Technetium-99m-Tetrofosmin, Technetium-99m-MIBI and Thallium-201 Uptake in Rat Myocardial Cells

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The mechanisms of uptake and intracellular distribution of ^{99m}Tc-tetrofosmin, ^{99m}Tc-MIBI and ^{201}Tl and the behaviors of ^{99m}Tc-tetrofosmin and ^{99m}Tc-MIBI in relation to Na^+ were studied with primary cultures of myocardial cells. **Methods:** Both the uptake and the washout of the tracers were sequentially measured. The cells were treated with ouabain, bumetanide, tetrodotoxin, dimethyl amiloride (DMA), nigericin and carbonyl cyanide m-chlorophenylhydrazone (CCCP) to observe the effects of the uptake and intracellular distribution of the traders. Cells equilibrated in buffers with or without Na^+ were treated with monensin and DMA to evaluate the effect of Na^+ on the accumulation of the tracers. **Results:** Despite the similarities in uptake kinetics, there was a higher level of retention of ^{99m}Tc-tetrofosmin inside the cells. Ouabain, bumetanide and tetrodotoxin did not show any inhibitory effect on the uptake of ^{99m}Tc-tetrofosmin and ^{99m}Tc-MIBI, whereas they produced various degrees of inhibition of ^{201}Tl uptake. DMA produced approximately 35% inhibition of ^{99m}Tc-tetrofosmin uptake and 50% inhibition of ^{99m}Tc-MIBI uptake. Nigericin increased the uptake of ^{99m}Tc-MIBI by the cells. The addition of CCCP produced the release of 38% of the accumulated ^{99m}Tc-tetrofosmin and 52%–70% of the accumulated ^{99m}Tc-MIBI, indicating that these percentages of accumulation were related to mitochondrial uptake. Neither Na^+-free buffer nor monensin had any significant effect on ^{99m}Tc-tetrofosmin accumulation, but they both caused increased accumulation of ^{99m}Tc-MIBI. **Conclusion:** The uptake of both ^{99m}Tc-tetrofosmin and ^{99m}Tc-MIBI through the cell membrane is partly related to the Na^+/H^+ antiporter system. Only part of the accumulated ^{99m}Tc-tetrofosmin inside the cells enters into mitochondria; most of the accumulated ^{99m}Tc-MIBI is related to mitochondrial uptake. This uptake may be related to Na^+.

Key Words: technetium-99m-tetrofosmin; technetium-99m-MIBI; thallium-201; myocardial cell; ion transport inhibitors

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Both ^{201}Tl-chloride and ^{99m}Tc-MIBI are monovalent cations that are widely used as myocardial perfusion agents (*1,2*). The uptake by cells of ^{201}Tl, which is a K^+ analog, is related to cell membrane potential, to Na^+, K^+ and adenosine triphosphatase activity, and to other K^+ channels through the cell membrane (*3–5*). The accumulation of ^{99m}Tc-MIBI in the cell also is related to cell membrane potential, and passage through this membrane is thought to involve passive diffusion (*6–8*). Whether there is involvement of ion transport systems through the cell membrane has not yet been clearly determined. Furthermore, the behaviors of these tracers inside cells seem to be different. Technetium-99m-MIBI is localized mostly inside mitochondria because of negative mitochondrial membrane potential, whereas ^{201}Tl remains in the cytosolic compartment (*4,6,9*). Technetium-99m-tetrofosmin, a newly developed compound of the diphosphin group, is currently being used as a myocardial perfusion agent (*10,11*). It is a monovalent lipophilic cation that rapidly enters myocardial cells because of its lipophilic properties (*10,12*), although these properties alone may not be the sole determinant and various channels may be involved in its uptake through the cell membrane.

To determine the possible involvement of cell membrane ion transport systems and the intracellular distribution of ^{99m}Tc-tetrofosmin, we used the following ion transport and metabolism inhibitors; ouabain, an Na^+, K^+ and adenosine triphosphatase inhibitor (*13*); dimethyl amiloride (DMA), a selective and potent Na^+/H^+ antiporter inhibitor (*14,15*); bumetanide, an Na^+-K^+-$2Cl^-$ cotransport inhibitor (*16*); tetrodotoxin, a voltage-sensitive Na^+ channel blocker (*17*); nigericin, and ionophore that increases mitochondrial inner membrane potential (*18*); and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), an uncoupler of oxidative phosphorylation that depolarizes mitochondrial membrane potential (*19*). To study the effect of Na^+ flux on the accumulation of ^{99m}Tc-tetrofosmin and ^{99m}Tc-MIBI and whether their uptake is proportional to the intracellular concentration of Na^+, we used buffers with and without Na^+, DMA and monensin, an Na^+ ionophore that increases Na^+ uptake into cells (*18*). The uptake and washout kinetics of ^{99m}Tc-tetrofosmin were studied also, and the results were compared with those for ^{99m}Tc-MIBI and ^{201}Tl.

MATERIALS AND METHODS

Cells

Primary cultures of rat myocardial cells were prepared by the modified method of McCall (*20*). Whole hearts, excluding the great vessels, from 1- to 2-day-old Sprague-Dawley rats were used. After collection under sterile conditions, the hearts were cut and washed with growth medium (Eagle's minimum essential medium supplemented with 10% fetal calf serum and bicarbonate) to clean away all blood. Then, the hearts were smashed, cut into small pieces (<0.5 mm) and broken down to single cells by repeated trypsinization (0.25% trypsin in phosphate-buffered saline). One cycle of trypsinization lasted 7–10 min, and the products of the first two cycles were discarded. Subsequently acquired supernatant layers were transferred into sterilized centrifuge tubes and centrifuged at 1000 rpm for 3–4 min. The supernatant layers were discarded, and the cell pellets were resuspended in fresh growth medium. The resulting cell suspensions were transferred to a 225-cm^2 tissue culture flask (Costar, Cambridge, MA) containing growth medium and incubated at 37°C in a humidified 5% CO_2–95% air atmosphere for 2.5–3 hr. During this incubation period, most fibroblasts attach to and spread out on the culture flask (*21*). After the end of the incubation, medium containing myocardial cells was poured into another culture flask, and fresh growth medium was added to obtain the desired concentration of cells. Then, the cells were transferred to 22-mm cell culture wells (Corning Glass Works, Corning, NY) at 1.5×10^5 to 2×10^5 cells

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in 2 ml of medium per well and kept at 37°C in a humidified 5% CO_2–95% air atmosphere for 3 days. For a single batch, the number of cells was the same in all wells. All experiments were performed after 3 days, by which time a synchronously contracting monolayer of myocardial cells was observed in each well. After 3 days, the old medium was discarded and, after the cells were washed once, replaced with 0.5 ml of Hanks' solution (containing, in millimoles per liter, the following: KCL, 5.36; NaCl, 136.89; KH_2PO_4, 0.44; Na_2HPO_4, 0.33; $CaCl_2$, 1.26; $MgCl_2$, 0.49; $MgSO_4$, 0.4; and $NaHCO_3$, 2.38; pH 7.4–7.5), except in experiments done to evaluate the effect of Na^+ on the accumulation of ^{99m}Tc-tetrofosmin and ^{99m}Tc-MIBI, in which cells were washed and the medium was replaced with 0.5 ml of either Na^+-containing buffer [buffer A, containing, in millimoles per liter, the following: NaCl, 140; KCl, 2; KH_2PO_4, 0.7; KOH, 2.3; glucose, 5.6; and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 5; pH 7.3 or Na^+-free buffer (buffer B, containing, in millimoles per liter, the following: *N*-methyl-D-glucamine chloride, 140; KCl, 2; KH_2PO_4, 0.7; KOH, 2.3; glucose, 5.6; and HEPES, 5; pH 7.3). The cells were then kept at 37°C for 30 min for equilibration.

Radiotracer Preparation

Tetrofosmin was purchased from Amersham Co. Ltd., (Tokyo, Japan) as a lyophilized kit preparation. Each vial contains 230 μg of tetrofosmin. After labeling with 185–370 MBq ^{99m}Tc, tetrofosmin was diluted with saline to 0.5 μg/50 μl and added to each well containing cells.

A lyophilized MIBI kit was purchased from Daiichi Radiopharmaceuticals Co. Ltd., (Tokyo, Japan). Each vial contains 1 mg of MIBI. After being labeled with 185–370 MBq ^{99m}Tc, MIBI was diluted with saline to 0.5 μg/50 μl and added to wells.

Thallium-201, purchased from Daiichi Radiopharmaceuticals Co. Ltd., was diluted with saline and added to each well at a final dose of 74–111 kBq (2–3 μCi)/ml.

To evaluate the effect of Na^+ on the accumulation of ^{99m}Tc-tetrofosmin and ^{99m}Tc-MIBI, both of these radiotracers were diluted with an Na^+-free solution (buffer B) to 0.5 μg/10 μl and added to wells.

Chemical Preparation

All chemicals were dissolved in their respective solvents (DMA, bumetanide, nigericin, and CCCP [all from Sigma Chemical Co., St. Louis, MO] in dimethyl sulfoxide (DMSO); monensin [Sigma Chemical Co.] in methanol; ouabain [Wako Pure Chemical Industries Ltd., Japan] in phosphate-buffered saline; and tetrodotoxin [Sigma Chemical Co.] in distilled water) at 1000-fold concentrations and diluted with normal saline to obtain the final concentrations. In experiments done to evaluate the effect of Na^+, however, both DMA and monensin were diluted with an Na^+-free solution (buffer B). The final concentration of DMSO and of methanol in each preparation was <0.1%. The control study revealed no significant effect of DMSO or methanol on the uptake of the tracers.

Uptake Studies

After incubation with the radiotracers, wells containing cells were quickly washed twice with 1cc cold solutions or buffers, and the cells were disrupted with Triton X-100, transferred into tubes and counted with an auto gamma-well counter (ARC-380; Aloka, Japan). The uptake in control cells after 60 min of incubation with the radiotracers was taken as the control uptake. For the evaluation of inhibitors with normal or low Na^+ concentrations, the uptake in control cells after 60 min of incubation with an Na^+-containing solution (buffer A) was taken as the control uptake.

Evaluation of Kinetics

Uptake Kinetics. Cells were incubated with the radiotracers under basal conditions for 10, 30 and 60 min. After these incubation periods, they were collected, and their radioactivity was counted.

Washout Kinetics. After 60 min of incubation with the radiotracers, the old Hanks' solution was replaced with fresh tracer-free solution. The cells were washed and collected at 10, 30 and 60 min, and their radioactivity was counted.

Evaluation of Effects of Ion Transport Inhibitors

Cells were preincubated with (final concentration) 1 mmol of ouabain per liter, 10 μmol of tetrodotoxin per liter, 100 μmol of bumetanide per liter or DMA alone or each in combination with ouabain for 15 min and then were incubated with each radiotracer for 60 min. The dose of each of these agents used was sufficient to cause its greatest effects (*3,14,22,23*).

Evaluation of Effects of Mitochondrial Potential

Cells were preincubated with 5 μg of nigericin per ml for 15 min before the addition of radiotracers. The total incubation time with the radiotracers was 60 min. After 55 min of incubation with radiotracers, 10 μmol of CCCP per liter (final concentration) was added to the incubated cells (cells under basal conditions or cells after preincubation with nigericin), and incubation was continued for an additional 5 min.

Evaluation of Effects of Na^+ on Accumulation of Technetium-99m-MIBI and Technetium-99m-Tetrofosmin

After equilibration with either Na^+-containing buffer (buffer A) or Na^+-free buffer (buffer B with an equimolar substitution with the nonpermeating cation *N*-methyl-D-glucamine), cells were treated with either 100 μmol of DMA per liter or 10 μmol of monensin per liter or a combination of both. DMA was added at 15 min before and monensin was added at the time of incubation with the tracers.

Assessment of Viability

Because of difficulties in harvesting total cells from the culture wells with trypsin, the contractility and the morphology of the cells before and after trypan blue addition were checked, under a microscope, in each experiment without harvesting of the cells from the culture wells. The contractility and the morphology were visually compared with those of control cells in culture wells.

Data Analysis

Uptake by the cells, both under basal conditions and after the chemical challenges in the respective experiments, was expressed as a percentage of the control uptake values obtained for that batch. Data are expressed as the mean ± s.e.m., and tests of significance were performed with a one-way analysis of variance; a p value of <0.05 was considered statistically significant.

RESULTS

Because the numbers of cells were the same in all wells for a single batch in an experiment and the uptake after various chemical challenges was expressed as a percentage of the control uptake values obtained for that batch, there were no significant interculture variations for the results.

Uptake and Washout Kinetics

Figure 1 shows the uptake and washout kinetics of each tracer. All of the tracers showed similar uptake patterns, although ^{201}Tl showed significantly higher uptake within 30 min. All tracers also showed similar washout patterns, although there were differences in cell-associated activities. Thallium-201 showed the lowest cell-associated activity from 10–60 min. Although the difference was not significant at 60 min, ^{99m}Tc-

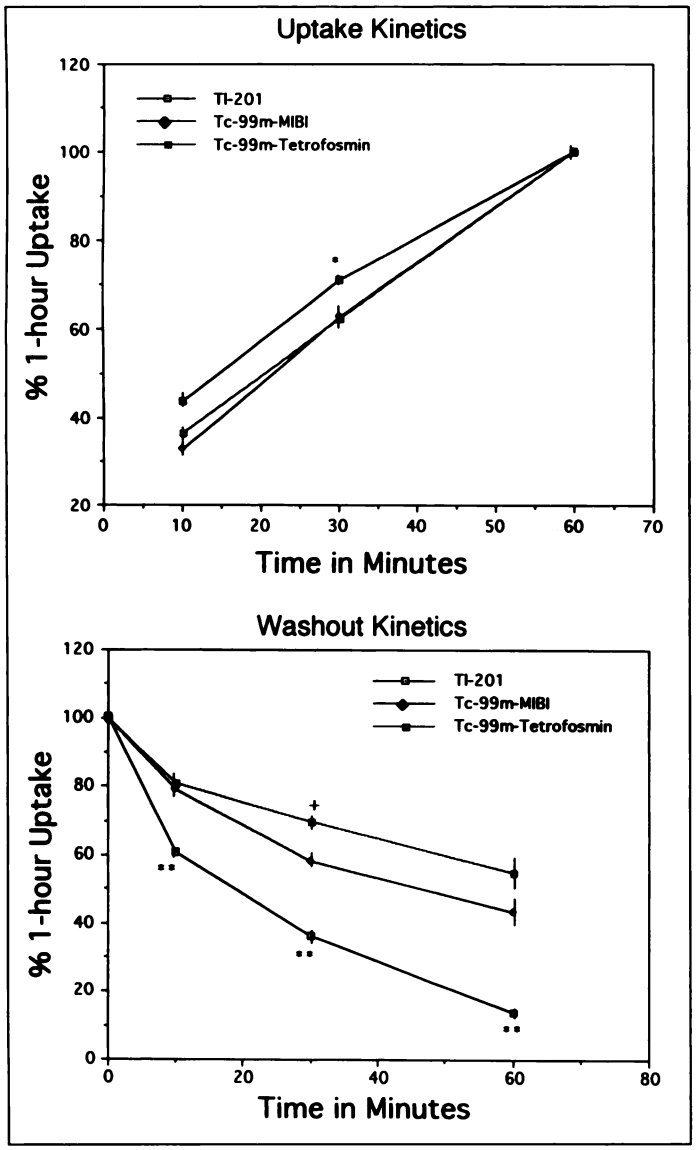

FIGURE 1. Uptake and washout kinetics of each tracer. Note that despite similar uptake kinetics, cell-associated activity of ^{99m}Tc-tetrofosmin was higher than that of ^{99m}Tc-MIBI. Thallium-201 showed higher uptake than either of them at 30 min, with rapid washout. Each point represents mean ± s.e.m. for 4–10 measurements. * and ** = significant difference compared with ^{99m}Tc-MIBI and ^{99m}Tc-tetrofosmin; + = significant difference between ^{99m}Tc-MIBI and ^{99m}Tc-tetrofosmin. * and + = $p < 0.05$; ** = $p < 0.01$.

tetrofosmin showed higher cell-associated activity at 30 and 60 min than did ^{99m}Tc-MIBI during washout.

Effects of Ion Transport Inhibitors

Table 1 shows the results for the effects of ion transport inhibitors and their significant differences. After preincubation with ouabain, only 42% of the control uptake of ^{201}Tl was observed; that is, 58% of the ^{201}Tl uptake was inhibited by ouabain. Bumetanide inhibited the uptake of ^{201}Tl by 25%. After preincubation with both ouabain and bumetanide in combination, the combined inhibitory effect (82%) was equivalent to the sum of the inhibitory effects of ouabain alone (58%) and of bumetanide alone (25%). On the other hand, ouabain and bumetanide, either alone or in combination, did not show any inhibitory effect on the uptake of ^{99m}Tc-tetrofosmin or of ^{99m}Tc-MIBI, and ouabain stimulated the uptake of ^{99m}Tc-tetrofosmin and ^{99m}Tc-MIBI. DMA inhibited the uptake of both ^{99m}Tc-tetrofosmin (35% inhibition) and ^{99m}Tc-MIBI (50% inhibition). The inhibitory effect of DMA was observed even after preincubation with ouabain, which showed a stimulatory effect. Although DMA alone inhibited the uptake of ^{201}Tl (25% inhibition), a combined inhibitory effect was not observed with preincubation with ouabain and DMA, as was observed with ouabain and bumetanide. Tetrodotoxin alone or in combination with ouabain did not affect the uptake of ^{99m}Tc-tetrofosmin or of ^{99m}Tc-MIBI. However, the uptake of ^{201}Tl was inhibited (24% inhibition) by tetrodotoxin, although a combined inhibitory effect with ouabain was not observed.

Effects of Mitochondrial Potential

Preincubation with nigericin significantly increased the uptake of ^{99m}Tc-MIBI but did not affect that of ^{99m}Tc-tetrofosmin (Table 2). The addition of CCCP under basal conditions released 38% and 52% of the accumulated ^{99m}Tc-tetrofosmin and ^{99m}Tc-MIBI, respectively. However, 38% release and 70% release of the accumulated ^{99m}Tc-tetrofosmin and ^{99m}Tc-MIBI, respectively, were observed when CCCP was added to cells preincubated with nigericin. No definite effect of nigericin and CCCP on the uptake of ^{201}Tl was observed.

Evaluation of Effects of Na^+ on Accumulation of Technetium-99m-MIBI and Technetium-99m-Tetrofosmin

In the absence of Na^+, there was a significant increase in ^{99m}Tc-MIBI uptake in cells. Monensin, an ionophore that increases intracellular Na^+ concentrations, stimulated the uptake of ^{99m}Tc-MIBI (Fig. 2A), although a significant difference was observed only with buffer A. DMA inhibited the uptake of ^{99m}Tc-MIBI in both Na^+-containing and Na^+-free buffers even in the presence of monensin. In contrast, no significant change in uptake was observed with ^{99m}Tc-tetrofosmin, although DMA inhibited ^{99m}Tc-tetrofosmin uptake to a lesser extent than it did ^{99m}Tc-MIBI uptake (Fig. 2B). However, DMA showed no effect in the presence of monensin.

Viability of the Cells

The rate of contraction of the myocardial cells changed after the addition of the chemicals and in the Na^+-free buffer, but the morphology of the cells was similar to that of control cells. However, our previous studies revealed no significant change in cell viability after treatment with these chemicals, as examined by a trypan blue dye exclusion test after harvesting of cells with trypsin (*24,25*).

DISCUSSION

Kinetics

All of the tracers showed similar uptake kinetics, with a gradual increase in uptake into myocardial cells. A similar pattern of gradual increase in uptake was observed in our previous study with tumor cell lines (*24*), and the kinetics were also in accord with those noted by other researchers (*5,26*). Although there was a gradual decrease in accumulated activity, the cell-associated activities were different for the tracers. Technetium-99m-tetrofosmin showed the highest retained cell-associated activity (55%), as was also seen in another investigation (*27*). Both uptake and washout were in agreement with those reported elsewhere (*4,5,27*).

Effect of Ion Transport Systems

There are several systems for ion transport through the cell membrane in which ^{201}Tl has been found to behave like K^+. The ouabain-sensitive fraction of ^{201}Tl uptake was 58%. To observe the possible mechanism of uptake of the remaining ouabain-resistant fraction of ^{201}Tl, we pretreated cells with bumetanide, DMA or tetrodotoxin alone or in combination with ouabain. Bumetanide inhibited 25% of the ^{201}Tl uptake, but when bumetanide was combined with ouabain, there was 82%

TABLE 1
Effects of Ion Transport Inhibitors

Parameter	Control	Ouabain	Bumetanide	Bumetanide + ouabain	DMA	DMA + ouabain	Tetrodotoxin	Tetrodotoxin + ouabain
% Uptake								
^{99m}Tc-tetrofosmin	99.9 ± 2.1 (10)	115.9 ± 3.1 (7)*	98.5 ± 2.6 (9)	109.1 ± 2.9 (8)†	65.4 ± 4.3 (8)*	71.8 ± 2.3 (8)*	101.6 ± 2.4 (6)	99.4 ± 2.7 (6)
^{99m}Tc-MIBI	100.0 ± 1.6 (8)	115.7 ± 2.8 (5)*	105.0 ± 3.3 (7)	111.5 ± 4.1 (7)†	50.2 ± 1.9 (7)*	62.7 ± 2.9 (7)*	98.2 ± 2.6 (4)	114.9 ± 4.9 (4)‡
^{201}Tl	100.1 ± 3.0 (5)	42.2 ± 1.5 (5)*	74.7 ± 3.6 (5)*	18.4 ± 2.7 (5)*§	75.2 ± 2.5 (5)*	43.4 ± 3.4 (5)*	75.5 ± 2.5 (5)*	45.3 ± 0.7 (5)*
% Inhibition								
^{99m}Tc-tetrofosmin		Stimulated	ns	Stimulated	34.6 ± 4.3 (8)	28.2 ± 2.3 (8)	ns	ns
^{99}Tc-MIBI		Stimulated	ns	ns	49.8 ± 1.9 (7)	37.3 ± 2.9 (7)	ns	Stimulated
^{201}Tl		57.8 ± 1.5 (5)	25.3 ± 3.6 (5)	81.6 ± 2.7 (5)	24.8 ± 2.5 (5)	56.6 ± 3.4 (5)	24.5 ± 2.5 (5)	54.7 ± 0.7 (5)

* $p < 0.001$ versus control.
† $p < 0.05$ versus control.
‡ $p < 0.01$ versus control.
§ $p < 0.001$ versus corresponding oubain-only pretreatment.
Cells were preincubated with each chemical agent for 15 min before incubation with radiotracers. All data are expressed as mean ± s.e.m. Numbers in parentheses indicate number of measurements. ns = nonsignificant; MIBI = hexakis-methoxyisobutyl-isonitrile; DMA = dimethyl amiloride.

inhibition, indicating that the uptake of ^{201}Tl, a K^+ analog, depends on the Na^+-K^+ pump and on Na^+-K^+-$2Cl^-$ cotransport systems, as also was observed in our previous study with tumor cells (*25*). The slight inhibition of the uptake of ^{201}Tl by DMA and tetrodotoxin alone may have been attributable to secondary reduction of sodium pump activity, as has also been suggested by others (*6*), because intracellular Na^+ levels and Na^+ influx are reported to be decreased by DMA and tetrodotoxin, respectively (*6,22*). When DMA and tetrodotoxin were used with ouabain, however, only the effect of ouabain was observed, indicating that the Na^+/H^+ antiporter system and voltage-sensitive Na^+ channels may not influence the uptake of ^{201}Tl.

Consistent with the results obtained in other investigations (*6,28*), ouabain stimulated the uptake of ^{99m}Tc-MIBI. Ouabain also stimulated the uptake of ^{99m}Tc-tetrofosmin. The stimulatory effect of ouabain may be explained as follows. After blocking of the sodium pump by ouabain, there is a loss of the sodium gradient inside the cells, and the protons (H^+) cannot be exchanged through the Na^+/H^+ antiporter, which decreases the pH inside the cells and thereby may increase the mitochondrial potential secondarily. A similar phenomenon is observed after nigericin incubation; intracellular K^+ is exchanged with extracellular H^+, and the pH inside the cells decreases (*14*). The increased mitochondrial potential may stimulate the uptake of ^{99m}Tc-MIBI and ^{99m}Tc-tetrofosmin because both of the tracers showed uptake related to mitochondrial potential (*24*). Moreover, if the uptake of ^{99m}Tc-tetrofosmin and ^{99m}Tc-MIBI involves the Na^+/H^+ antiporter, then the intracellular concentrations of these tracers may increase after blocking of the sodium pump, as the intracellular Na^+ concentration is reported to be increased after ouabain preincubation (*29*).

The effects of other ion transport inhibitors, either alone or in combination, indicate the possible involvement of other ion transport systems in the uptake of ^{99m}Tc-tetrofosmin and ^{99m}Tc-MIBI. The presence of an Na^+/H^+ antiporter system in myocardial cells is established, and this amiloride-sensitive Na^+/H^+ antiporter system has been shown to be important in regulating intracellular pH (*30*). Used alone, DMA, a more selective and potent Na^+/H^+ antiporter inhibitor than amiloride (*14,15*), inhibited 35% and 50% of ^{99m}Tc-tetrofosmin and ^{99m}Tc-MIBI activities, respectively. When DMA was used in combination with ouabain, the inhibitory effects were observed despite the stimulatory effects of ouabain. These DMA effects indicate that the Na^+/H^+ antiporter system plays an important role in the uptake of both ^{99m}Tc-tetrofosmin and ^{99m}Tc-MIBI in myocardial cells. Other investigations (*6*) as well as our previous report on tumor cells (*25*) showed similar results for the effects of amiloride on the uptake of ^{99m}Tc-MIBI and ^{99m}Tc-tetrofosmin. However, there is a conflicting report (*27*)

TABLE 2
Effects of Metabolic Inhibitors

Parameter	Control	Nigericin	CCCP	Nigericin + CCCP
% Uptake				
^{99m}Tc-tetrofosmin	99.9 ± 2.1 (10)	102.5 ± 3.4 (7)	61.4 ± 8.8 (6)*	62.7 ± 2.8 (5)*
^{99m}Tc-MIBI	100.0 ± 2.7 (5)	127.9 ± 4.7 (5)*†	47.9 ± 5.3 (6)*	29.8 ± 4.1 (5)*†
^{201}Tl	100.1 ± 3.0 (5)	85.9 ± 2.42 (5)‡	82.8 ± 2.9 (4)‡	84.4 ± 1.8 (2)§
% Inhibition				
^{99m}Tc-tetrofosmin		ns	38.6 ± 8.8 (6)	38.3 ± 2.8 (5)
^{99m}Tc-MIBI		Stimulated	52.1 ± 5.3 (6)	70.2 ± 4.1 (5)
^{201}Tl		14.1 ± 2.42 (5)	19.2 ± 2.9 (4)	15.6 ± 1.8 (2)

* $p < 0.001$ versus control.
† $p < 0.001$ for ^{99m}Tc-tetrofosmin versus ^{99m}Tc-MIBI.
‡ $p < 0.01$ versus control.
§ $p < 0.05$ versus control.
Nigericin was added to the cells 15 min before incubation with radiotracers. Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) was added 55 min after the start of incubation with radiotracers. All data are expressed as mean ± s.e.m. Numbers in parentheses indicate number of measurements. ns = nonsignificant; MIBI = hexakis-methoxyisobutyl-isonitrile.

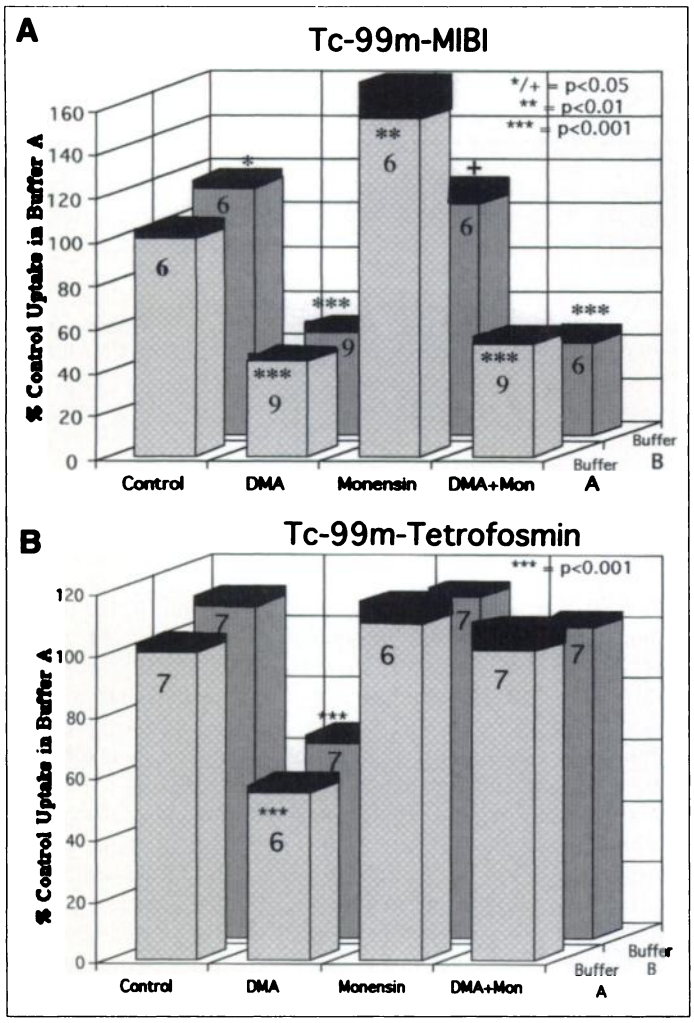

FIGURE 2. Effects of monensin (Mon) and dimethyl amiloride (DMA) in buffers with and without Na^+ on uptake of (A) ^{99m}Tc-hexakis-methoxyisobutyl-isonitrile (^{99m}Tc-MIBI) and (B) ^{99m}Tc-tetrofosmin. Number shown in each column represents number of measurements. Black area at top of each column represents s.e.m. *, **, *** = comparison with control uptake in buffer A; + = comparison between uptake with monensin in buffer A and monensin in buffer B.

that amiloride had no effect on the uptake of ^{99m}Tc-tetrofosmin in myocardial cells. This disagreement may be attributable to either the use of the more potent analog (DMA) of amiloride or the longer preincubation time with this inhibitor in our study. The sensitivity of amiloride or its analogs has been found to be dependent on the cell type and the plasma membrane location of the Na^+/H^+ antiporter (*15*).

The stimulatory effect of nigericin on the uptake of ^{99m}Tc-MIBI may be explained in either of two ways: involvement of the Na^+/H^+ antiporter or uptake of ^{99m}Tc-MIBI that is proportional to the intracellular concentration of Na^+. Because nigericin is known to decrease the pH of cells (*14*), it secondarily increases the mitochondrial potential (*6,18*). After preincubation with nigericin, the increased uptake of ^{99m}Tc-MIBI by myocardial cells may be attributable to an exchange of intracellular H^+ with extracellular ^{99m}Tc-MIBI along with Na^+ through the Na^+/H^+ antiporter system; in this respect, ^{99m}Tc-MIBI behaves like Na^+. While entering the cells, ^{99m}Tc-MIBI accumulates inside the mitochondria because of the higher mitochondrial potential. After preincubation with nigericin, there may be an exchange of intracellular H^+ with extracellular Na^+ through the Na^+/H^+ antiporter system, and the intracellular concentration of Na^+ may be increased, in turn possibly stimulating the electrogenic sodium pump to hyperpolarize membrane potentials (*6*). Therefore, the uptake of ^{99m}Tc-MIBI will be increased because its uptake is also dependent on the cell membrane potential (*6,8*).

Because there was no effect of bumetanide and tetrodotoxin, either alone or when combined with ouabain, on the uptake of ^{99m}Tc-tetrofosmin or ^{99m}Tc-MIBI, the Na^+-K^+-$2Cl^-$ cotransport system and voltage-sensitive Na^+ channel may not play any role in the uptake of these tracers.

Effects of Mitochondrial Potential

The addition of CCCP, which depolarizes the mitochondrial membrane potential and may affect cell membrane potential (*19*), caused 52%–70% release of accumulated ^{99m}Tc-MIBI from the cells, indicating that the percentage released was related to mitochondrial accumulation. The amount of ^{99m}Tc-MIBI released was not as great as in previous reports (*6,24,25*). Because ^{99m}Tc-MIBI does not bind to any nondiffusable cytosolic protein (*9*), the remaining fraction of ^{99m}Tc-MIBI in the cells may be attributable to the effect of cell membrane potential (*6,8*), which may not have been totally diminished after the addition of CCCP in our experimental model. However, only 38% release of accumulated ^{99m}Tc-tetrofosmin was observed after CCCP challenge. This finding may indicate that only a fraction of intracellular ^{99m}Tc-tetrofosmin, unlike ^{99m}Tc-MIBI, is related to mitochondrial accumulation, as was also asserted in our previous reports (*24,25*). The difference in intracellular distribution between ^{99m}Tc-MIBI and ^{99m}Tc-tetrofosmin may have influenced the washout of ^{99m}Tc-tetrofosmin when the cell-associated activity of ^{99m}Tc-tetrofosmin was greater than that of ^{99m}Tc-MIBI (Fig. 1). After entering the cell, ^{99m}Tc-tetrofosmin may bind to a nondiffusable cytosolic compartment because our previous study with tumor cells showed no nonspecific binding to cell membranes (*24*). Further clarification of the compartment inside the cells that ^{99m}Tc-tetrofosmin may enter is required.

Effects of Sodium Ion on Uptake of Technetium-99m-MIBI and Technetium-99m-Tetrofosmin

Our previous study (*25*) as well as this study revealed the involvement of the Na^+/H^+ antiporter system in the uptake of ^{99m}Tc-MIBI and ^{99m}Tc-tetrofosmin, although there was less involvement of the latter. To study the effect of Na^+ flux on the accumulation of ^{99m}Tc-MIBI and ^{99m}Tc-tetrofosmin and whether their uptake is proportional to the intracellular concentration of Na^+, we used buffers with and without (with an equimolar substitution with the nonpermeating cation *N*-methyl-D-glucamine) Na^+, and Na^+ ionophore (monensin) and DMA. The uptake of ^{99m}Tc-MIBI was significantly increased in Na^+-free buffer and after incubation with monensin, indicating that ^{99m}Tc-MIBI behaves like Na^+ during its uptake. The inhibition of uptake by DMA with or without monensin also supports our assumption as to the behavior of ^{99m}Tc-MIBI and the involvement of the Na^+/H^+ antiporter system. On the other hand, the uptake of ^{99m}Tc-MIBI also changed with the intracellular concentration of Na^+. Uptake in Na^+-containing buffer was greater than that in Na^+-free buffer with monensin. Because it is not possible to make a buffer absolutely free of Na^+ (because of the release of intracellular Na^+), monensin may have affected the concentration of intracellular Na^+ and thereby the different increases in the uptake of ^{99m}Tc-MIBI in the two buffers. An increased intracellular concentration of Na^+ may stimulate ^{99m}Tc-MIBI uptake by increasing cell membrane potential (as discussed above), and part of its uptake that involves the Na^+/H^+ antiporter system may be blocked by DMA. The insignificant change in the uptake of ^{99m}Tc-tetro-

fosmin in Na^+-free buffer and after incubation with monensin may indicate that its behavior is not similar to that of ^{99m}Tc-MIBI despite the inhibition of uptake by DMA.

CONCLUSION

Despite the similar uptake kinetics, ^{99m}Tc-tetrofosmin shows slower washout than do either of the other tracers. In myocardial cells, both ^{99m}Tc-tetrofosmin and ^{99m}Tc-MIBI show uptake related to the Na^+/H^+ antiporter system. The uptake of ^{99m}Tc-tetrofosmin also depends on the mitochondrial potential, and only a fraction of ^{99m}Tc-tetrofosmin accumulates inside mitochondria, whereas most ^{99m}Tc-MIBI accumulation occurs inside mitochondria. Either ^{99m}Tc-MIBI behaves like Na^+ or its uptake may depend on the intracellular concentration of Na^+. The uptake of ^{201}Tl by cells is partly independent of the Na^+-K^+ pump and involves the Na^+-K^+-$2Cl^-$ cotransport system; ^{201}Tl remains in the cytosol.

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