Divergent Kinetics of $^{201}$Tl and $^{99m}$Tc-SESTAMIBI in Cultured Chick Ventricular Myocytes During ATP Depletion

David Piwnica-Worms, MD, PhD; Mary L. Chiu, BS; and James F. Kronauge, PhD

**Background.** Thallous chloride ($^{201}$Tl) and hexakis(2-methoxyisobutyl isonitrile) technetium (I) ($^{99m}$Tc-SESTAMIBI) are myocardial perfusion imaging agents with biological properties that also reflect tissue viability. Initial myocellular uptake rates of $^{201}$Tl reflect activity of Na,K-ATPase, whereas those of $^{99m}$Tc-SESTAMIBI reflect mean plasma membrane potential.

**Methods and Results.** To better understand the mechanistic responses of these tracers to myocardial injury, cultured chick embryo cardiac myocytes were metabolically inhibited in iodoacetate (1 mM) and rotenone (10 μM) for up to 2 hours, and initial uptake rates of each agent were determined at successive intervals along with correlative cellular contents of ATP, sodium, and potassium and lactate dehydrogenase release. ATP content fell from 30.5±1.4 to 2.7±0.9 nmol ⋅ (mg protein)$^{-1}$ within 2 minutes, whereas sodium and potassium contents ran down their thermodynamic gradients more slowly ($t_{1/2}$ 60 minutes). Modestly severe cell injury was produced at 2 hours as estimated by lactate dehydrogenase release (18% of total). Initial uptake rates of $^{201}$Tl declined from 6.9±0.8 to 4.0±0.4 fmol ⋅ (mg protein)$^{-1}$ ⋅ (nM)$^{-1}$ ⋅ (min)$^{-1}$ by 20 minutes and remained depressed and ouabain (100 μM)-insensitive at 30±13% of control. Conversely, initial uptake rates of $^{99m}$Tc-SESTAMIBI increased from 10.6±0.8 to 15.0±0.6 fmol ⋅ (mg protein)$^{-1}$ ⋅ (nM)$^{-1}$ ⋅ (min)$^{-1}$ within 10 minutes, remained elevated for 40–60 minutes, and later declined to low values. Injury-induced enhancement of initial uptake rates of $^{99m}$Tc-SESTAMIBI were insensitive to ouabain (100 μM), carbonyl cyanide-m-chlorophenyl hydrazone (5 μM), and valinomycin (1 μg/ml) but were significantly inhibited by 130 mM K$_{\text{e}}$ buffer, Ba$^{2+}$ (1 mM), glybenclamide (100 μM), and quinacrine (10 μM).

**Conclusions.** Uptake rates of $^{201}$Tl monotonically decline, correlating with Na-K pump inhibition from ATP depletion. Conversely, uptake rates of $^{99m}$Tc-SESTAMIBI at first increase above control for 40–60 minutes, indicating a mean plasma membrane hyperpolarization possibly resulting from opening of ATP-sensitive and arachidonic acid-activated potassium channels, before declining to low values with more severe cell injury. Correlative non-flow-dependent relations between $^{201}$Tl and $^{99m}$Tc-SESTAMIBI contain information regarding the degree of myocardial injury. (*Circulation* 1992;85:1531–1541)

**KEY WORDS** • membrane potentials • mitochondria • potassium channels • arachidonic acid • metabolism • glybenclamide, quinacrine, nordihydroguaiaretic acid • ATP • sodium

Assessment of myocardial viability in conjunction with myocardial perfusion has become an important clinical parameter in this era of aggressive thrombolytic and surgical interventional therapy. Evaluation and definition of salvagable myocardium, regions at risk, and areas of reversible dysfunction have both diagnostic and prognostic value in managing patients with revascularization procedures.$^{1-3}$ Of the broad range of technologies currently under various stages of development and clinical use for the assessment of myocardial perfusion and viability (cardiac ultrasound, magnetic resonance imaging, rapid acquisition computed tomography, and scintigraphy),$^{4-8}$ significant experience has been gained with positron emission tomography$^{9-11}$ and single photon emission computed tomography (SPECT).$^{12-16}$ To better understand the biological properties and mechanisms of accumulation of radiopharmaceuticals used in SPECT for evaluation of myocardial perfusion and viability, this study was conducted to assess quantitatively, independent of perfusion, the comparative cellular kinetics of the cation $^{201}$Tl and the lipophilic cation hexakis (2-methoxyisobutyl isonitrile) technetium (I) ($^{99m}$Tc-SESTAMIBI) during metabolic inhibition in a heart preparation. $^{201}$Tl and $^{99m}$Tc-SESTAMIBI are both perfusion and viability markers$^{17-30}$; the fundamental myocardial mechanisms of uptake and retention of these two radiotracers under steady-state conditions, however, are quite different.$^{17}$ Approximately 50–60% of myocardial influx of $^{201}$Tl is mediated by Na,K-ATPase.$^{18,20}$ with additional contributions from the Na,K–2 Cl cotransporter.$^{29}$ In contrast, the fundamental mechanism of myocardial accumulation of $^{99m}$Tc-SESTAMIBI involves passive distribution across sarcolemmal and mitochondrial membranes in response to transmembrane potentials.$^{29,31,32}$ A three-compartment
model of myocellular accumulation of $^{99m}$Tc-SESTAMIBI has been developed\(^{29}\) in which extracellular space, cytosol, and the lumped aggregate of mitochondria are in series. Initial uptake rates of $^{99m}$Tc-SESTAMIBI in cells under physiological conditions reflect primarily potential-dependent diffusion across the plasma membrane, whereas steady-state or maximal accumulation levels reflect the ability of the mean mitochondrial and plasma membrane potentials to concentrate the majority of total cell-associated tracer within the mitochondrial inner matrix.\(^{29,33}\) The fundamental myocellular uptake and retention mechanisms of $^{201}$Tl and $^{99m}$Tc-SESTAMIBI might predict differential responses of these two diagnostic pharmaceuticals to ischemic injury or metabolic inhibition and may have clinical utility in defining myocardial energetics and severity of myocellular injury.

**Methods**

**Cultured Heart Cells**

Techniques for preparation of monolayers of spontaneously contractile chick ventricular myocytes from 10- or 11-day-old chick embryo hearts disaggregated with trypsin have recently been described.\(^{29}\) For these experiments, 25-mm circular glass coverslips served as substrate for cell growth, and confluent preparations were used for experiments only on day 3 or 4 in culture.

**Solutions**

Control buffer for these experiments was a modified Earle’s balanced salt solution (MBS) with the following composition (mM): Na\(^+\), 145; K\(^+\), 5.4; Ca\(^2+\), 1.2; Mg\(^2+\), 0.8; Cl\(^-\), 152; H\(_2\)PO\(_4\)-, 0.8; SO\(_4^{2-}\), 0.8; dextrose, 5.6; HEPES, 4.0; and bovine calf serum, 1% (vol/vol); pH 7.4±0.03; 37°C. In a few experiments, 26 mM NaHCO\(_3\) replaced an equimolar concentration of NaCl, and solutions were then maintained at pH 7.4 in a 5% CO\(_2\)/95% air atmosphere. A 130 mM K/20 mM Cl solution was made by equimolar substitution of potassium methanesulfonate for NaCl as described.\(^{34}\) Na\(^+\)-free, K\(^+\)-free rinsesolution contained choline chloride (150 mM) replacing NaCl, KCl, NaH\(_2\)PO\(_4\), and serum. BaCl\(_2\) (1 mM) was added directly to control buffer in one series of experiments.

Rotenone, iodoacetic acid (IAA), carbonyl cyanide-m-chlorophenyl hydrazone (CCCP), ouabain, glibenclamide, quinacrine, and valinomycin (Sigma Chemical Co., St. Louis, Mo.) were dissolved in dimethyl sulfoxide (DMSO) before they were added to solutions. Ruthenium red (Sigma) was dissolved directly into buffer. Final DMSO concentration was typically <0.5%, which has been found to have no effect on $^{99m}$Tc-SESTAMIBI net uptake\(^{29}\) or electrophysiological characteristics of cultured heart cells.\(^{35}\)

Loading solutions were made by addition of [$^{99m}$Tc]SESTAMIBI (final concentration, 50–100 μCi/ml; 5–9 pmol/mCi) or [$^{201}$Tl] thallous chloride (final concentration, 20–25 μCi/ml; 15–30 pmol/mCi) to the indicated buffer. $^{99m}$Tc[SESTAMIBI was synthesized with a one-step kit formulation (kindly provided by T.R. Carroll, E.I. DuPont, Medical Products Division, North Billerica, Mass.) as previously described in detail.\(^{29}\) Radiochemical purity was found to be greater than 95% in all preparations by reverse thin-layer chromatography using Baker-flex Al\(_2\)O\(_3\) IB-F strips (J.T. Baker Chemical Co., Phillipsburg, N.J.) and ethanol (absolute) as the mobile phase (R\(_f\)=0.9). [$^{201}$Tl] thallous chloride was obtained in saline as a sterile, pyrogen-free, clinically injectable radiopharmaceutical (Dupont Medical Products, Billerica, Mass.).

**Cellular Kinetic Studies**

Cellular tracer analysis was performed by minor modification of previously described methods.\(^{29}\) To determine the effect of ATP depletion on the uptake kinetics of tracer, coverslips with confluent cells were removed from culture media and rinsed for 30–60 seconds to clear extracellular spaces in control buffer alone or in buffer containing the metabolic inhibitors IAA (1 mM) and rotenone (10 μM) as indicated in the text. Cells were then incubated for various times in 35-mm plastic petri dishes containing MBS buffer and the same inhibitors. After metabolic inhibition for the times indicated in each figure, tracer uptake kinetics were determined by immediate immersion of cells in 60-mm glass Pyrex dishes containing the buffer, additional transport or metabolic inhibitors, and $^{99m}$Tc SESTAMIBI or $^{201}$Tl as indicated. Initial uptake rates were derived from a 2-minute incubation of cell preparations with tracer\(^{19,33}\) corrected for nonspecific binding of tracer to substrate as described below. Cells were removed from the load solution, rinsed three times in 25-ml volumes of ice-cold (2°C) isotope-free control buffer for 8 seconds each to clear extracellular tracer, and placed in 35-mm plastic petri dishes for analysis of $^{99m}$Tc. Cell preparations, aliquots of loading buffer, and stock solutions were assayed for radioactivity in a standard well-type sodium iodide gamma counter (Omega 1, Canberra, Meriden, Conn.). Protein determination of cells extracted from each coverslip in 1% sodium dodecyl sulfate (SDS) with 10 mM sodium borate was then made by the method of Lowry et al.\(^{38}\) with bovine serum albumin as the protein standard.

Nonspecific binding of $^{99m}$Tc-based agents to cell-free coverslips was typically <3% of the total cell-associated activity. We found, however, that $^{201}$Tl binding to some blank glass coverslips was comparable to cell-associated activity, which made determination of cell-specific $^{201}$Tl activity highly variable when the above protocol was used. Therefore, for $^{201}$Tl, cells previously rinsed in ice-cold isotope-free buffer were scraped off coverslips with a rubber spatula and then extracted in 1.5 ml of 1% SDS/10 mM sodium borate solution in a 75-mm test tube before gamma counting and protein determination. Control experiments showed that the nonspecific $^{201}$Tl activity that could be scraped off blank glass coverslips was <11% of the plateau cell-associated activity; values of nonspecific tracer binding were subtracted from total activity to yield corrected cell-associated counts. These values were approximately 25% lower than previously reported by our laboratory because of this difference in determination of nonspecific binding. Furthermore, for $^{99m}$Tc analysis, no significant differences were found in cell-associated activity between cell scraping or nonscraping techniques (data not shown). Appropriate geometric corrections and activity standards were combined with generator equilibrium equations in the case of $^{99m}$Tc (see Reference 37) or commercially available specific activity determinations in the case of $^{201}$Tl to calculate the
absolute concentration of each agent in the loading solution. Within clinically relevant concentrations (10^{-11} to 10^{-8} M), the t_{1/2} of accumulation of both 201Tl and 99mTc-SESTAMIBI is independent of extracellular tracer concentration^{18,29}; therefore, tracer kinetics can be described by the equation \( y = Kx \), where \( y \) represents tracer uptake rate [fmol \cdot (mg protein)^{-1} \cdot (min)^{-1}] and \( x \) represents extracellular concentration of the agent. Most data are presented as \( K \), which represents the nonsaturable uptake rate in fmol \cdot (mg protein)^{-1} \cdot (min)^{-1} \cdot (nM_e)^{-1}.

To determine the effect of metabolic inhibition on net accumulation of 99mTc-SESTAMIBI and 201Tl, preparations were immersed at time zero in buffer containing the metabolic inhibitors and either 99mTc-SESTAMIBI or 201Tl, removed after long times of incubation, and processed as described above. To determine the effect of ATP depletion on net retention of the agents, preparations were loaded to plateau in control buffer containing 99mTc-SESTAMIBI (60 minutes) or 201Tl (20 minutes), and then the metabolic inhibitors together were added directly to the loading buffer. Preparations were removed from the loading solution at various times and processed as above. Control experiments showed that addition of rotenone (10 \( \mu \)M) alone or drug carrier (DMSO, <0.25%) alone to MEBSS had no significant effect on net accumulation of 99mTc-SESTAMIBI (\( p > 0.025 \)).

**Analytical Measurements**

Cell ATP content during the course of metabolic inhibition was assayed fluorometrically by a standard hexokinase reaction\(^{38} \) on cells that had simultaneously been analyzed for 99mTc-SESTAMIBI uptake. After tracer loading and rinsing in ice-cold isotope-free buffer as above, preparations were extracted in perchloric acid, neutralized with potassium carbonate, immediately assayed for gamma activity, and frozen (\(-20^\circ\)C) as previously described in detail.\(^{26} \) Cell protein was determined by Lowry assay. Previously frozen cell extracts were then thawed within 5 days of the experiment and assayed for ATP content fluorometrically (SFM 25, Kontron Instruments, Zurich, Switzerland). ATP is expressed as nanomoles \cdot (mg protein)^{-1}.

Lactate dehydrogenase (LDH) released by cells into buffer was measured spectrophotometrically with a standard LDH kit (340-UV, Sigma). Monolayers of cells grown on 100-mm culture dishes were incubated in 3 ml of buffer containing IAA and rotenone for various times; 250 \( \mu \)l of the buffer was removed and diluted into 2.65 ml of 0.1 M potassium phosphate buffer (pH 7.5) and allowed to sit for 20 minutes (25\(^{\circ}\)C); then sodium pyruvate (100 \( \mu \)l of a 22.7 mM stock) was added. The rate of absorbance change at 340 nm was determined (Spectronic 610, Milton Roy Co., Rochester, N.Y.). Total cell LDH was determined in preparations disrupted in distilled water for 10 minutes. LDH release was normalized to milligrams of cell protein on each culture dish and expressed as a percent of total LDH.

Atomic absorption spectrophotometry (3030, Perkin-Elmer, Norwalk, Conn.) was used to measure sodium and potassium content during metabolic inhibition in cells that had simultaneously been analyzed for 99mTc-SESTAMIBI uptake. Briefly, preparations were incubated in metabolic inhibitors for various times, then exposed to the same buffer containing 99mTc-SESTAMIBI for 2 minutes. After

<table>
<thead>
<tr>
<th>AGENT CONTENT [fmol (mg protein)^{-1} \cdot (min)^{-1}]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
</tr>
<tr>
<td>500</td>
</tr>
<tr>
<td>250</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>50</td>
</tr>
<tr>
<td>0</td>
</tr>
</tbody>
</table>

**Statistics**

Values are presented as mean±SEM. Statistical significance was determined by one-way ANOVA, unpaired Student’s \( t \) test, or Mann-Whitney rank-sum test\(^{39} \) as indicated in the text. Values of \( p \leq 0.05 \) were considered significant.

**Results**

Cultured chick heart cells exposed to tracer in control buffer accumulated both 201Tl and 99mTc-SESTAMIBI asymptotically, which implied that a cellular steady state was achieved for each agent (Figure 1). The kinetics of myocardial accumulation have been shown to be faster for 201Tl (\( t_{1/2}=5 \) minutes\(^{18,30} \)) than for 99mTc-SESTAMIBI (\( t_{1/2}=9.5 \) minutes\(^{30} \)); however, Figure 1 demonstrates that final net accumulation for 99mTc-SESTAMIBI was approximately fivefold greater [206.7±14.0 versus 43.8±7.2 fmol \cdot (mg protein)^{-1} \cdot (nM_e)^{-1} \]; \( p < 0.001 \)] when normalized to equal extracellular concentrations for both agents.

**Metabolic Inhibition**

To evaluate the kinetic responses of 201Tl and 99mTc-SESTAMIBI to ATP depletion, heart cells were incubated in buffer containing IAA (1 mM) to inhibit glycolysis and rotenone (10 \( \mu \)M) to inhibit mitochondrial respiration (site I).\(^{40} \) Correlative analytical data on ATP, sodium, potassium, and LDH release were obtained throughout the course of metabolic inhibition. Cell content of ATP fell rapidly from a control value of 30.5±1.4 nmol/mg protein to 2.7±0.9 nmol/mg protein within 2 minutes of exposure to combined IAA and rotenone (\( p < 0.001 \)) and in three independent experi-
ments remained low for the duration of the experiment (Figure 2A); the decline was faster than previously reported in IAA (1 mM) alone. 30 Cell content of potassium monotonically declined and sodium monotonically increased throughout the course of inhibition at a net rate significantly slower than the depletion of ATP (Figure 2B). Cell contents of potassium and sodium equalized after 60 minutes and inverted by 120 minutes of metabolic inhibition. Despite prolonged ATP depletion, cell injury manifest as LDH release was modest in three independent experiments until times greater than 60 minutes (Figure 2C).

At successive intervals during metabolic inhibition, 2-minute uptakes of the agents were determined. Within the first 20 minutes of metabolic inhibition, initial uptake rates of 201Tl rapidly declined from 6.9±0.8 to 4.0±0.4 fmol·(mg protein)⁻¹·(min⁻¹) (n=3; p<0.05) (Figure 3A). For times greater than 20 minutes in three independent experiments, 201Tl uptake rates remained depressed at 30±13% of control, and no further decline of the initial uptake rate was observed for as long as 90 minutes. Sodium pump inhibition with ouabain (100 μM) had no additional effect on the injury-induced reduction of 201Tl uptake rates (ouabain-sensitive 201Tl uptake rate, 48±1.4% of control before metabolic inhibition versus 0.5±0.9% of control at 30 minutes of metabolic inhibition; n=3; p<0.001 comparing before and after metabolic inhibition). Conversely, within 10 minutes of metabolic inhibition, initial uptake rates of 99mTc-SESTAMIBI increased significantly from 10.6±0.8 to 15.0±0.6 fmol·(mg protein)⁻¹·(min⁻¹) (p<0.02) (Figure 3B) and remained above or near control values until approximately 60 minutes into metabolic inhibition, when initial uptake rates of 99mTc-SESTAMIBI declined to low values. The increase in initial uptake rates of 99mTc-SESTAMIBI was observed in 11 of 12 independent experiments and peaked between 10 and 20 minutes into the course of metabolic inhibition at 144.1±8.0% of control (p=0.005).

The effect of ATP depletion on net accumulation of the agents is shown in Figure 4. For these experiments, 201Tl or 99mTc-SESTAMIBI was added to the incubation buffer along with IAA and rotenone throughout. The first few minutes of metabolic inhibition did not significantly affect net accumulation of 201Tl, but thereafter inhibited accumulation of the tracer relative to control (Figure 4A). Metabolic inhibition also greatly reduced net uptake of 99mTc-SESTAMIBI (Figure 4B).

To fully document the altered kinetics of the agents, heart cells were first allowed to accumulate 201Tl or 99mTc-SESTAMIBI in control buffer to plateau levels, then metabolic inhibition was initiated with addition of IAA.

**Figure 2.** Plots of the effect of duration of metabolic inhibition with iodoacetate (IAA) (1 mM) and rotenone (10 μM) on ATP content (panel A), sodium (●) and potassium (■) contents (panel B), and lactate dehydrogenase (LDH) release from chick cardiac myocytes (panel C). For panels A and B, preparations were preincubated in metabolic inhibitors for the times indicated and rinsed in ice-cold buffer, and contents were determined as described in “Methods.” For panel C, LDH released from cells into the buffer after the indicated time of metabolic inhibition was determined (■) and expressed as a percentage of total cell LDH (325 units/mg protein); □, LDH released by cells incubated in control buffer. Each point with error bars is the mean±SEM of three determinations; points without bars are the average of two determinations. Data at time zero were determined on control preparations incubated in media.

**Figure 3.** Plots of effects of duration of metabolic inhibition with iodoacetate (1 mM) and rotenone (10 μM) on initial uptake rates of 201Tl (panel A) and hexakis (2-methoxyisobutyryl isonitrile) technetium (I) (99mTc-SESTAMIBI) (panel B). Data are mean±SEM (n=3) of successive initial uptake rate determinations after the indicated minutes of metabolic blockade. Data at time zero are the uptake rates in control buffer.
and rotenone to the incubation buffer. Both $^{201}$Tl and $^{99m}$Tc-SESTAMIBI demonstrated significant net efflux during ATP depletion (Figure 5), but the rapidity and extent of the response was greater with $^{99m}$Tc-SESTAMIBI ($t_{1/2}$ 7 minutes; residual activity, 4.6±0.8% of control compared with $^{201}$Tl: $t_{1/2}$ 15 minutes; residual activity, 24±2% of control; $p<0.001$). Addition of ruthenium red (15 $\mu$M), an inhibitor of the mitochondrial Ca$^{2+}$ uniporter, to the $^{99m}$Tc-SESTAMIBI incubation buffer 30 seconds before addition of the metabolic inhibitors had no effect on the rate of net efflux (data not shown; $p>0.5$).

Characterization of $^{99m}$Tc-SESTAMIBI Kinetics

The transport data thus far indicated that the kinetics of $^{201}$Tl and $^{99m}$Tc-SESTAMIBI responded differently to ATP depletion. To better understand the mechanisms of the increase in initial uptake rates of $^{99m}$Tc-SESTAMIBI during the course of metabolic inhibition, several interventions directed at altering membrane conductances and transport properties of myocardial cells were undertaken.

To test whether the enhanced uptake rates reflected a plasma membrane hyperpolarization attributable to electronegic Na-K pump stimulation, cells were exposed to IAA and rotenone as before, and then $^{99m}$Tc-SESTAMIBI uptake rates were determined in the absence or presence of ouabain (100 $\mu$M) in the loading buffer. Previous experiments have shown that the time of onset of action of ouabain is less than 6 seconds and that this concentration maximally inhibits Na-K pump activity in cultured chick heart cells.$^{42}$ Consistent with the data indicating depletion of ATP, Figure 6 shows that sodium pump inhibition had no significant effect on the injury-induced enhancement of $^{99m}$Tc-SESTAMIBI uptake rates.

To determine the relative influence of the plasma membrane potential ($E_m$) on $^{99m}$Tc-SESTAMIBI initial uptake rates during the time course of injury, heart cells were metabolically inhibited as before and then assayed successively for $^{99m}$Tc-SESTAMIBI uptake rates in buffer containing either 5.4 mM K$_o$ or 130 mM K$_i$ in the absence (Figure 7A) or in the presence (Figure 7B) of a

![Figure 4](http://circ.ahajournals.org/)

**Figure 4.** Plot of net accumulation of $^{201}$Tl (panel A) and hexakis (2-methoxyisobutyl isonitrile) technetium (I) ($^{99m}$Tc-SESTAMIBI) (panel B) into cardiac myocytes incubated with tracer in control buffer (○,●) or in the presence of iodoacetic acid (1 mM) and rotenone (10 $\mu$M) (○,□). Data are mean±SEM of three determinations each.

![Figure 5](http://circ.ahajournals.org/)

**Figure 5.** Plot of effect of metabolic inhibition on net retention of $^{201}$Tl (panel A) and hexakis (2-methoxyisobutyl isonitrile) technetium (I) ($^{99m}$Tc-SESTAMIBI) (panel B). Myocytes were preincubated to plateau in control buffer containing $^{201}$Tl (20 minutes) or $^{99m}$Tc-SESTAMIBI (60 minutes) before addition of iodoacetic acid (1 mM) and rotenone (10 $\mu$M) directly to the loading buffer. Net content of tracer was determined after the indicated times of inhibition (○,●) or continued incubation in control buffer (○,□). Each point with error bars is mean±SEM of three determinations. Points without error bars are the mean of two determinations.
high dose of the potassium ionophore valinomycin (1 µg/ml). High-K<sub>a</sub>-induced depolarization of E<sub>m</sub> inhibited up to 93% of the initial uptake rate of <sup>99</sup>mTc-SESTAMIBI both early and late in the course of metabolic inhibition. The presence of valinomycin in the high-K<sub>a</sub> assay buffer, which eliminated any possible contribution of mitochondrial potential (ΔΨ),<sup>29,43</sup> reduced the residual uptake rate observed in high-K<sub>a</sub> buffer in the absence of metabolic inhibition but had no additional effect during inhibition (Figure 7B and Table 1). The small residual uptake in high-K<sub>a</sub> buffer plus valinomycin therefore represented potential-independent diffusion of <sup>99</sup>mTc-SESTAMIBI.<sup>29,33</sup> Furthermore, addition of CCCP (5 µM), a mitochondrial uncoupler that depolarizes ΔΨ,<sup>29</sup> to HCO<sub>3</sub> uptake buffer had no significant effect on <sup>99</sup>mTc-SESTAMIBI initial uptake rates during ATP depletion (initial uptake rate at 20 minutes of metabolic inhibition [% control], without CCCP, 142±7.7%; with CCCP, 144±14%; n=6 each, p>0.5; at 40 minutes, without CCCP, 154±30%; with CCCP, 117±17%; n=6 each, p>0.3). Conversely, addition of valinomycin to normal-K<sub>a</sub> buffer tended to produce a slight increase in the uptake rates of <sup>99</sup>mTc-SESTAMIBI (Figure 7B), consistent with hyperpolarization of the mean E<sub>m</sub> toward the potassium reversal potential (E<sub>K</sub>). In summary, these data indicated that the enhanced initial uptake rates of <sup>99</sup>mTc-SESTAMIBI primarily reflected successive changes in the mean E<sub>m</sub> during ATP depletion.

To further characterize the plasma membrane potassium conductances contributing to the injury-induced enhancement of <sup>99</sup>mTc-SESTAMIBI uptake rates, the K<sup>+</sup> channel blocker Ba<sup>2+</sup> was added to the uptake buffer. As shown in Figure 8A, enhanced uptake rates were reduced to control levels by 1 mM Ba<sup>2+</sup> (p<0.05). Glybenclamide, an inhibitor of ATP-sensitive potassium channels,<sup>44,45</sup> significantly inhibited the enhanced uptake rates at a concentration of 100 µM (Figure 8B) but had no effect at 10 µM (data not shown; p>0.5). In addition, 10 µM quinacrine (Figure 8C) or 20 µM nordihydroguaiaretic acid, inhibitors of phospholipase A<sub>2</sub>,<sup>46,47</sup> significantly reduced injury-enhanced uptake rates of <sup>99</sup>mTc-SESTAMIBI. The inhibitors had no effect, however, at times greater than 30 minutes (data not shown; p>0.5) when <sup>99</sup>mTc-SESTAMIBI uptake rates had declined below control.

**Discussion**

Although <sup>201</sup>Tl and <sup>99</sup>mTc-SESTAMIBI are diffusible myocardial imaging agents with properties in vivo that primarily reflect regional myocardial blood flow,<sup>16</sup> both agents also behave as probes of tissue viability.<sup>17–30</sup> This property becomes especially important during reperfusion imaging when flow (agent delivery) and tissue viability (extraction) are uncoupled and assessment of

![Figure 6](#)  
**FIGURE 6.** Plot of effect of ouabain (100 µM) on hexakis (2-methoxyisobutyl isonitrile) technetium (I) (<sup>99</sup>mTc-SESTAMIBI) initial uptake rates during metabolic inhibition. Preparations were preincubated in the presence of iodoacetic acid (1 mM) and rotenone (10 µM) for the times indicated followed by determination of <sup>99</sup>mTc-SESTAMIBI uptake rates in buffer of the identical composition in the absence (■) or presence (□) of ouabain. Data at time zero were determined in control buffer without inhibitors.

![Figure 7](#)  
**FIGURE 7.** Plots of effects of high K<sub>a</sub> buffer and valinomycin on initial uptake rates of hexakis (2-methoxyisobutyl isonitrile) technetium (I) (<sup>99</sup>mTc-SESTAMIBI) during metabolic inhibition. Preparations were preincubated in normal K<sub>a</sub> buffer containing iodoacetic acid (1 mM) and rotenone (10 µM) for the times indicated in each panel. For panel A, successive <sup>99</sup>mTc-SESTAMIBI uptake rates were determined in buffer of identical composition (■) or 130 mM K<sub>a</sub> buffer plus the metabolic inhibitors (□). For panel B, uptake rates were determined in normal K<sub>a</sub> buffer plus inhibitors (■), in normal K<sub>a</sub> buffer plus inhibitors and valinomycin (1 µg/ml) (□), or in 130 mM K<sub>a</sub> buffer plus inhibitors and valinomycin (1 µg/ml) (○). Values are the mean±SEM of three determinations when error bars are present and the mean of two determinations when no error bars are present. Each panel shows results from different cultures. Data at time zero were determined in buffers in the absence of metabolic inhibitors.
TABLE 1. Effect of ATP Depletion on 99mTc-SESTAMIBI Initial Uptake Rates in Cardiac Myocytes in Various Ko Buffers

<table>
<thead>
<tr>
<th>Uptake buffers</th>
<th>Preincubation buffer</th>
<th>5.4 mM Ks</th>
<th>130 mM Ks</th>
<th>130 mM Ks + valinomycin (1 µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control MEBSS (fmol/mg protein · nM Ks · min)</td>
<td>17.7±1.0</td>
<td>6.9±0.6</td>
<td>1.2±0.2</td>
<td></td>
</tr>
<tr>
<td>MEBSS + IAA + rotenone (fmol/mg protein · nM Ks · min)</td>
<td>20.1±1.2*</td>
<td>1.4±0.1*</td>
<td>1.2±0.1</td>
<td></td>
</tr>
</tbody>
</table>

MEBSS, modified Earle's balanced salt solution; IAA, iodoacetic acid. Values are mean±SEM of four determinations each. *p<0.05 compared with control MEBSS by one-way ANOVA and modified t test.

Preparations were removed from media, rinsed in tracer-free control MEBSS preincubation buffer for 1 minute, then evaluated for 99mTc-SESTAMIBI uptake rates in each of the Ks buffers as described in "Methods," or preparations were exposed to IAA (1 mM) and rotenone (10 µM) for 20 minutes before determination of uptake rates.

regional metabolic status of myocardium can have important therapeutic and prognostic implications.

Divergent Response of 203Tl and 99mTc-SESTAMIBI Kinetics to ATP Depletion

The present study was designed to evaluate and compare the effect of metabolic interventions that mimic components of ischemic injury on 203Tl and 99mTc-SESTAMIBI kinetics in a non-flow-dependent preparation of myocardial cells. Oxidative phosphorylation and glycolysis were inhibited simultaneously by rotenone (10 µM) and IAA (1 mM), respectively, producing a precipitous decline in myocardial ATP content but modest cell injury for times less than 60 minutes as estimated by release of LDH (Figure 2). As discussed by Murphy et al.,40 LDH release appears to be a late event in the course of cell injury, being preceded by significant ion content changes and ultrastructural alterations. Under these conditions, initial uptake rates of 203Tl and 99mTc-SESTAMIBI responded in remarkably divergent ways to ATP depletion. Initial uptake rates of 203Tl declined within 20 minutes of metabolic inhibition by 50% to 70% and remained depressed for the duration. Previous studies in cultured rat and chick myocardial cells have shown that 50% to 60% of thallium influx is mediated by Na,K-ATPase,18,30 consistent with the portion of thallium uptake inhibitable by depletion of high-energy phosphates in this study. Furthermore, ouabain had no additive effect during metabolic inhibition in the present study.

Conversely, initial uptake rates of 99mTc-SESTAMIBI increased significantly by 10–20 minutes and remained elevated for the first 40–60 minutes of metabolic inhibition. Only later did 99mTc-SESTAMIBI uptake rates decline to values lower than control (Figure 3B). Because initial unidirectional uptake rates (influx) of 99mTc-SESTAMIBI respond predominantly to the Ecm,33 the data indicated that the mean Ecm was hyperpolarized during the course of injury. Consistent with this was the marked reduction in uptake rates induced by high-Ks buffer and only slight enhancement of 99mTc-SESTAMIBI uptake rates by addition of valinomycin to nor-

![Figure 8](https://circ.ahajournals.org/figure/8)

**Figure 8.** Plots of effects of alterations in potassium conductance on initial uptake rates of hexakis (2-methoxyisobutyl isonitrile) technetium (I) (99mTc-SESTAMIBI) during metabolic inhibition. Preparations were preincubated in normal Ks buffer containing iodoacetic acid (1 mM) and rotenone (10 µM) for the times indicated followed by determination of initial uptake rates of 99mTc-SESTAMIBI in buffer of the identical composition in the absence (●) or presence (○) of 1 mM Ba2+ (panel A), 100 µM glybenclamide (panel B), or 10 µM quinacrine (panel C). For the quinacrine determinations, preparations were preincubated in media containing the drug for 60–90 minutes before initiation of metabolic inhibition.47 Data at time zero were determined in control buffer in the absence of metabolic inhibitors or channel blockers. Each panel represents a different culture. *p<0.05 for individual time points compared with control. p<0.05 for entire treatment group at times ≤30 minutes in each panel by the Mann-Whitney rank-sum test.
Vol 85, No 4 April 1992

Physiological Consequences

The kinetics of 99mTc-SESTAMIBI accumulation are slow compared with action potential durations and probably allow the probe to respond only to the mean driving force of the time-averaged $E_m$. Therefore, any metabolically induced arrhythmias or electromechanical dysfunctions would contribute to uptake of the agent in proportion to their contribution to the time-averaged $E_m$. Because steady-state time-averaged $E_m$ in these beating preparations is $-41$ mV, injury-induced shortening of action potential duration would appear as hyperpolarizations of mean $E_m$. An additional direct effect could be mediated by longer-term hyperpolarization of maximum diastolic potentials toward $E_K$. Although inhibition of glycolysis and mitochondrial electron transport has previously been reported to acutely depolarize the plasma membrane in cultured chick heart cells, this effect is seen within the first 120 seconds of exposure; our model examined much longer-term events. The data indicated that mean $E_m$ hyperpolarized toward $E_K$ during the course of injury and depolarized later in parallel with $E_K$ as intracellular calcium content fell.

Our data begin to identify the plasma membrane conductances contributing to the mean hyperpolarization produced during metabolic blockade. Glybenclamide, a sulfonylurea that blocks $I_{K,ATP}$ in cardiac myocytes, significantly inhibited the enhanced initial uptake rates of 99mTc-SESTAMIBI induced by ATP depletion in a dose-dependent manner. These time-independent channels are inhibited by intracellular ATP and open in response to metabolic inhibition or anoxia. Our results further support a physiologically significant contribution by $I_{K,ATP}$ to mean $E_m$ during metabolic inhibition in a whole-cell preparation and, in addition, suggest that 99mTc-SESTAMIBI may be a useful probe for the in vivo study of these channels.

In addition, quinacrine and nordihydroguaiaretic acid significantly inhibited injury-induced enhancement of 99mTc-SESTAMIBI uptake rates. Quinacrine, a potent inhibitor of phospholipase A$_2$, has been shown to provide protection against reperfusion injury in the ischemic pig heart and to inhibit or delay arachidonic acid release from cultured rat myocardial cells exposed to a metabolic blockade similar to the present study. An ATP-insensitive potassium channel activated by arachidonic acid ($I_{K,AA}$) has been reported recently in rat atrial membrane and rat ventricular cells and has been proposed to contribute to extracellular K$^+$ accumulation in later phases of ischemic injury. The inhibitory effect of quinacrine on 99mTc-SESTAMIBI uptake rates is consistent with a contribution by $I_{K,AA}$ to mean $E_m$ in this model of myocardial injury. However, quinacrine has also been reported to inhibit phospholipase C-dependent cellular reactions as well as Na$^+$-Ca$^+$ exchange; therefore, the exact mechanism of 99mTc-SESTAMIBI inhibition is not established. Nonetheless, both $I_{K,ATP}$ and $I_{K,AA}$ are known to be blocked by Ba$^{2+}$, consistent with the large inhibitory effect on 99mTc-SESTAMIBI kinetics produced by Ba$^{2+}$ in this model. Note the temporal dissociation in the present study between changes in bulk ATP content and in subsequent tracer kinetics. This would be consistent with evidence supporting functional compartmentation of intracellular ATP pools for instance, in the cases of sodium pump-mediated 201Tl influx and $I_{K,ATP}$ activation.

Limitations of the Metabolic Inhibition Model

Cultured chick heart cells represent a model myocardial preparation that minimizes the extracellular diffusion delays that hinder evaluation of tracer kinetics in whole heart preparations. Cultured heart cell preparations have been validated by several investigative groups as model systems of cell injury to obtain fundamental information regarding changes in ion transport, electrical activity, contractility, metabolism, and morphology during reversible, and irreversible injury. It has been documented, however, that qualitative differences exist in the interactive mechanisms and contributions of ATP depletion, cell Ca$^{2+}$ membrane phospholipids, and oxygen free radicals to cell injury during pharmacological metabolic inhibition compared with oxygen and substrate deprivation characterizing ischemic injury in vivo. In particular, global ischemia in perfused heart preparations generally shows slower rates of depletion of high-energy phosphates by nuclear magnetic resonance, taking 20–30 minutes compared with <5 minutes in this model of metabolic blockade. Furthermore, the favorable property of mini-
Possible Consequences for Clinical Imaging

These kinetic observations lend themselves to a scheme correlating the relative extraction efficiencies of $^{201}$TI and $^{99m}$Tc-SESTAMIBI to degrees of myocardial injury in vivo. Increased incubation time in IAA and rotenone in our model of non-flow-dependent metabolic inhibition would correspond to increased depth of ischemic injury in myocardium in vivo. Three zones of relative rates of agent uptake would then characterize the degree of non-flow-dependent myocardial injury (Figure 9). Zone I represents very mild injury characterized by near-normal uptake rates of $^{201}$TI and mildly enhanced uptake rates of $^{99m}$Tc-SESTAMIBI (matched activity on dual tracer images). ATP levels might be depressed, but not markedly, and mean $E_{\text{pi}}$ would be normal or hyperpolarized. Zone II would represent moderate injury characterized by depressed uptake rates of $^{201}$TI (SPECT defect) but increased or near-normal uptake rates of $^{99m}$Tc-SESTAMIBI ($^{201}$TI/$^{99m}$Tc-SESTAMIBI mismatch). This level may correspond to reversibly injured or salvageable myocardium in which ATP levels are severely depleted but the plasma membrane remains intact and polarized. Zone III would then represent severe or irreversible injury characterized by concordant SPECT defects. This tissue would be devoid of high-energy phosphates, and membrane integrity would be lost. Note that this zone may actually reveal images in which the $^{99m}$Tc-SESTAMIBI defect contrasts more greatly with normal tissue than the $^{201}$TI defect does, because residual injury-insensitive uptake rates of $^{99m}$Tc-SESTAMIBI are significantly less than those of $^{201}$TI. The discordant uptake profile of both tracers during the time course of metabolic injury could be transposed to represent the border zone around a region of reperfused infarcted myocardium resulting in $^{99m}$Tc-SESTAMIBI imaging defect sizes smaller than $^{201}$TI defects. Indeed, the data and proposed grading system could explain on a metabolic basis alone the clinical observation that $^{99m}$Tc-SESTAMIBI defects are consistently smaller than $^{201}$TI defects (for example, see Reference 15).

Dissociating the contribution of flow from the influence of tissue viability on accumulation of $^{99m}$Tc-SESTAMIBI in ischemic or infarcted regions has complicated the interpretation of many previous protocols with intact heart models or patients. Nonetheless, many results could be additionally interpreted in terms of the divergent flow-independent responses of $^{201}$TI and $^{99m}$Tc-SESTAMIBI to myocardial injury. For example, several reports of mild to moderate injury in perfused heart or open-chest canine models have produced tissue activities of $^{99m}$Tc-SESTAMIBI greater than the flow determined by microspheres consistent with Zones I and II patterns. Conversely, severe injury in whole-organ models produces significant reductions in $^{99m}$Tc-SESTAMIBI activity relative to flow, consistent with Zone III pattern.

In addition, in isolated blood-perfused rabbit hearts, injection of $^{99m}$Tc-SESTAMIBI and $^{201}$TI after 10 minutes of reperfusion after a 60-minute episode of coronary occlusion showed by quantitative autoradiography that relative $^{99m}$Tc-SESTAMIBI activity was significantly less than $^{201}$TI activity in the central defect area corresponding to Zone III injury pattern. Preliminary data of activity profiles of $^{99m}$Tc-SESTAMIBI and $^{201}$TI in border zones around reperfusion injury were remarkably similar to the injury profile delineated in Figure 9. $^{99m}$Tc-SESTAMIBI activity in border regions exceeded activity of both $^{201}$TI and control region $^{99m}$Tc-SESTAMIBI. Moving toward the center of the defect, $^{201}$TI activity fell off more rapidly than $^{99m}$Tc-SESTAMIBI activity, but retained a higher residual count in the central necrotic zone, also similar to Zone III in our model. Whether these differences in tracer activity can be discerned by external imaging, given the current limitations in spatial resolution and activity in severe defects, remains to be determined.

Thus, in summary, non-flow-dependent cellular kinetics of $^{201}$TI and $^{99m}$Tc-SESTAMIBI respond differently to ATP depletion. As the degree of myocardial injury progresses, initial uptake rates of $^{99m}$Tc-SESTAMIBI first increase, then later decline, in large part reflecting alterations in mean $E_{\text{pi}}$. Physiological mechanisms contributing to the enhancement of non-flow-SESTAMIBI uptake rates during ATP depletion appear to involve ATP-sensitive and ATP-insensitive potassium channels. Conversely, initial uptake rates of $^{201}$TI decline monotonically earlier but less completely than $^{99m}$Tc-SESTAMIBI. The divergent
responses may allow dual-tracer scintigraphic mapping of the severity of myocardial injury.

Acknowledgments

We thank Georgia Washington for her secretarial assistance and Jeff Cone and Kathleen Taylor for their assistance in preparing 99mTc and 1540 Circulation Beller hexakis for tomographic technetium-99m-hexakis-2-methoxyisobutyl-1438-1444 P, Gibbons Efficacy R, left ventricular viable 235-247 pharmacologic predicted myocardial GA: necrosis within the framework of ischemic bed size (myocardium at risk) and collateral flow. Lab Invest 1979;40:633–644


64. Kim D, Clapham DE: Potassium channels in cardiac cells activated by arachidonic acid and phospholipids. Science 1989;244:1174–1176


68. Findley I: ATP-sensitive K⁺ channels in rat ventricular myocytes are blocked and inactivated by internal divalent cations. Pflugers Arch 1987;410:313–320


Divergent kinetics of 201Tl and 99mTc-SESTAMIBI in cultured chick ventricular myocytes during ATP depletion.
D Piwnica-Worms, M L Chiu and J F Kronauge

Circulation. 1992;85:1531-1541
doi: 10.1161/01.CIR.85.4.1531

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/85/4/1531