Effect of Metabolic Inhibition on Technetium-99m-MIBI Kinetics in Cultured Chick Myocardial Cells

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Cellular uptake characteristics of hexakis(methoxyisobutylisonitrile)technetium(I) ([^{99m}Tc]MIBI), a myocardial perfusion imaging agent, were evaluated in cultured chick embryo heart cells. Myocyte net uptake of ^{99m}Tc-MIBI approached a plateau with a half-time of 9.3 ± 1.5 min (mean ± s.e.m.; n = 10). Tracer [^{99m}Tc]MIBI showed apparent competitive displacement by carrier [^{99}Tc]MIBI at relatively high molar ratios ([^{99m}Tc]MIBI/[^{99}Tc]MIBI) indicating a low affinity cellular retention process (apparent $K_D \sim 7 \times 10^{-5}$). Metabolic inhibition induced by pre-incubation of cells for 2.5 hr in rotenone (10 μM), iodoacetate (1 mM), or both metabolic inhibitors together reduced 1-min [^{99m}Tc]MIBI uptake to 74.1% ± 8.0% ($p < 0.05$), 6.2% ± 3.4% ($p < 0.01$), and 10.1% ± 3.6% of control ($p < 0.01$), respectively (n = 11–12). Half-maximal inhibitory concentration of iodoacetate was ~5 μM. Iodoacetate inhibition of [^{99m}Tc]MIBI uptake kinetics was time-dependent; no significant effect on [^{99m}Tc]MIBI uptake was seen during the first 60 min of metabolic inhibition despite significant depletion of ATP content determined on the same preparations (control ATP: 40.2 nmoles/mg protein versus iodoacetate incubation: 2.8 nmoles/mg protein; $p < 0.01$). However, prolonged metabolic blockade did eventually depress 1-min [^{99m}Tc]MIBI uptake. These data indicate that a late component of myocardial cell injury can depress [^{99m}Tc]MIBI cellular uptake.

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Hexakis(alkylisonitrile)technetium(I) complexes are a class of potential myocardial perfusion imaging agents that exploit the favorable scintigraphic properties of technetium (^{99m}Tc) (*1*). Although these agents are lipophilic cations, cellular studies have demonstrated that the lipophilic properties alone are an important but insufficient determinant of their myocardial uptake properties (*2,3*). For example, selective synthesis of various ^{99m}Tc-isonitrile complexes and correlative structure-uptake analysis with cultured heart cells have shown that agents of increased lipophilicity in general have greater myocardial uptake (*2*). However, a few highly lipophilic agents demonstrate poor myocyte uptake suggesting that lipophilicity alone does not fully characterize ^{99m}Tc-isonitrile cellular uptake. Other factors such as the cationic charge, ligand branching pattern, and molecular size of the ^{99m}Tc-isonitrile complex also appear to influence myocardial uptake. Thus, cellular mechanisms of myocardial uptake remain unresolved.

Hexakis (methoxyisobutylisonitrile)technetium-99m ([^{99m}Tc]MIBI) and hexakis(carbomethoxyisopropylisonitrile)Tc-99m ([^{99m}Tc]CPI) are two members of this class that have undergone the most extensive clinical testing to date (*4,5,6*). Cellular studies have shown [^{99m}Tc]CPI myocyte net uptake to be linearly proportional to extracellular concentration of the complex and no competitive displacement of tracer by carrier [^{99}Tc]CPI up to a concentration of 10^{-7} M (*7*). These data indicate the absence of a high affinity cell receptor for [^{99m}Tc]CPI. Myocardial net uptake of [^{99m}Tc]MIBI has been determined to be greater than that of [^{99m}Tc]CPI in cultured chick heart cells (*2*), as well as in rabbit and guinea pig heart preparations (*2,8,9*). Myocardial blood flow is linearly related to [^{99m}Tc]MIBI distribution in normal dog (*10*) and good correlation between [^{99m}Tc]MIBI and thallium (^{201}Tl) distribution in vivo has been reported in phase II clinical trials (*6*).

Since uptake mechanisms will affect the appropriate clinical applications of the ^{99m}Tc-isonitriles, we used chick heart cells in monolayer culture to characterize myocardial cellular uptake and washout kinetics of [^{99m}Tc]MIBI. To determine whether the metabolic state of the heart may modulate cellular uptake of [^{99m}Tc]

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MIBI, conditions associated with ischemia were produced by metabolic blockade with rotenone and iodoacetate and found to markedly inhibit [^{99m}Tc]MIBI cellular uptake.

MATERIALS AND METHODS

Cultured Heart Cells and [^{99m}Tc]MIBI Kinetic Studies. The techniques for preparing monolayers of spontaneously contracting chick myocytes from 10- to 11-day-old chick embryo ventricles disaggregated with trypsin have been described (*11, 12,13*). Twenty-five-millimeter circular glass coverslips served as substrate for attachment of the cells. Some cultures were grown to confluence in media containing L-[4,5-^{3}H(N)]leucine for 3–4 days (*14*). Calibration of cellular ^{3}H counts with protein determination by the method of Lowry (*15*) allowed normalization of each coverslip to cell protein (typically 0.1–0.2 mg protein per coverslip). Protocols for cellular uptake kinetic and washout studies in cultured preparations as well as in vivo biodistribution studies in newborn chicks have been previously described (*7*). Control experiments showed that [^{99m}Tc]MIBI 1-min binding to blank glass coverslips was 14.9% ± 2.9% of the total 1-min activity obtained with cell-containing coverslips (n = 4); cell uptake was determined by subtracting this value from total uptake. Net cellular accumulation of radioactivity in Figures 5 and 6 were normalized to extracellular concentration of the isotope by dividing cell content of ^{201}Tl or [^{99m}Tc]MIBI by the activity determined in an aliquot of extracellular loading buffer. This allowed direct comparison of net cell uptakes independent of subcellular compartmentation despite the use of different isotopes or different extracellular concentrations of radioactivity. These values, when divided by cell-water space (6.6 μl/mg protein; recalculated from Ref. 11), could yield intracellular/extracellular concentration ratios for each agent; however, such analysis would assume homogeneous distribution of tracer throughout the cytosol. Therefore, to avoid confusion regarding the subcellular distribution of the agents, data were normalized simply as cell content divided by extracellular concentration of radioactivity. Equilibrium calculations were used to estimate total moles of ^{99m}Tc and ^{99}Tc for some experiments (*16, 17*).

Experimental Solutions. Experiments were performed in HEPES-buffered physiologic salt solution with the following composition (m*M*): NaCl, 137; KCl, 4.5; $MgCl_2$, 0.5; $CaCl_2$, 0.9; HEPES, 4.0; dextrose, 5.6; pH 7.35 in air; 37°C. Loading solutions were obtained by addition of [^{99m}Tc]MIBI (final concentration: 50–150 μCi/ml; 3–10 fmol/μCi) or ^{201}Tl (final concentration: 10–15 μCi/ml; ~25 fmol/μCi). Rotenone, 2-deoxyglucose (2DG), and iodoacetic acid (IAA) were dissolved in dimethylsulfoxide (DMSO) prior to addition to solutions. Final DMSO concentration was typically 0.1%. DMSO concentrations up to 1% do not significantly alter contractile activity or action potential configuration of cultured chick heart cells (*18*). Control experiments also showed no effect of DMSO on [^{99m}Tc]MIBI cellular uptake.

Ouabain, amiloride, bumetanide, and verapamil were dissolved in HEPES buffer directly from powder. Thallium-201 was obtained from E.I. Dupont, Billerica, MA. Technetium-99m-MIBI was synthesized from neat (free) ligand or kit preparations (Cardiolite, E.I. Dupont) and purified as described (*2,8*). Carrier-added [^{99}Tc]MIBI was synthesized from 8–10 mg (8–10 mmoles) of ^{99}Tc $(MIBI)_6$ hexafluorophosphate powder dissolved in 0.5 ml of tracer [^{99m}Tc]MIBI in 95% ethanol solution.

ATP Content. In several series of experiments, cell ATP content was assayed fluorometrically by a standard hexokinase reaction (*19*) on cells which had simultaneously been analyzed for [^{99m}Tc]MIBI uptake. Following incubation in IAA for the appropriate time and subsequent exposure to [^{99m}Tc]MIBI-containing buffer for 1 min also in the presence of inhibitor, cells on coverslips were extracted in 300 μl 14% perchloric acid for 10–15 min in 35 mm plastic petri dishes on ice. A 250-μl aliquot was then transferred to a microfuge tube, neutralized with 200 μl 1.6 *M* K_2CO_3 on ice, and the sample assayed for [^{99m}Tc]MIBI content in a well-type gamma counter prior to freezing (−20°C). Cell debris remaining in the petri from the extraction procedure was also assayed for gamma activity, then dissolved in 1.9 ml of solution containing 1% sodium dodecyl sulfate and 10 m*M* sodium borate for protein determination by the method of Lowry (*15*). Appropriate geometric corrections allowed total cell-associated gamma activity to be calculated from the paired microfuge tubes and petri dishes. Previously frozen extracts were then thawed, spun for 5 min in an air centrifuge, and 150 μl of the supernatant assayed fluorometrically for ATP content (Kontron Instruments SFM 25, Zurich, Switzerland; excitation 340 nm, emission 460 nm). Reactions were initiated by addition of hexokinase. ATP standards were assayed every third to fourth sample to allow internal calibration. ATP content is expressed as nmol/mg cell protein.

Statistics. Values are presented as mean ± s.e.m. Statistical significance was determined by one-way analysis of variance (*20*) or the two-tailed Student's t-test (*21*) as indicated in the text.

RESULTS

Technetium-99m-MIBI net uptake into spontaneously contractile chick heart cells in monolayer culture approached a plateau by 40 min with a half-time ($t_{1/2}$) for cellular uptake of 9.3 ± 1.5 min (n = 10) (Fig. 1). Plateau accumulation was maintained for at least 90 min, implying a cellular steady-state or equilibrium condition had been achieved. Saturable net uptake has also been previously observed with [^{99m}Tc]CPI, but the lack of competitive displacement of tracer [^{99m}Tc]CPI by carrier [^{99}Tc]CPI (concentration range: 10^{-11} to 10^{-7} *M*) mitigated against a high-affinity ^{99m}Tc-isonitrile receptor (*7*). Similar net uptake experiments were performed with [^{99m}Tc]MIBI including a range with higher concentrations of [^{99}Tc]MIBI (Fig. 2). Half-maximal accumulation of [^{99m}Tc]MIBI (apparent K_D) was observed at a molar ratio ([^{99m}Tc]MIBI/[^{99}Tc]MIBI) of ~7 × 10^{-5} indicating the presence of a low-affinity retention process.

Cells pre-equilibrated in [^{99m}Tc]MIBI load solution for 20 min and then switched to isotope-free solution (37°C) showed [^{99m}Tc]MIBI unidirectional washout kinetics with a mean $t_{1/2}$ of 8 ± 2 min (n = 4) assuming

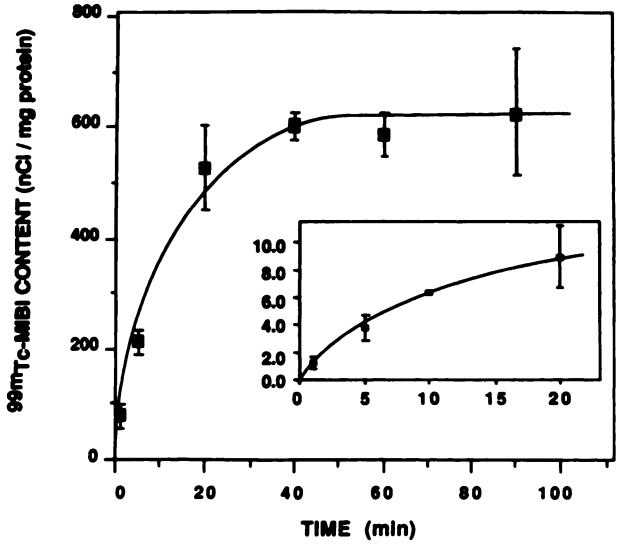

FIGURE 1
Technetium-99m-MIBI net uptake into cultured chick heart cells. Cells were incubated in solution containing [^{99m}Tc]MIBI [typically 50–150 μCi/ml (3–10 fmole total Tc-MIBI/μCi)] for various times before washing in ice-cold isotope-free solution to clear extracellular spaces. Each point represents the mean ± s.e.m. of 3–4 determinations. Solid line was drawn by eye assuming a saturable process with $T_{1/2}$ of 10 min. Inset: [^{99m}Tc]MIBI net uptake in another cultured preparation better resolving early time points. Same axis legends apply.

a single-compartment for curve fitting (Fig. 3). Two experiments showed evidence of two compartments; the faster compartment (65% of activity) had a $t_{1/2}$ of ~6 min and the slower compartment a $t_{1/2}$ of ~90 min. Total washout varied from culture to culture; mean 60-min washout was 84% ± 2% of plateau activity determined from four series of experiments involving 11 preparations. However, one additional experiment showed no significant washout. (Other than biologic variability, the cause of this one aberrant washout experiment could not be determined. This experiment was one of two washouts performed with [^{99m}Tc]MIBI synthesized from ligand de novo rather than from a kit formulation. However, reverse-phase high performance liquid chromatography (HPLC) analysis of this [^{99m}Tc]MIBI sample was identical to kit formulations [data not shown]). Another series of experiments directly compared [^{99m}Tc]MIBI and [^{99m}Tc]CPI washout from preparations of the same culture. Total [^{99m}Tc]CPI washout at 60 min was greater than [^{99m}Tc]MIBI washout (92% ± 2.8% washout versus 80% ± 1.8%, respectively; n = 3; p = 0.02).

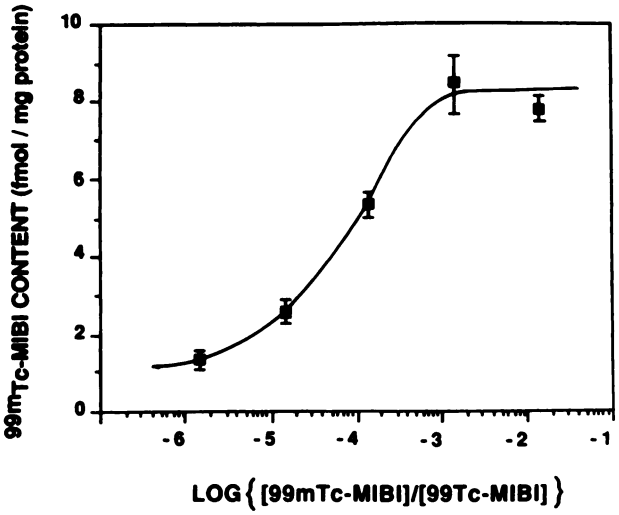

FIGURE 2
Equilibrium content of tracer [^{99m}Tc]MIBI (30 min incubation) as a function of carrier [^{99}Tc]MIBI. Load solutions were prepared by addition of equal aliquots of [^{99m}Tc]MIBI (1.9 fmoles/μCi) with increasing aliquots of [^{99}Tc]MIBI. [^{99m}Tc]MIBI/[^{99}Tc]MIBI molar ratios are shown on the abscissa. Each point represents four preparations. Line was drawn by eye.

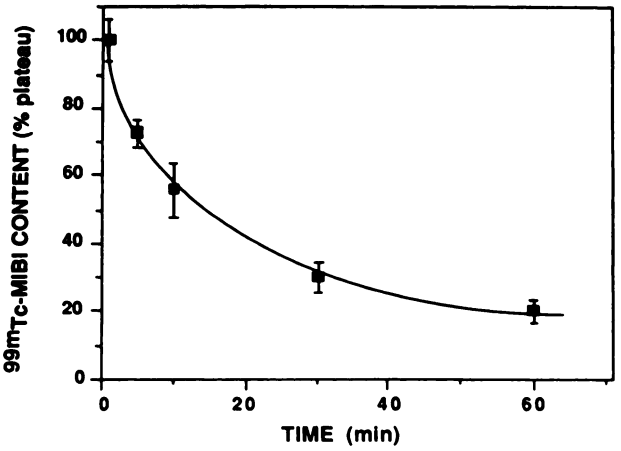

FIGURE 3
Technetium-99m-MIBI unidirectional washout from cultured heart cells. All preparations were incubated for 20 min in a loading solution containing [^{99m}Tc]MIBI and switched to [^{99m}Tc]MIBI-free control solution for washout. Each point represents three determinations. Solid line was drawn by eye.

Biodistribution studies on new born chicks in vivo demonstrated significant net [^{99m}Tc]MIBI myocardial clearance. Heart activity 30 min postinjection (2.9% ± 0.2% of injected dose/g; n = 5) was less than heart activity 5 min postinjection (4.3% ± 0.4%; n = 4) (p = 0.02).

Technetium-99m-MIBI is a cation. To evaluate if [^{99m}Tc]MIBI uptake occurs via defined sarcolemmal cation transport mechanisms, the effect of myocardial cationic membrane transport inhibitors on [^{99m}Tc]MIBI uptake kinetics was determined. Pre-treatment for 1 min with saturating concentrations of ouabain (100 μM; Na/K ATPase inhibitor) (*14*), amiloride (100 μM; Na/H exchange blocker at this concentration) (*22,23*), bumetanide (10 μM; Na+K+2Cl co-transport inhibitor) (*24*), or verapamil (1 μM; Ca^{2+} channel blocker) (*25*) showed no significant effect on subsequent 1-min [^{99m}Tc]MIBI uptake (p > 0.5) (Fig. 4A). (Control experiments have demonstrated 95% exchange of extra-

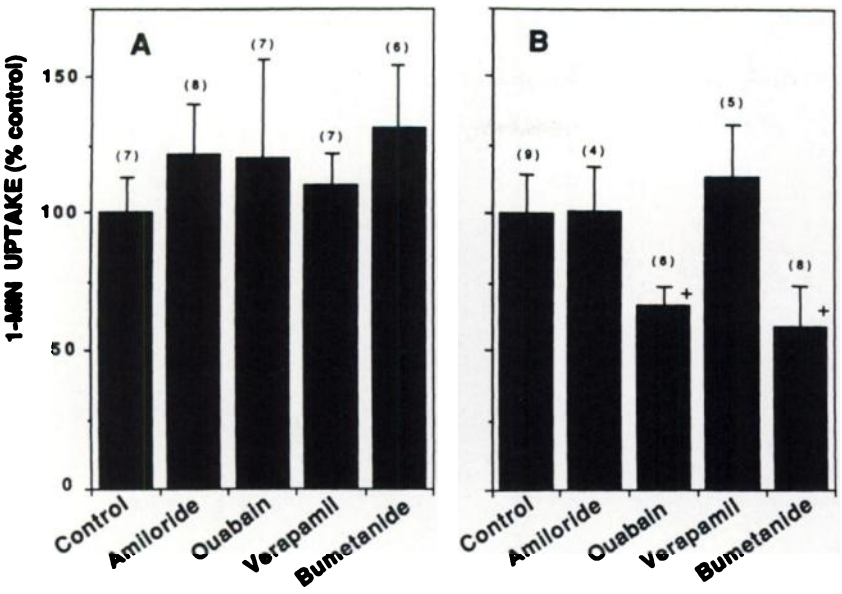

FIGURE 4
Effect of cationic membrane transport inhibitors on 1-min [^{99m}Tc]MIBI uptake (A) and ^{201}Tl uptake (B). Cells were pre-incubated in solutions containing inhibitors for 1 min prior to determination of tracer uptake in the continued presence of inhibitors. The following concentrations were used: amiloride (100 μM); ouabain (100 μM); bumetanide (10 μM); verapamil (1 μM). Values are mean ± s.e.m. Number of determinations are shown in parentheses. There were no significant differences between [^{99m}Tc]MIBI values based on one-way analysis of variance (*18*). For ^{201}Tl values, + denotes $p < 0.05$ compared to control.

cellular spaces in <30 sec in these cultured preparations thereby producing rapid onset of action of these transport inhibitors secondary to the simple extracellular spaces (*12,23*)). This contrasted with the transport inhibition profile determined for ^{201}Tl uptake in these preparations. Unlike [^{99m}Tc]MIBI, 1-min ^{201}Tl uptake was significantly inhibited by ouabain and bumetanide (Fig. 4B), consistent with the known physiologic similarities between thallium and potassium (K) (*26,27*). The different transport inhibition profiles of 1-min uptakes between [^{99m}Tc]MIBI and ^{201}Tl could not be accounted for by vastly different compartment sizes and uptake rate constants (thereby limiting application of the 1-min uptake assay) since net cell uptakes for both agents were comparable for 10 min when normalized to constant extracellular concentration of tracer (Fig. 5). As opposed to unidirectional uptake, steady-state accumulation of [^{99m}Tc]MIBI in myocytes could be altered by prolonged cationic transport inhibition (Fig. 6). Plateau levels of [^{99m}Tc]MIBI were not significantly different from control in buffers containing bumetanide or amiloride, but were actually increased in buffers containing ouabain ($p = 0.05$) or verapamil ($p < 0.01$).

Since one primary clinical function of scintigraphic myocardial perfusion imaging agents is to probe ischemic myocardium, the effect on [^{99m}Tc]MIBI cellular uptake kinetics of conditions associated with ischemia were determined (Fig. 7). Site I of mitochondrial electron transport was inhibited with rotenone (10 μM) while glycolysis was inhibited with iodoacetate (IAA; 1 mM) or 2-deoxyglucose (2DG; 1 mM) (*28*). Pre-incubation for 2.5 hr in rotenone alone partially inhibited 1-min [^{99m}Tc]MIBI cellular uptake ($p < 0.05$). Pre-incubation in IAA, either alone or with rotenone, nearly completely inhibited [^{99m}Tc]MIBI uptake ($p < 0.01$). Pre-incubation in 2DG alone showed no significant effect; however, 2DG plus IAA markedly inhibited [^{99m}Tc]MIBI uptake ($p < 0.01$). Control experiments revealed no evidence of uptake inhibition ($p > 0.5$) in cells pre-incubated in HEPES-buffered control solution for 2.5 hr compared with serum-containing culture media prior to determination of [^{99m}Tc]MIBI uptake. Thus, the absence of serum in the test solutions could not explain the metabolic effect on [^{99m}Tc]MIBI uptake.

Correlative ATP contents were determined after 2.5 hr of metabolic inhibition (Table 1). Compared to ATP content of control cells pre-incubated in HEPES buffer (30.1 ± 1.4 nmoles ATP/mg protein), rotenone (10 μM) alone partially depressed ATP content and IAA

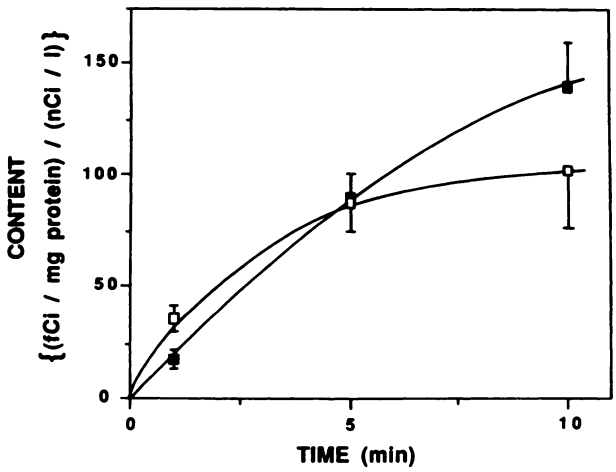

FIGURE 5
Comparative [^{99m}Tc]MIBI (■) and ^{201}Tl (□) net uptake into cultured chick myocytes. Data were normalized to constant extracellular concentration of tracer and expressed as fCi cellular tracer content/mg protein per nCi extracellular tracer/liter (see methods). Each point is the mean ± s.e.m. of 3–4 determinations. Solid lines were drawn by eye.

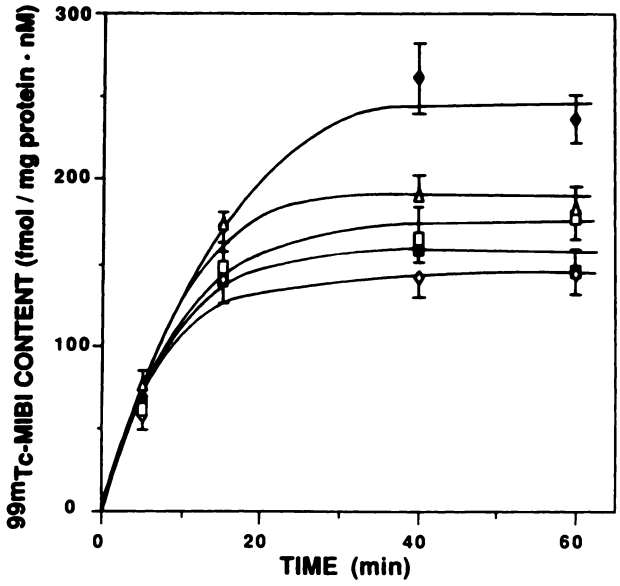

FIGURE 6
Effect of membrane transport inhibitors on steady-state [^{99m}Tc]MIBI content. Cells were incubated in HEPES buffer containing inhibitors and tracer for the times indicated and tracer content determined as described in legend to Figure 1. Data are normalized to constant extracellular concentration of [^{99m}Tc]MIBI and expressed as fmol cellular tracer content/mg protein · n*M* extracellular [^{99m}Tc]MIBI. Each point is the mean ± s.e.m. of four determinations. Solid lines were drawn by eye. (■) control; (◇) amiloride (100 μM); (△) ouabain (100 μM); (□) bumetanide (10 μM); (◆) verapamil (1 μM).

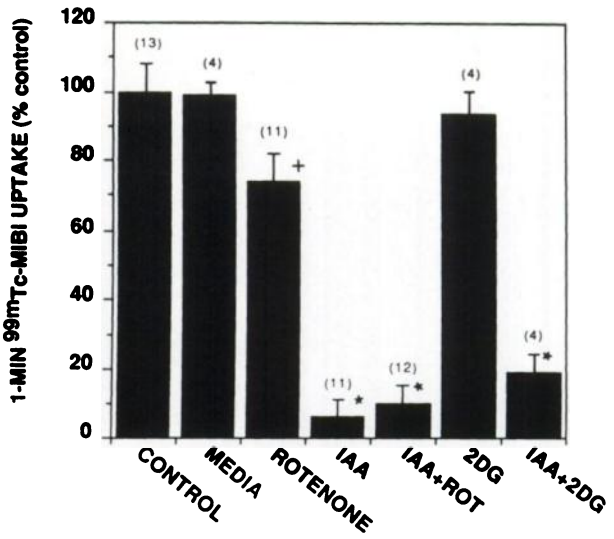

FIGURE 7
Effect of metabolic inhibitors on 1-min [^{99m}Tc]MIBI uptake. Cells were pre-incubated for 2.5 hr in HEPES buffer alone (control) or containing combinations of 2-deoxyglucose (1 m*M*), rotenone (10 μM), and iodoacetate (1 m*M*) prior to determination of 1-min [^{99m}Tc]MIBI uptake in HEPES buffer in the continued presence of inhibitors. Cells maintained in serum-containing culture media were also subsequently assayed for [^{99m}Tc]MIBI uptake in HEPES buffer. Number of determinations are shown in parentheses. * denotes $p < 0.01$ and + denotes $p < 0.05$ compared to control uptake.

(0.1 m*M*), either alone or in combination with rotenone (10 μM), completely depleted cell ATP. 2DG (1 m*M*) alone had no effect on ATP content.

The concentration-effect curve for IAA inhibition of 1-min [^{99m}Tc]MIBI uptake is shown in Figure 8. Half-maximal inhibition was determined to occur at ~5 μM. Onset of the inhibitory action of IAA was time-dependent (Table 2). Pre-incubation in saturating concentrations of IAA (1 m*M*) for up to 60 min was without significant effect on 1-min [^{99m}Tc]MIBI uptake. However, ATP content determined on the *same* cells was depressed to less than 10% of control values after 60 min of exposure to IAA (Table 2). Longer incubations then eventually did depress 1-min [^{99m}Tc]MIBI uptakes.

DISCUSSION

To evaluate overall kinetic and cellular processes involved in myocardial extraction and retention of [^{99m}Tc]MIBI, a minimal compartmental analysis would require a three-component model exchanging in series. The usual physiologic compartments assumed for this analysis allows [^{99m}Tc]MIBI exchange between intravascular spaces and interstitial (extracellular) spaces and subsequent exchange between extracellular spaces and myocytes. More complex compartmental models also have been proposed (*29*). Whole-animal and perfused organ techniques may be best suited to evaluate overall exchange kinetics of [^{99m}Tc]MIBI between vascular and interstitial spaces, but these techniques only indirectly examine exchange at the cellular level. The complex extracellular spaces and diffusion delays of whole-organ models is well established in the physiologic literature to severely limit evaluation of cellular exchange processes (*12,30*). Cultured chick heart cells are a physiologically stable myocardial preparation that has minimal extracellular diffusion distances (*12,31*) and lacks endothelial and neural elements that complicate tracer analysis in whole animal or intact tissue preparations. This study therefore examined cellular uptake kinetics and net accumulation of [^{99m}Tc]MIBI utilizing this well characterized preparation (*31*). These data can potentially be combined with results from other techniques that examine vascular exchange to generate an overall scheme of cardiac extraction processes for ^{99m}Tc-isonitriles.

Steady-State Analysis. Net cellular accumulation of [^{99m}Tc]MIBI approached a plateau by 40 min with a $t_{1/2}$ of 9.3 ± 1.5 min; mean $t_{1/2}$ of washout was 8 ± 2 min. The presence of a plateau implied that the cellular mechanisms of unidirectional influx and efflux were in a steady-state at plateau levels. A similar observation has been made for [^{99m}Tc]CPI; however the uptake and washout kinetics are approximately twice as rapid as [^{99m}Tc]MIBI (*7*). Exposure of cultured myocyte prepa-

TABLE 1
Cell ATP Content After Metabolic Inhibition

Inhibitor	ATP content (nmoles/mg cell protein)
Control	30.1 ± 1.4
2DG (1 m*M*)	34.5 ± 2.3
rotenone (10 μM)	* 20.6 ± 1.4
IAA (0.1 m*M*)	* 0.4 ± 0.3
IAA + rot	* 2.1 ± 0.3

Cells were pre-incubated in various inhibitors for 2.5 hr prior to assay for ATP content. Each value was determined in triplicate and shown as mean ± s.e.m.

* denotes $p < 0.05$.

TABLE 2
Effect of Pre-Treatment Time in Iodoacetate on 1-Min [^{99m}Tc]MIBI Uptake and ATP Content in Cultured Heart Cells

Pretreatment time (min)	1-min [^{99m}Tc]MIBI uptake (nCi/mg cell protein)	ATP content (nmoles/mg cell protein)
0	118 ± 29 (n = 3)	40.2 ± 7.1 (3)
15	129 ± 26 (3)	29.4 ± 1.8 (3)
30	173 ± 16 (3)	26.7 ± 4.6 (3)
60	123 ± 41 (4)	* 2.8 ± 1.1 (4)
105	† 43 ± 9.8 (3)	* ND (3)
150	† 34 ± 4.5 (3)	* 0.4 ± 0.3 (3)

Time-dependence of IAA inhibition of [^{99m}Tc]MIBI uptake and ATP depletion in heart cells. Cells were pre-incubated in IAA (1 m*M*) for various times prior to determination of 1-min [^{99m}Tc]MIBI uptake and ATP content in the same preparations. Values are mean ± s.e.m. (n = number of determinations). Two additional series of experiments on separate cultures confirmed the lack of effect of IAA (0.1 m*M* and 1 m*M*) on [^{99m}Tc]MIBI uptake during pre-incubation periods of up to 60 min, but inhibition thereafter.

* $p < 0.01$.

† $p < 0.05$.

ND = none detectable.

rations to constant extracellular concentrations of [^{99m}Tc]MIBI can assist in analyzing cellular mechanisms and kinetics of myocyte uptake and retention, but it is emphasized that the plateau level of net cellular uptake observed in this study was isolating only a component of the dynamic behavior of whole-heart tissue in vivo. For example, following an intravenous bolus injection of [^{99m}Tc]MIBI, myocardial tissue activity peaks during the first 30 to 120 sec in vivo (*2,8,10*), in large part secondary to the rapid intravascular clearance of the agent. Time-activity curves for [^{99m}Tc]MIBI in the myocardial interstitial spaces are likely to reflect to some degree the more easily measured time-activity curves of the intravascular pool of [^{99m}Tc]MIBI. Therefore, myocytes in vivo would be exposed to a transient bolus of extracellular activity rather than a constant concentration of [^{99m}Tc]MIBI as in this study. None the less, steady-state analysis will yield quantified information regarding cellular exchange processes. Furthermore, these steady-state data indicated that the 1-min uptake times used in later cellular kinetic analysis were only 1%–2% of the time required to achieve net equilibration of [^{99m}Tc]MIBI (40–60 min), and therefore, these short times could be used to isolate cellular unidirectional uptake kinetics relatively uncontaminated by cellular efflux (back flux) (*32*).

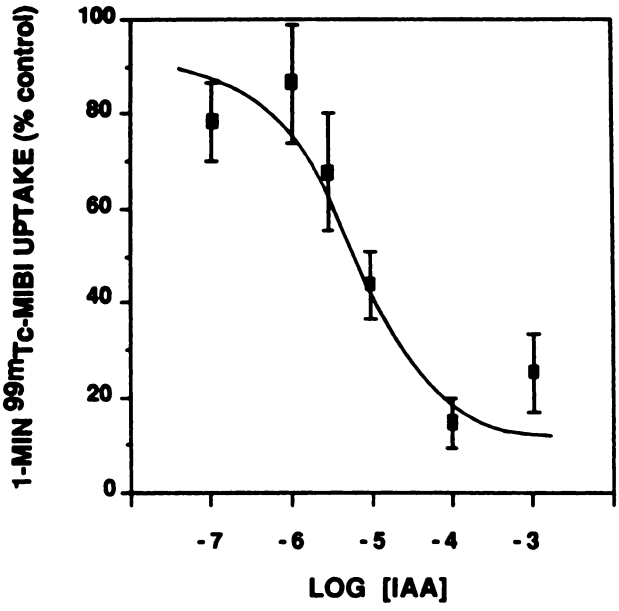

FIGURE 8
Concentration-effect curve for IAA inhibition of 1-min [^{99m}Tc]MIBI uptake. Cells were pre-incubated for 2.5 hr in HEPES buffer containing IAA of various concentrations prior to determination of [^{99m}Tc]MIBI uptake. Results are expressed as percent of control uptake. Each point represents four determinations. Solid line was drawn by eye assuming an IC_{50} of 5 μM.

Washout. Total unidirectional washout of [^{99m}Tc]MIBI from cultured heart cells (84% at 60 min) was greater than net myocardial clearance in newborn chick in vivo (33% at 30 min) and significantly greater than net fractional clearance previously reported for dogs in vivo (11% at 4 hr) (*10*). Net myocardial clearance of [^{99m}Tc]MIBI in human is 27% at 3 hr (*6*), intermediate between values found in dog and chick. Note that the large fractional washout from cultured heart cells represented a unidirectional efflux into isotope-free buffer originating from cells previously equilibrated in [^{99m}Tc]MIBI. Aside from species differences, these experiments in vitro therefore isolated myocellular washout kinetics, whereas studies of the slower net myocardial clearance in vivo reflect a complex interaction of washout, retention and uptake between myocytes, interstitial spaces, blood vessels, and other organs.

Displacement. Apparent competitive displacement of tracer by carrier [^{99}Tc]MIBI occurred at high molar

ratios (apparent $K_D \sim 7 \times 10^{-5}$), which implied a low-affinity cellular retention process. Apparent competitive displacement of tracer [^{99m}Tc]MIBI by high concentrations of carrier [^{99}Tc]MIBI also has been observed in isolated guinea pig heart slices (*3*). Previous experiments with [^{99m}Tc]CPI in cultured heart cells similarly demonstrated a low-affinity or nonspecific process of cellular retention (*7*). Although these data could be construed to reflect a low-affinity receptor(s) for ^{99m}Tc-isonitriles, several alternative mechanisms are *equally* consistent with the data. Also possible are:

1. The onset of cellular toxicity at high extracellular concentrations of [^{99}Tc]MIBI, thereby decreasing net cellular content. (However, note that clinical imaging is performed far from these saturating concentrations of tracer; typical extracellular concentrations of ^{99m}Tc-isonitriles during clinical imaging are approximately 10^6-fold lower at 10^{-11} to 10^{-10}M (*7*).)
2. The presence of a high capacity subcellular or pericellular compartment that sequestered [^{99m}Tc]MIBI. In this regard, preliminary data from cultured cells point to the presence of subcellular compartmentation of [^{99m}Tc]MIBI within mitochondria (*33*). This finding would also further justify the use of the term "uptake" in the context of cellular tracer studies with [^{99m}Tc]MIBI.

Transport Inhibition. The tested cation transport blockers did not inhibit [^{99m}Tc]MIBI uptake kinetics and, furthermore, no transport inhibitor decreased plateau accumulation of [^{99m}Tc]MIBI. These findings would be incompatible with direct inward transport of [^{99m}Tc]MIBI by a cationic binding site on these membrane transport proteins. However, prolonged exposure of myocytes to ouabain or verapamil actually increased [^{99m}Tc]MIBI steady-state accumulation suggesting that indirect effects of Na/K pump inhibition or Ca^{2+} channel blockade either increased a myocellular compartment for [^{99m}Tc]MIBI or decreased [^{99m}Tc]MIBI efflux.

Metabolic Inhibition. Controversy exists as to whether distribution of [^{99m}Tc]MIBI in vivo is a simple function of blood flow or whether it is responsive to myocardial metabolic function. Preliminary data in isolated guinea pig heart slices indicated that [^{99m}Tc]MIBI uptake was inhibited by hypoxia produced by pre-equilibration in buffer gassed with nitrogen/carbon-dioxide (*3*). First-pass extraction fractions in isolated rabbit hearts have been reported to increase after 30 min of reperfusion preceeded by global ischemia (*34*). The investigators concluded that this reflected in some way myocardial damage and was independent of coronary flow. In addition, [^{99m}Tc]MIBI washout from isolated rat heart was increased by ischemic flow rates (3 ml/min) relative to control rates (12 ml/min) suggesting a component of [^{99m}Tc]MIBI myocardial retention sensitive to myocardial metabolism (*35*). However, other investigators report that 15 min of metabolic blockade with cyanide and iodoacetate in cultured rat myocytes had no effect on [^{99m}Tc]MIBI uptake (*36*).

Our data demonstrate strong inhibition of [^{99m}Tc]MIBI uptake kinetics by metabolic blockade. Nearly complete inhibition of 1-min [^{99m}Tc]MIBI uptake occurred after pre-incubation of heart cells for 2.5 hr in IAA (1 m*M*) or IAA plus rotenone (10 μM). The half-maximal inhibitory dose of IAA was ~5 μM. However, onset of [^{99m}Tc]MIBI uptake inhibition was markedly time-dependent; pre-incubation periods of up to 60 min ([IAA] = 0.1–1 m*M*) caused no significant inhibition, but thereafter markedly reduced [^{99m}Tc]MIBI uptake. In agreement with the early time points, Maublant et al. (*36*) previously reported no effect of a 15-min pre-incubation period in IAA (0.1 m*M*) or IAA plus cyanide (5 m*M*) on subsequent 20-min [^{99m}Tc]MIBI uptake in cultured rat myocytes. However, it was likely that their 15-min pre-incubation period was too short to elicit the marked inhibitory effect of prolonged metabolic blockade observed in this report from cultured chick myocytes. Relatively long incubation times in the presence of metabolic inhibitors before induction of cell injury is a characteristic previously reported for cultured rat and chick heart cell models in the study of ischemia-related conditions (*28,37*).

Although IAA is generally used as an inhibitor of glycolysis (glyceraldehyde-3-phosphate dehydrogenase), other mechanisms of action may occur and could involve sulfhydral carboxymethylation of other proteins within the cytosol, cellular membranes and organelles (*38*). However, the relatively slow onset of IAA effects were most consistent with depletion of a substrate or enzyme. The lack of effect of 2DG on [^{99m}Tc]MIBI uptake may be due to greater specificity of action on glycolysis or less efficacy as a metabolic inhibitor at concentrations of 1 m*M* (*39*). Note that the lack of effect of 2DG or short exposures to IAA on 1-min [^{99m}Tc]MIBI uptake does not exclude a possible effect of the inhibitors on steady-state levels of accumulation of [^{99m}Tc]MIBI. Further experiments would be required to fully explore changes in unidirectional myocellular uptake rates, washout rates, and cellular compartment sizes of [^{99m}Tc]MIBI during prolonged membrane transport inhibition or metabolic blockade to clarify potential effects of these manipulations on clinical images.

What is the source of the metabolic effect on [^{99m}Tc]MIBI cellular uptake? Myocyte ATP content (alone or in part) does not fully account for the observations. For example, [^{99m}Tc]MIBI uptake was unaffected by 60 min of metabolic blockade in IAA (1 m*M*) at a time when ATP *simultaneously* determined in the same preparations was <10% of control values (Table 2). However, with more prolonged metabolic inhibition, [^{99m}Tc]MIBI

uptake eventually declined. In addition, Murphy et al. (*28*) have examined ATP content, lactate dehydrogenase (LDH) release, and intracellular Na, K, and Ca contents in cultured chick heart cells exposed to IAA (1 m*M*) and rotenone (0.1 m*M*) for up to 2.5 hr. After 60 min of metabolic inhibition at normal extracellular Ca concentrations, ATP content was <2 nmole/mg protein (control: 40 nmole/mg protein) and intracellular Na and K contents had approached their extracellular values. No further changes in these parameters occurred up to 2.5 hr. LDH release into the supernatant, on the other hand, was <4% of total cellular LDH at 60 min, but reached nearly 50% of total LDH at 2.5 hr. One-minute [^{99m}Tc]MIBI uptake values from our study under similar conditions appeared to correlate inversely with LDH release and again correlated poorly with ATP or intracellular cation content, suggesting that a component of "late injury" or cell death caused inhibition of [^{99m}Tc]MIBI myocellular uptake.

The delayed effect of metabolic inhibition on [^{99m}Tc]MIBI uptake stands in contrast to the earlier effect of metabolic inhibition on Tl uptake in similar models. McCall et al. (*40*) reported that ^{204}Tl influx into cultured rat myocytes decreased during the first 30–40 min of pre-treatment with 2,4-dinitrophenol (1 m*M*) or IAA (0.1 m*M*) in a time course that paralleled the decline in ATP content during metabolic inhibition determined in this study and by others (*37,39,41*). This supported the proposal that 60%–70% of ^{204}Tl uptake was mediated by the plasma membrane Na/K ATPase. The divergent responses of ^{204}Tl and [^{99m}Tc]MIBI uptakes to transport and metabolic inhibition add further evidence for differing mechanisms of cellular uptake and retention. Additional experiments are required to explore these complex relationships between perfusion agent uptake and myocardial cell injury.

In summary, [^{99m}Tc]MIBI net uptake into cultured heart cells approached a plateau level, which implied an equilibrium or steady-state process involved in net cellular accumulation of the agent. Conditions associated with ischemia induced by prolonged metabolic blockade with IAA and rotenone markedly inhibited [^{99m}Tc]MIBI cellular uptake kinetics. Although [^{99m}Tc]MIBI has been shown to be a flow-dependent tracer, our data also indicate that myocellular net accumulation and uptake kinetics can be affected, respectively, by pharmacologic alterations in membrane transport and metabolic status.

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