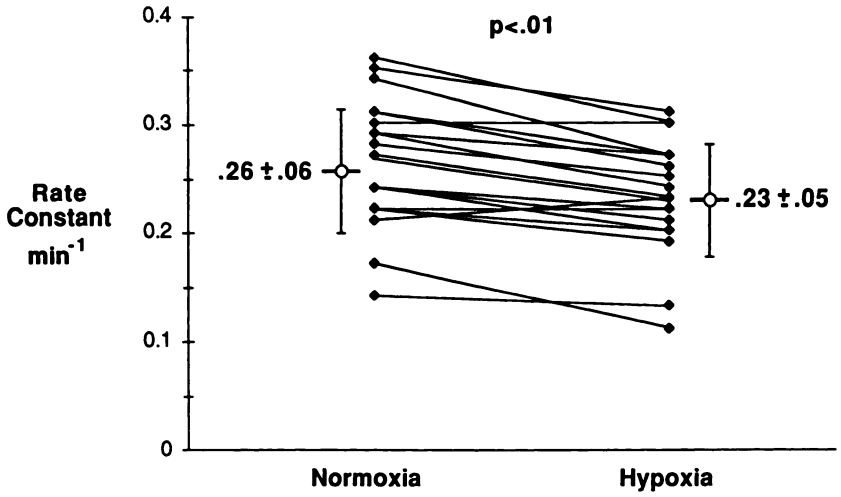

FIGURE 6
Transport rate constant (k_{21}) expressed as min^{-1} for cellular thallium washout during normoxia and hypoxia.

due to difficulties in distinguishing compartments with similar elimination rate constants (λ_i) as well as to differences in the models used in these studies.

Effects of Hypoxia and Ischemia on Cellular Thallium Uptake

Previous studies (*12,23*) have suggested that initial myocardial uptake of thallium primarily reflects coronary blood flow and cellular viability. This premise forms the basis of clinical imaging. Weich et al. (*12*) reported that thallium uptake in dogs was minimally affected by hypoxia. They found a small (11%) decrease in the extraction fraction of thallium along with a 70% increase in mean coronary flow at an oxygen concentration of 30 mmHg. They also reported that increased coronary flow in excess of myocardial demands was associated with a decrease in extraction fraction making it difficult to separate the effects of coronary blood flow and intrinsic cellular activity.

Goldhaber et al. (*11*) using fetal mouse hearts demonstrated a nonsignificant reduction in thallium content relative to control after 60 min of ischemia, followed by a progressive decrease in thallium content during ischemia for up to 10 hr. At 3 hr thallium content in isolated hearts was 64% of that in control hearts. Our study showed only a 14% decrease (0.22 versus 0.19 of asymptote) in cellular thallium uptake after 60 min of hypoxia (oxygen concentration gradually decreasing for 30 min and then maintained <12 mmHg for 30 min) and similarly a 13% decrease (0.23 versus 0.20 of asymptote) in cellular thallium uptake during hypoxia and mild acidosis. Therefore, during prolonged hypoxia, these findings are concordant with those of Goldhaber et al. during ischemia. Leppo et al. (*24*) using a dual isotope technique in isolated rabbit hearts reported that peak myocardial thallium extraction decreased insignificantly during hypoxia but increased significantly during ischemia.

Rate of Thallium Washout and Uptake

The suppression of the rate of thallium washout during chronic hypoxia was concordant with clinical observations of redistribution and other published data (*25,26*). Grunwald (*25*) reported delayed intrinsic myocardial cellular thallium washout during persistently diminished perfusion in dogs. Okada (*26*) reported more rapid thallium loss from the normal as compared with the reduced flow zone as the mechanism of redistribution in dogs with severe flow reduction.

Krivokapich et al. (*27*) used the isolated rabbit interventricular septum to demonstrate that perfusion with media equilibrated with 98% N_2-2% CO_2 for 20 to 40 min caused a decrease in thallium tissue uptake due to an increased efflux of thallium with a smaller increase in thallium influx. During 40 and 60 min of perfusion with anoxic media, the increased efflux of thallium was reversed and the increase in thallium influx was absent. Our experimental design did not permit the evaluation of the effects of hypoxia on thallium kinetics during the first 40 min of hypoxia. However, our data do concur with the findings as reported by Krivokapich (*27*) for the period between 40 and 60 min.

Methodological Considerations

The use of monolayers of cells allows direct study of cellular metabolism without the added complexities of interstitial and vascular spaces. On the other hand, the presence of diffusion barriers to oxygen in "intact tissues" as the superfused papillary muscle or arterially perfused muscle result in the effects of hypoxia being evident with less stringent reductions in perfusate oxygen. There is some evidence (*28–30*) that due to an enhanced capacity for metabolism via anaerobic glycolysis, cultured heart cells may be resistant to the effects of hypoxia. The level of hypoxia (<12 mmHg) used in this experiment was selected because it has been shown to be the critical oxygen concentration at which

contractile function was impaired in these cells (*14*). This was also the oxygen concentration recorded in the midwall of the left ventricle of dogs subjected to critical coronary artery stenosis (*31*). The difficulty obtaining low oxygen concentrations rapidly with this model limits its use in studies of acute hypoxia.

The cultured cell model has been more conducive to the study of hypoxia than the complex phenomenon of ischemia. These experiments were designed for the study of hypoxia. However, an ischemia-like state in cultured myocardial cells can be produced by markedly limiting the volume of extracellular medium combined with total oxygen and glucose deprivation (*32*). Previous studies have demonstrated that factors secondary to in vivo hypoxia as the intracellular accumulation of free fatty acids (*33*) and the development of acidosis (*34*) were more injurious to cultured rat myocardial cells than hypoxia alone. Several studies (*24,27*) have demonstrated differences in the effects of hypoxia and ischemia on thallium exchange. In this study there was no apparent difference in the effects of hypoxia versus hypoxia combined with mild acidosis on cellular thallium uptake. These results would not be applicable to a more severe acidosis.

Clinical Relevance

Numerous questions remain to be answered before thallium kinetics are fully understood and can be monopolized to improve the accuracy of thallium imaging, including tomography and quantitative analysis. Ischemia during thallium scintigraphy results both in depressed total accumulation (*12,25*) and in delayed disappearance (*25,26*) of thallium. Similarly, the present study is the first to demonstrate a small decrease both in cellular thallium uptake and washout during chronic hypoxia without the confounding effects of extracellular compartments. The 14% decrease in cellular thallium uptake observed in this study during hypoxia does not account for clinically observed perfusion defects. Certainly this effect is insignificant compared with that of reduced myocardial perfusion. In an individual human study, for example, if determination of thallium transport were to be used in an attempt to determine hypoxic alteration of cell membrane transport, the effects would be virtually unobservable and probably overwhelmed by flow-related effects. However, if thallium is to be used in a quantitative manner or for the study of cellular viability or complex phenomena as reperfusion, chronic ischemia, and myocardial infarction, these basic changes need to be considered.

NOTES

* Grand Island Biological Co., Grand Island, NY.
† Sykes-Moore Chamber, Bellco Glass, Vineland, NY.
‡ Instrumentation Laboratory, Lexington, MA.
§ Medi-Physics, Emeryville, CA.

ACKNOWLEDGMENTS

The authors thank Darleen Chamberlain and Jo Ann Earnest for their expert secretarial assistance in the preparation of the manuscript.

This study was supported by a grant from the American Heart Association, Kentucky Affiliate and the College of Medicine PSP Fund, Lexington, Kentucky.

REFERENCES

1. Lowry OH, Krayer O, Hastings AB, et al. Effect of anoxemia on myocardium of isolated heart of the dog. *Proc Soc Exptl Biol Med* 1972; 49:670–674.
2. Sybers HD, Helmer PR, Murphy QR. Effects of hypoxia on myocardial potassium balance. *Am J Physiol* 1971; 220:2047–2050.
3. Hill JL, Gettes LS. Effect of acute coronary occlusion on local myocardial extracellular K activity in swine. *Circulation* 1980; 61:768–778.
4. Rau EE, Shine KI, Langer GA. Potassium exchange and mechanical performance in anoxic mammalian myocardium. *Am J Physiol* 1977; 232:H85–H94.
5. Mullins LJ, Moore RD. The movement of thallium ions in muscle. *J Gen Physiol* 1960; 43:759–773.
6. Gehring PJ, Hammond P. The uptake of thallium by rabbit erythrocytes. *J Pharmacol Exp Ther* 1964; 200:215–221.
7. Britten JS, Blank M. Thallium activation of the Na,K activated ATPase of the rabbit kidney. *Biochem Biophys Acta* 1968; 159:160–166.
8. Gehring PJ, Hammond P. The interrelationship between thallium and potassium in animals. *J Pharmacol Exp Ther* 1968; 155:187–201.
9. Llaurado JG, Smith GA, Madden JA, et al. Partition of thallium-201 in isolated myocardial tissue of rats previously injected at rest or after exercise. *J Nucl Med* 1979; 20:1136–1141.
10. Krivokapich J, Shine KI. Effects of hyperkalemia and glycoside on thallium exchange in rabbit ventricle. *Am J Physiol* 1981; 240:H612–H619.
11. Goldhaber S, Newell JB, Alpert N, et al. Effects of ischemic-like insult on myocardial T1-201 accumulation. *Circulation* 1983; 67:778–786.
12. Weich H, Strauss H, Pitt B. The extraction of T1-201 by the myocardium. *Circulation* 1977; 56:188–191.
13. DeHaan RL. Regulation of spontaneous activity and growth of embryonic chick heart cells in tissue culture. *Develop Biol* 1967; 16:216–249.
14. Barry WH, Pober J, Marsh J, et al. Effects of graded hypoxia on contraction of cultured chick embryo ventricular cells. *Am J Physiol* 1980; 239:H651–H657.
15. Wagner JG. Fundamentals of clinical pharmacokinetics. Hamilton, IL: Drug Intelligence Pub., 1975: 59.
16. Sedman AS, Wagner JG. CSTRIP, a Fortran IV computer program for obtaining initial polyexponential parameter estimates. *J Pharm Sci* 1976; 65:1006–1010.
17. Metzler CM, Elfring GL, McEwan AJ. A package of computer programs for pharmacokinetic modeling. *Biometrics* 1974; 30:562–580.
18. Wagner JG. Fundamentals of clinical pharmacokinetics. Hamilton, IL: Drug Intelligence Pub., 1975: 288.
19. Boxenbaum HG, Riegelman S, Elashoff RM. Statistical estimations in pharmacokinetics. *J Pharmacokinet Biopharm* 1974; 2:123–148.

20. Berman M, Schoenfeld R. Invariants in experimental data on linear kinetics and the formulation of models. *J Appl Phys* 1956; 27:1361–1370.
21. Loo TL, Tanner B, Housholder G, et al. Some pharmacokinetic aspects of 5-(dimethyltriazeno)-imidazole-4-carboxamide in the dog. *J Pharm Sci* 1968; 57:2126–2131.
22. McCall D, Zimmer LS, Katz A. Kinetics of thallium exchange in cultured rat myocardial cells. *Circ Res* 1985; 56:370–376.
23. Strauss HW, Harrison K, Langan JK, et al. Thallium-201 for myocardial imaging: relation of thallium-201 to regional myocardial perfusion. *Circulation* 1975; 51:641–645.
24. Leppa JA, Moring AF. The effects of reperfusion, ischemia and on myocardial thallium extraction [Abstract]. *Circulation* 1985; 72:III-467.
25. Grunwald A, Watson D, Holzgrefe H, et al. Myocardial thallium kinetics in normal and ischemic myocardium. *Circulation* 1981; 64:610–617.
26. Okada R, Leppo J, Strauss W, et al. Mechanisms and time course for the disappearance of thallium-201 defects at rest in dogs. *Am J Cardiol* 1982; 49:699–706.
27. Krivokapich J, Watanabe CR, Shine KI. Effects of anoxia and ischemia on thallium exchange in rabbit myocardium. *Am J Physiol* 1985; 249:H620–H628.
28. Acosta D, Puckett M. Ischemic myocardial injury in cultured heart cells preliminary observations on morphology and beating activity. *In Vitro* 1977; 13:818–823.
29. Martinez TT, Walker JA. The actions of ouabain dinitrophenol, anoxia, and ionic manipulation on cultured heart cells. *Proc West Pharmacol Soc* 1977; 20:269–273.
30. Harary I, Slater E. Studies in vitro on single beating heart cells. The effect of oligomycin, dinitrophenol and ouabain on the beating rate. *Biochem Biophys Acta* 1965; 99:227–233.
31. O'Riordan JG, Flaherty JH, Khuri SF, et al. Effect of atrial pacing on regional myocardial gas tensions with critical coronary stenosis. *Am J Physiol* 1977; 232:H49–H53.
32. Vemuri R, Heller M, Pinson A. Studies of oxygen and volume restriction in cultured cardiac cells. II. The glucose effect. *Basic Res Cardiol* 1985; 80 (suppl 2):165–168.
33. Acosta D, Wenzel D. Injury produced by free fatty acids to lysosomes and mitochondria in cultured heart muscle and endothelial cells. *Atherosclerosis* 1974, 20:417–426.
34. Wenzel D, Acosta D. Labilization of lysosomes and mitochondria in situ by hypoxia related factors. *Res Commun Chem Path Pharmacol* 1975; 12:173–176.