Effect of Mitochondrial and Plasma Membrane Potentials on Accumulation of Hexakis (2-Methoxyisobutylisonitrile)Technetium(I) in Cultured Mouse Fibroblasts

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Hexakis(2-methoxyisobutylisonitrile)technetium(I) (Tc-MIBI) is representative of a class of ^{99m}Tc-based lipophilic cationic mycocardial perfusion imaging agents. To test the hypothesis that the mechanism of cellular uptake may involve distribution across biologic membranes in response to membrane potential, Tc-MIBI net uptake and retention were determined in cultured mouse BALB/c 3T3, NIH 3T3, and v-src transformed NIH 3T3 fibroblasts as well as in cultured chick embryo heart cells. Isovolumic depolarization of plasma membrane potentials with 130 m*M* K 20 m*M* Cl buffer decreased Tc-MIBI net cell uptake in all preparations. In BALB/c 3T3 cells, depolarizing mitochondrial membrane potential with valinomycin in high K buffer or with the protonophore CCCP inhibited net uptake and retention of Tc-MIBI while hyperpolarizing mitochondrial and plasma membrane potentials with the K^+/H^+ exchanger nigericin increased Tc-MIBI net uptake. These results indicated that net cellular uptake and retention of Tc-MIBI in fibroblasts were determined by both mitochondrial and plasma membrane potentials; the gamma-emitting properties of Tc-MIBI may therefore raise the possibility of monitoring membrane potential in vivo.

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Hexakis(alkylisonitrile)technetium(I) complexes are a class of low valence technetium (^{99m}Tc) coordination compounds empirically designed as clinical myocardial perfusion imaging agents (*1–4*). Conceived to be used in a manner similar to thallium (^{201}Tl) for the noninvasive evaluation of coronary artery disease, the compounds exploit the more favorable emission characteristics of ^{99m}Tc for applications in clinical imaging (*5,6*). Chemical analysis of these complexes with the ground state ^{99}Tc isotope shows them to be monovalent cations with a central Tc(I) core octahedrally surrounded by six identical ligands coordinated through the isonitrile carbon (Fig. 1). The terminal alkyl groups, when bound to the technetium, encase the metal with a sphere of lipophilicity (*1,7*). Of the many agents synthesized in this class, ^{99m}Tc complexes of the 2-methoxyisobutylisonitrile ligand (Tc-MIBI) have emerged as the most promising for clinical application (*4,8–11*).

In the course of investigating mechanisms of cellular retention of these agents, studies have shown that neither the lipophilic properties nor the cationic charge alone are sufficient to characterize the biologic properties of these complexes (*12*). The requirement of lipophilicity and cationic charge for myocardial localization raises the possibility that their cellular uptake and retention mechanisms are at least in part determined by mitochondrial and plasma membrane potentials in a manner analogous to several other known permeant cationic probes of membrane potential such as tetraphenylphosphonium (TPP) and derivatives (*13–15*), safranine-O (*16*), or rhodamine-123 (*17–19*). Typically, such probes are sufficiently lipophilic to partition into or through the hydrophobic core of biologic membranes, but have a delocalized charge allowing passive transmembrane equilibration based on membrane potential in accordance with the Nernst equation (*20*). Since negative plasma membrane potentials (E_m) should accumulate the lipophilic cation in the cytoplasm relative to the extracellular spaces and strongly negative mitochondrial membrane potentials ($\Delta\psi$) should further concentrate the probe within the inner mitochondrial matrix relative to cytoplasm, the net concentration in the mitochondria would theoretically be a function of both E_m and $\Delta\psi$:

$$[\text{mito Tc-MIBI}] = [\text{extracellular Tc-MIBI}]\, e^{-(E_m+\Delta\psi)F/RT}, \quad (1)$$

where RT/F = 26 mV at 37°C.

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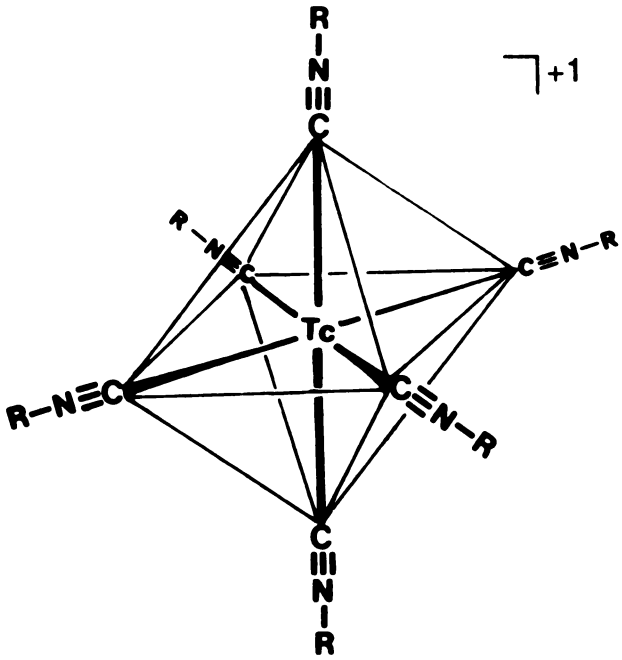


FIGURE 1
General structure of the hexakis (2-methoxyisobutyl isonitrile) technetium(I) complex emphasizing its octahedral configuration. R represents $CH_2C(CH_3)_2OCH_3$.

Cells in monolayer culture provided convenient preparations that could be physiologically and pharmacologically manipulated to evaluate the cellular uptake and retention mechanisms of the gamma-emitting Tc-MIBI complex. Net cell uptake and retention was found to be a predictable function of both mitochondrial and plasma membrane potentials.

METHODS

Cells

BALB/c 3T3 mouse fibroblasts were obtained from Brian Druker, Dana-Farber Cancer Institute, Boston, MA; NIH 3T3 cells were obtained from Helen Piwnica-Worms, Tufts University Medical School, Boston, MA; methods for obtaining v-src transformed NIH 3T3 cells have been described (*21*). Cells were grown to confluence in approximately four days on 25-mm glass cover slips placed on the bottom of 100-mm plastic culture dishes in Dulbecco's modified Eagle's medium (Grand Island Biological Co., Grand Island, New York) supplemented with 10% calf serum at 37°C in a 10% CO_2/90% air-humidified environment. Serial passage was performed by gently shaking the cells off the culture dish during exposure to 0.05% trypsin solution (GIBCO) for 3–5 min (room temperature) and diluting the cell suspension 1:5 in growth medium. Techniques for preparing monolayers of spontaneously contracting chick myocytes from 10- to 11-day old chick embryo ventricles disaggregated with trypsin have been described (*22,23*). Twenty-five-millimeter circular glass cover slips also served as substrate for attachment of heart cells. Freeze-thawed preparations were made by removing culture medium from the cells and incubating plates at −20°C for 6–12 hr. Plates were allowed to warm to room temperature for 10–15 min prior to uptake experiments.

Preparation of Tc-MIBI

Synthesis of the radiolabeled compound [^{99m}Tc]MIBI was performed using a one-step kit formulation (kindly provided by T.R. Carroll, E.I. DuPont, Medical Products Division, North Billerica, MA) containing solid stannous chloride (0.075 mg) as a reducing agent for the technetium and MIBI as the $Cu(MIBI)_4BF_4$ salt. Kits were prepared and purified with minor modification of previously described methods (*10, 24*). Radiochemical purity was found to be greater than 98% by thin-layer chromatography (aluminum oxide plates, J.T. Baker, Phillipsburg, NJ) using ethanol (absolute) as the mobile phase.

Tc-MIBI Cell Accumulation

Cover slips with confluent cells were pre-equilibrated for 1 min in control buffer containing 144 m*M* NaCl, 5.4 m*M* KCl, 1.2 m*M* $CaCl_2$, 0.8 m*M* NaH_2PO_4, 0.8 m*M* $MgSO_4$, 5 m*M* glucose, 5.4 m*M* HEPES, and 1% calf serum (pH 7.4, 37°C). High K low Cl buffer was made by equimolar substitution of K-methanesulfonate for NaCl. K-methanesulfonate was made by titration of methanesulfonic acid (Eastman Kodak, Rochester, NY) with KOH prior to addition to buffer (*25*). Uptake experiments were initiated by immersion of cover slips in 60-mm glass Pyrex dishes containing 4 ml of loading solution consisting of control buffer with 0.2–5.0 n*M* Tc-MIBI (0.04–0.67 Ci/nmole). Cells on cover slips were removed from the loading buffer at various times, rinsed three times in 25 ml volumes of ice-cold (2°C) isotope-free buffer for 8 sec each to clear extracellular spaces and placed in 35-mm plastic petri dishes. Two hundred-microliter samples of the loading buffer were then obtained for standardizing cellular data with extracellular concentration of Tc-MIBI. Preparations and extracellular samples were assayed for gamma activity in a well-type sodium iodide gamma counter (Omega 1, Canberra, Meriden, CT) after which cells were extracted in 1.5 ml 1% sodium dodecylsulfate with 10 m*M* Na-borate prior to protein assay by the method of Lowry using albumin as the protein standard (*26*). Nonspecific binding of Tc-MIBI to blank glass cover slips was ≤7% of the total activity associated with cell-containing cover slips. Appropriate geometric corrections and activity standards were obtained and combined with generator equilibrium equations (*27*) to calculate the absolute concentration of total Tc-MIBI in the buffers.

Cell Water Determination

Intracellular water space was measured by a modification of the method of Kletzein et al. (*28*). Cells on cover slips were incubated in glucose-free normal K or high K low Cl buffer containing 2, 5, or 10 m*M* [^{3}H]3-O-methylglucose (MeGlc) (1μCi/ml; 65Ci/mmol) for 1 hr at 37°C. Control experiments in cultured heart cells showed complete equilibration of the non-metabolizable hexose across cell membranes by 40 min. Cells were rinsed three times in 25 ml volumes of ice-cold buffer to clear extracellular MeGlc, extracted in 1% SDS for protein determination as previously described, and assayed for tritium-3 activity by standard liquid scintillation techniques along with a 200-μl sample of the extracellular solution. MeGlc uptake/mg protein increased linearly with extracellular [MeGlc] ($r \geq 0.99$) and intersected the ordinate at a point not

significantly different from the origin ($p > 0.25$). Cell water (μl/mg protein) was the slope of the linear regression of a plot of MeGlc uptake (nmole/mg protein) versus extracellular [MeGlc] (mmol/l).

Ionophores and Transport Inhibitors

Ouabain, nigericin, valinomycin and carbonylcyanide-m-chlorophenylhydrazone (CCCP) (all reagents from Sigma Chemical Co., St. Louis, MO) were dissolved in dimethylsulfoxide (Me_2SO) prior to addition to buffers. Final Me_2SO concentration was typically <0.25%.

RESULTS

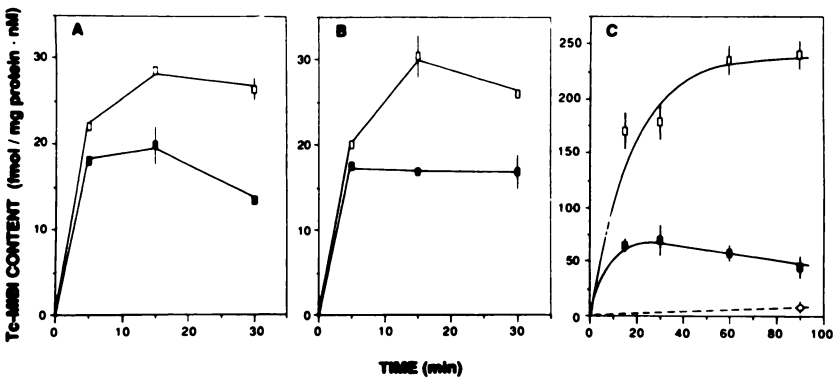
NIH 3T3 cells in monolayer culture exposed to Tc-MIBI-containing control buffer accumulated the lipophilic cation to a plateau level within ~15 min (Fig. 2). Development of plateau uptake in the continued presence of extracellular Tc-MIBI implied that a steady-state or equilibrium condition had been achieved. Cells depolarized by exposure to 130 m*M* K, 20 m*M* Cl buffer also demonstrated plateau accumulation, but to a significantly lower level. Substitution of Cl by equimolar concentrations of the impermeant anion methanesulfonate was used to prevent the high K-induced swelling typically observed in vertebrate cells (see discussion). Cell water space measurements confirmed the lack of cell swelling in high K low Cl buffer (normal K buffer: 5.4 ± 0.5 μl/mg protein; high K low Cl buffer: 4.8 ± 0.2 μl/mg protein; $p > 0.3$).

V-src transformed NIH 3T3 fibroblasts and primary cultured chick embryo heart cells were similarly incubated in Tc-MIBI-containing buffer under normal K and high K, low Cl conditions (Fig. 2). All preparations demonstrated plateau Tc-MIBI accumulation to greater levels in normal K buffer compared to high K buffer. Cell water space did not change significantly in high K low Cl buffer (transformed 3T3 cells: 5.4 ± 0.3 (n = 7) versus 5.5 ± 0.5 (n = 9) μl/mg protein; chick heart cells: 6.9 ± 0.4 (n = 9) versus 7.3 ± 0.7 (n = 9) μl/mg protein in normal K versus high K buffer, respectively; $p > 0.5$). Both non-transformed and v-src transformed NIH 3T3 cells showed uptake half-times and plateau accumulation of Tc-MIBI similar to each other, but contracting myocytes showed greater net Tc-MIBI accumulation with longer half-times. Normalized to constant extracellular Tc-MIBI concentration (Fig. 2), heart cells demonstrated plateau uptake per milligram of protein nearly ten-fold over the other cells tested and did not obtain a steady-state until after ~40–60 min. Furthermore, using the cell water spaces of 5.4 μl/mg protein for fibroblasts and 6.9 μl/mg protein for heart cells, the uptake values in Figure 2 correspond to nominal cytosolic:buffer Tc-MIBI concentration ratios of 5 and 35 for fibroblasts and heart cells, respectively. However, other lipophilic cations have been reported to accumulate in mitochondria, which raises the possibility of subcellular compartmentation of Tc-MIBI in both preparations and hinders use of simple accumulation ratios for full characterization of cellular uptake processes. This hypothesis was further tested with the use of ionophores.

Effect of Ionophores

CCCP is a protonophore that collapses mitochondrial $\Delta\psi$ and has also been reported to collapse plasma membrane potentials in vertebral cells such as mouse NG108–15 (*14*). After allowing Tc-MIBI uptake to proceed to plateau in both non-transformed and v-src transformed NIH 3T3 cells, addition of CCCP (5 μM) to the external loading buffer collapsed the membrane potentials causing a rapid and near complete net washout within 1–2 min of the previously accumulated cellular Tc-MIBI (Fig. 3). The rapid rate of CCCP-induced net washout indicated that steady-state levels of Tc-MIBI retention probably represented a balance between influx and efflux of the cation rather than irreversible binding of the complex to cellular components. The CCCP-insensitive net uptake represented <10% of total net uptake in these cells suggesting that nonspecific binding of Tc-MIBI was present, but low. Furthermore, HPLC analysis showed no evidence of CCCP-induced dissociation or alteration of the [^{99m}Tc] MIBI complex (data not shown). A similar experiment was performed with BALB/c 3T3 cells which also demonstrated rapid loss of Tc-MIBI upon addition of CCCP

FIGURE 2
Net uptake of Tc-MIBI by cultured mouse NIH 3T3 fibroblasts (A), v-src transformed NIH 3T3 fibroblasts (B), and chick embryo heart cells (C). □, control accumulation in normal K buffer; ■, accumulation in 130 m*M* K, 20 m*M* Cl buffer; ○, accumulation by freeze-thawed heart cells in normal K buffer. Each point is the mean of 2–4 determinations. Bars where indicated represent s.e. Net uptake has been normalized to extracellular Tc-MIBI and is expressed as fmole cellular Tc-MIBI/mg protein per n*M* extracellular Tc-MIBI.



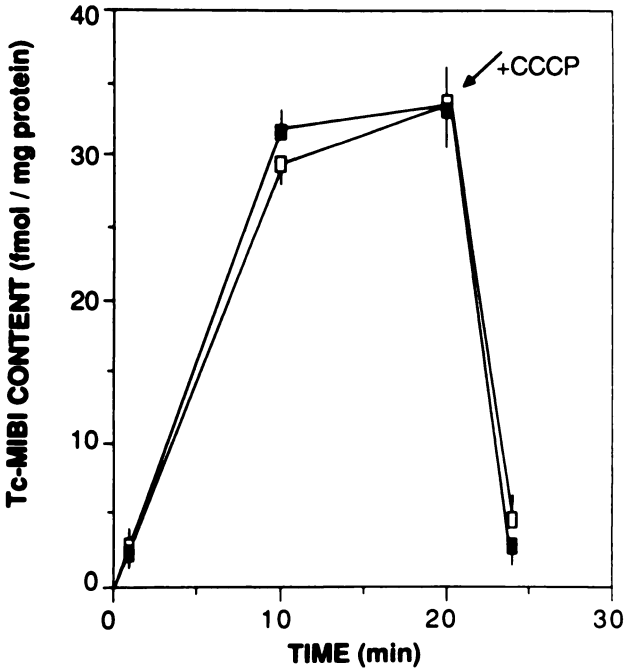


FIGURE 3
Net uptake of Tc-MIBI by NIH 3T3 cells (□) and v-src transformed NIH 3T3 cells (■) in normal K buffer. At the indicated time, CCCP (5 μM) was added to the buffer. Each point is the mean ± s.e. for 3–4 determinations.

to the extracellular loading medium (Fig. 4). In this series of experiments, freeze-thawed BALB/c 3T3 preparations were used as an estimate of nonspecific Tc-MIBI binding or adsorption into lipid membranes and debris. The CCCP-induced loss of Tc-MIBI reduced cellular Tc-MIBI retention in intact cells to approximately the levels found in freeze-thawed preparations. The slightly greater Tc-MIBI uptake in freeze-thawed

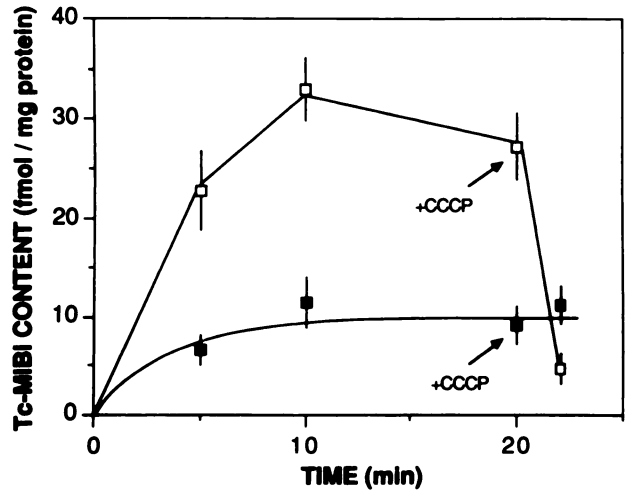


FIGURE 4
Net uptake of Tc-MIBI by BALB/c 3T3 cells (□) and freeze-thawed BALB/c 3T3 preparations (■) in normal K buffer. At the arrows CCCP (5 μM) was added to the buffer. Each point is the mean ± s.e. for 3–4 determinations.

TABLE 1
Effect of High K Buffer and Valinomycin on Tc-MIBI Accumulation

Buffer	Cell uptake (fmole/mg protein) NIH 3T3	v-src transformed NIH 3T3
5.4 mM K	16.3 ± 1.2 (4)	14.3 ± 1.3 (4)
130 mM K, 20 mM Cl	5.6 ± 0.4 (5)	5.7 ± 0.7 (5)
130 mM K, 20 mM Cl plus valinomycin (1 μg/ml)	2.0 ± 0.2 (5)	1.9 ± 0.2 (5)

Plateau uptake (30 min) of Tc-MIBI (fmole/mg protein) in NIH 3T3 and v-src transformed NIH 3T3 fibroblasts in various buffers. Results are expressed as mean ± s.e. (number of determinations are in parentheses). Extracellular Tc-MIBI concentration was 0.38 nM.

3T3 preparations compared to CCCP-treated cells may have represented residual uptake in surviving subcellular organelles, although addition of CCCP to freeze-thawed 3T3 preparations produced no further release of Tc-MIBI. The residual uptake in freeze-thawed myocyte preparations was also significantly reduced to <3% of control equilibrium uptake (Fig. 2C).

To begin to evaluate the interaction of E_m and $\Delta\psi$ on Tc-MIBI accumulation and retention, Tc-MIBI net uptake was determined in NIH 3T3 cells exposed to 130 mM K, 20 mM Cl buffer in the presence or absence of the potassium ionophore valinomycin (Table 1). Since E_m in high K buffer should have been approximately zero, increasing potassium permeability by addition of valinomycin (1 μg/ml) would have had no further affect on E_m (K_i approximately equaled K_o). Therefore, the further reduction of Tc-MIBI net uptake observed in high K buffer containing valinomycin probably represented effects mediated by collapse of mitochondrial $\Delta\psi$ alone.

To further investigate the contributions of E_m and $\Delta\psi$ to net cellular uptake and retention of Tc-MIBI, BALB/c 3T3 cells were exposed to normal K buffer containing the ionophore nigericin (5 μg/ml). This ionophore mediates electroneutral K^+/H^+ exchange and is known to collapse the pH gradient across mitochondrial inner membranes and produce a secondary increase in $\Delta\psi$ (*29*). Figure 5 shows a three-fold increase in 20-min Tc-MIBI accumulation induced by addition of nigericin to the loading medium at the start of the experiment. Similarly, after allowing Tc-MIBI uptake to proceed to equilibrium in control buffer, subsequent addition of nigericin further increased cellular Tc-MIBI accumulation (Fig. 6). However, the data indicated that the nigericin-induced enhancement of Tc-MIBI net uptake probably reflected the combined effects of both mitochondrial membrane hyperpolarization as well as plasma membrane hyperpolarization. For example, inhibition of the plasma membrane electrogenic Na^+/K^+

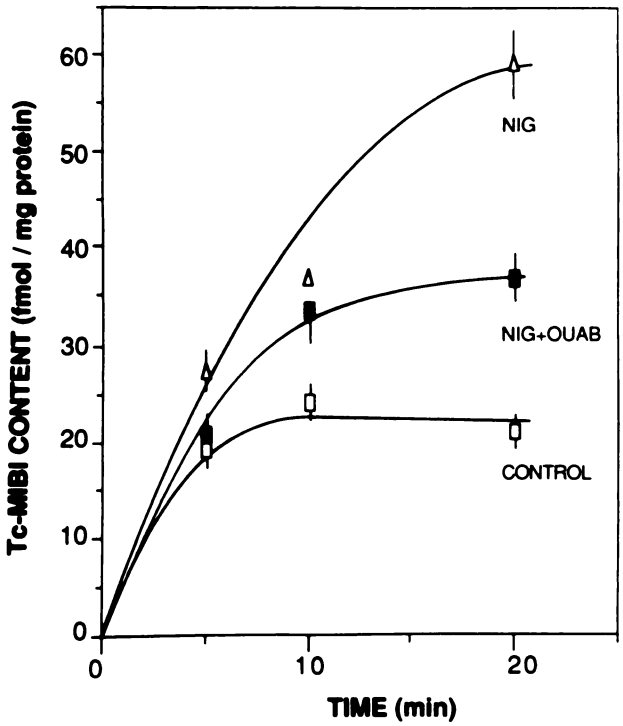


FIGURE 5
Net uptake of Tc-MIBI by BALB/c 3T3 cells in normal K buffer. (□) control buffer; (△) nigericin (5 μg/ml) or (■) nigericin (5 μg/ml) plus ouabain (100 μ*M*) added to buffer at start of experiment. Extracellular Tc-MIBI concentration was 0.86 nM. Each point is the mean ± s.e. for 3–4 determinations.

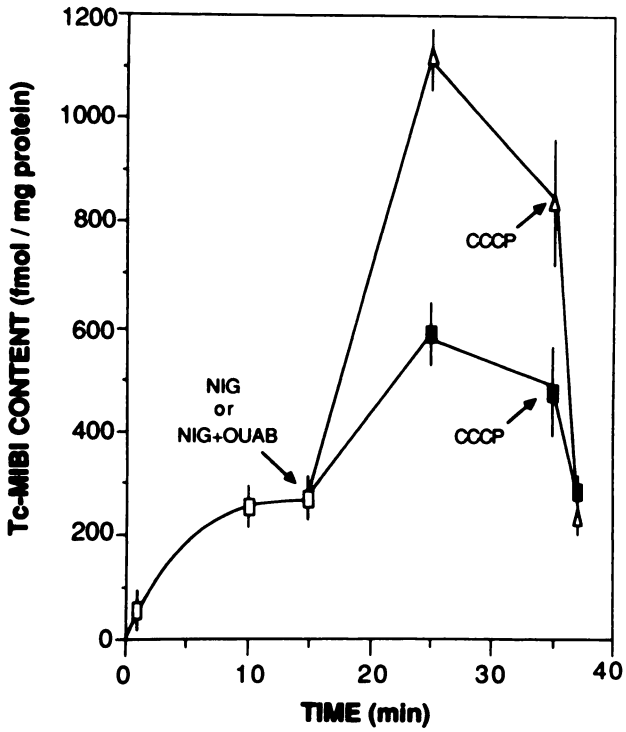


FIGURE 6
Net uptake of Tc-MIBI by BALB/c 3T3 cells in normal K buffer. Following accumulation in control buffer (□), nigericin (5 μg/ml) (△) or nigericin plus ouabain (100 μ*M*) (■) were added at the time indicated by the first arrow. CCCP (5 μ*M*) was subsequently added to buffers at the second set of arrows. Extracellular Tc-MIBI concentration was 5.8 n*M*, reflecting a lower than usual specific activity for this experiment. Each point is the mean ± s.e. for 3–4 determinations.

pump with ouabain (100 μ*M*) partially attenuated the nigericin-induced enhancement of Tc-MIBI net uptake whether added at the start of the experiment or following plateau accumulation. Ouabain alone does not affect the early kinetics (1-min uptake) of Tc-MIBI transport (*30*). As expected, addition of CCCP, thereby collapsing the nigericin-induced hyperpolarization, rapidly depleted cellular Tc-MIBI content (Fig. 6).

The effect of membrane hyperpolarization on Tc-MIBI unidirectional washout was also tested (Fig. 7). Retention time of Tc-MIBI in BALB/c 3T3 cells was increased by hyperpolarizing the mitochondrial membrane potential with nigericin plus ouabain in a manner similar to previous reports with other lipophilic cations sensitive to membrane potential (*19,31*).

DISCUSSION

The use of permeant lipophilic ions to measure electrical potentials across biologic membranes has been well established since the introduction of the technique in the early 1970s (*32*). Tritium-labeled cations exemplified by tetraphenylphosphonium (TPP) and triphenylmethylphosphonium (TPMP) have been applied to a wide variety of prokaryotic and eukaryotic systems generally too small for direct measurement of membrane potential with microelectrode techniques (*13–15*). Optical probes exemplified by the cyanine dyes DiS-C_3 (*5*) and DiO-C_5 (*3*), or the laser dye rhodamine-123 have similarly found wide application (*33–35*). In general, these ions are synthesized such that they are sufficiently lipophilic to partition into and through the hydrophobic core of biologic membranes, but also possess a charge which induces a potential-dependent transmembrane distribution (*34*).

Hexakis(alkylisonitrile)technetium(I) complexes are a class of cations originally synthesized for use as myocardial perfusion imaging agents to exploit the favorable gamma emission characteristics of ^{99m}Tc (*1*). The data presented in this report indicate that one of the most clinically promising agents of this class, Tc-MIBI, is a lipophilic cation that may serendipitously possess the unique combination of properties required to be a probe of biologic membrane potential.

Estimation of E_m

In the four cell culture preparations tested, net uptake of Tc-MIBI was easily detected using standard cellular physiologic techniques. E_m is primarily a potassium diffusion potential in cultured chick heart cells (*36*) and assuming this relation in fibroblast cells (*37,38*), the E_m in 130 m*M* K buffer is approximately zero. Further-

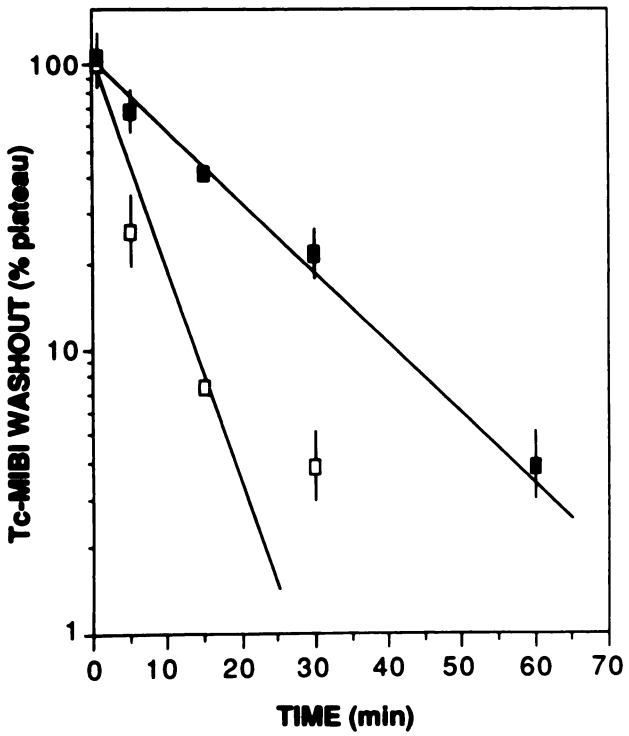


FIGURE 7
Unidirectional washout of Tc-MIBI from BALB/c 3T3 cells. After accumulation of Tc-MIBI to plateau (15 min) in control buffer (□) or buffer containing nigericin (5 μg/ml) plus ouabain (100 μM) (■), cells were transferred to Tc-MIBI-free buffer of the identical content and cell-associated activity assayed at various times. Data are normalized to plateau accumulation for each buffer. Each point is the mean ± s.e. for 3–4 determinations. Note logarithmic scale.

more, addition of an ionophore such as valinomycin to high K buffer should prevent mitochondrial accumulation of the lipophilic cation (*15*). Therefore, by analogy to methods used with TPP, E_m can be estimated with the Nernst equation from Tc-MIBI intracellular/extracellular concentration ratios after correcting the plateau accumulation of Tc-MIBI in normal K buffer by the potential-independent net uptake determined in high K buffer plus valinomycin (*15*). Within the errors inherent to this technique (*15,19*), the data in Table 1 and cell volumes were used to estimate E_m for NIH 3T3 and transformed NIH 3T3 cells as −51 ± 4 mV and −47 ± 4 mV, respectively. We therefore, found no difference in E_m between non-transformed and v-src transformed NIH 3T3 fibroblasts, confirming the results of others using rhodamine-123 applied to Rous sarcoma virus-transformed NIH 3T3 fibroblasts (*31*). In addition, these estimates of E_m were slightly less but comparable to other estimates of E_m for fibroblasts (*15, 38*), further supporting this model for Tc-MIBI uptake.

In applying the normal K minus high K correction to determine E_m, cell volumes have generally been directly estimated in order to calculate intracellular concentrations of the membrane potential probes (*14, 15*). Previous experiments in cultured chick heart cells have shown that balancing the transmembrane chemical gradients for Na, K, and Cl prevents high K-induced cell swelling by maintaining the volume-responsive ($Na^+ + K^+ + 2Cl^-$) and ($K^+ + Cl^-$) co-transporters near thermodynamic equilibrium (*39*). These transporters are quite ubiquitous and, therefore, as an alternative approach for determination of E_m, the present study prevented high K-induced cell swelling by lowering extracellular Cl to a concentration expected to thermodynamically balance the transporters. Cell water space determinations confirmed the inhibition of high K-induced cell swelling which would allow use of cell Tc-MIBI content rather than Tc-MIBI concentration for estimating E_m. While potentially simplifying the experiments, this method could still yield reasonable values for E_m, but further experiments are required to independently check the assumption that high K, low Cl solution does not also affect sarcolemmal and mitochondrial nonspecific binding of Tc-MIBI in order to fully verify the application of high K corrections for estimating E_m. In addition, we often observed a slow progressive decline in Tc-MIBI cell content in high K buffer which may have reflected cell injury and a loss of mitochondrial membrane potential under these conditions.

Influence of Mitochondria

The cellular data indicated that Tc-MIBI was responding to both mitochondrial and plasma membrane potentials in a manner identical to that reported for several other well characterized lipophilic cations. Differentiating the contribution from these two components is not straightforward, but two experimental protocols appeared to separate the influence of E_m and $\Delta\psi$ on Tc-MIBI net uptake:

1. The reduction of Tc-MIBI accumulation produced by depolarizing the plasma membrane potential with high K buffer was further decreased by the addition of valinomycin. Since the plasma membrane potential was nearly zero by equalizing the plasma transmembrane electrochemical gradient for K^+, the increase in K^+ permeability produced by valinomycin should have had little further effect on E_m. Thus, the valinomycin-induced reduction of Tc-MIBI net uptake in high K buffer should have reflected the consequence of collapse of the mitochondrial membrane potential alone. However, if K_i and K_o were not equal upon addition of valinomycin, then a component of the reduction in Tc-MIBI uptake could have resulted from a loss of E_m induced by valinomycin-mediated equilibration of K_i and K_o.
2. Ouabain, a specific inhibitor of the plasma membrane electrogenic Na^+/K^+ pump, significantly inhibited the nigericin-induced enhancement of

Tc-MIBI net uptake in BALB/c 3T3 cells. This implied that nigericin may have caused both plasma membrane as well as mitochondrial membrane hyperpolarizations. The well characterized lipophilic cation TPP demonstrates nearly identical nigericin-induced enhancement and ouabain-induced attenuation of net uptake in CV-1 cells (*19*). The mechanisms of the nigericin-induced mitochondrial membrane hyperpolarizations are well documented (*29*), but the apparent nigericin-induced plasma membrane hyperpolarization is less well understood. Although several possible mechanisms have been proposed (*19*), which focus on indirect stimulation of the Na^+/K^+ pump, changes in ionic content are also possible.

Nonetheless, the main conclusion of this series of experiments was that Tc-MIBI demonstrated changes in cellular accumulation and retention in response to alterations in membrane potential identical to that previously reported for other potential-sensitive lipophilic cations. This likely accounts for the mechanism of cellular distribution of the technetium organometallic complex.

Consequences of Potential-Dependent Accumulation

Equilibrium uptake of Tc-MIBI in cultured chick heart cells was nearly ten-fold greater per milligram of protein than the net uptake in BALB/c 3T3 and NIH 3T3 fibroblasts. Several factors could have contributed:

1. Plasma membrane potentials of heart cells are generally greater than fibroblasts (Fig. 2 and Ref. 36), therefore the increased myocardial cytosolic pre-concentration of Tc-MIBI could secondarily drive more of the agent into mitochondria compared to non-cardiac cells (see Equation 1).
2. Mitochondrial membrane potentials may be greater in myocytes relative to fibroblasts thereby resulting in increased mitochondrial sequestration of the agent in heart tissue. In this regard, the nigericin-induced increase of mitochondrial membrane potentials in BALB/c 3T3 cells caused Tc-MIBI uptake to approach the levels seen in heart cells (Fig. 5 and Fig. 6).
3. Mitochondrial volume could be greater in myocytes and contribute to overall increased accumulation of the agent. These mechanisms relating to relative levels of Tc-MIBI cellular uptake could contribute in part to the apparent myocardial specificity of Tc-MIBI in clinical imaging.

Use of fluorescent probes of membrane potential such as the cyanine or laser dyes are confined to optically transparent preparations such as isolated cells, organelles, cultured cells, or the surface of tissues. Similarly, application of ^3H-labeled compounds such as [^3H]TPP have also generally been confined to isolated cells, organelles, or cultured preparations where independent measure of intra- and extracellular compartments are feasible. Beta emissions from ^3H-labeled compounds localized within biologic tissues obviously cannot be detected within whole organs. Tc-MIBI, while suitable for cultured preparations as demonstrated in this study, can also be applied to whole organ or animal studies due to the high energy gamma photons which readily penetrate biologic tissues. This makes feasible the potential application of this new lipophilic cation for noninvasive monitoring of mitochondrial and plasma membrane potentials in vivo.

In summary, the data presented in this study indicate that Tc-MIBI, originally synthesized as a myocardial perfusion imaging agent, is in addition a lipophilic cation responsive to changes in mitochondrial and plasma membrane potentials in cultured fibroblasts. This organometallic complex may therefore possess some interesting biologic properties worth exploiting in physiologic studies and new clinical applications.

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