Biochimica et Biophysica Acta 812 (1985) 665–670
Elsevier

BBA 72419

Comparison of the transport of $^{42}K^+$, $^{22}Na^+$, $^{201}Tl^+$, and $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ using human erythrocytes

H. Sands, M.L. Delano, L.L. Camin and B.M. Gallagher

New England Nuclear Corporation, Immunopharmaceutical Research and Radiopharmaceutical Research Departments, 331 Treble Cove Road, North Billerica, MA 01862 (U.S.A.)

(Received April 26th, 1984)

Key words: Cation transport; Alkali ion; Synthetic monovalent cation; $(Na^+ + K^+)$-ATPase; (Human erythrocyte)

The ability of isolated human erythrocytes to exchange Na^+ for K^+ via $(Na^+ + K^+)$-ATPase was used to study the characteristics and interactions of the transport of both alkali metal and synthetic monovalent cations. Both efflux and influx studies were carried out and the results showed that: (1) Efflux of $^{22}Na^+$ from human erythrocytes was stimulated by the addition of either of K^+, or Tl^+ at 10 mM and inhibited by the addition of ouabain. Unlabeled K^+ and the addition of $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$ (dmpe, 1,2-bis(dimethylphosphino)ethane) at 5 mM had no effect on $^{22}Na^+$ efflux. (2) Influx of $^{42}K^+$ was inhibited by the addition of ouabain, unlabeled K^+, or Tl^+. $^{201}Tl^+$ influx was more rapid and of a greater magnitude than $^{42}K^+$ influx. $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$ had no effect on $^{42}K^+$ uptake. (3) Influx of $^{201}Tl^+$ was inhibited by ouabain and by the addition of unlabeled Tl^+. Addition of $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$ at 5 mM resulted in an inhibition of $^{201}Tl^+$ influx. (4) $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$ influx resembled that of $^{42}K^+$ with respect to rate and magnitude. Influx of $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ was shown to be unaffected by ouabain, unlabeled K^+ or Tl^+. Addition of 5 mM $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$ initially had no effect on $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ influx, however, a time-dependent stimulation of the influx of the $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ was observed. We conclude that the influx of the various alkali, metal and synthetic monovalent cations into erthyrocytes is mediated by different mechanisms. Most clearly, the influx of $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ is not by a mechanism similar to that of utilized by K^+ or Tl^+.

Introduction

$^{201}Tl^+$ has been used extensively in patients for the detection of acute myocardial infarction and ischemia [1]. Its main utility is for the in vivo determination of myocardial blood flow and tissue viability in humans under normal and stress-induced ischemic conditions. Because Tl^+ and K^+ shown many physiological biochemical and pharmacological similarities, it has been generally accepted that $^{201}Tl^+$ is acting physiologically as a K^+ analogue [2]. Support for this concept has been supplied by Skulskii and co-workers who demonstrated that: (1) except for the concentrations of Tl^+ needed, the $(Na^+ + K^+)$-ATPase were found to be identical to the $(Na^+ + Tl^+)$-ATPases; and (2) Tl^+ could substitute for K^+ in stimulating Na^+ efflux from erythrocytes [3–5]. Britten and Blank [6] came to similar conclusions using the $(Na^+ + K^+)$-ATPase of rabbit kidney membranes.

While $^{201}Tl^+$ has been shown to be useful in nuclear medicine [7], its expense, low energy, and relatively long physical half life (73 h) have resulted in the search for other cationic species. ^{99m}Tc is a widely available isotope in nuclear

Abbreviation: dmpe, 1,2-bis(dimethylphosphino)ethane.

medicine which is inexpensive, has optimal decay properties and a physical half life of 6 h. A ^{99m}Tc complex which is also a monovalent cation and acts physiologically similar to K^+ and Tl^+ has been suggested. Several such cationic ^{99m}Tc complexes have recently been reported as potential myocardial imaging agents and thus as clinical replacement for $^{201}Tl^+$ [8]. The efficacy of one, $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ complex to image the dog heart has been demonstrated recently [9–12].

The mechanism by which $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ is taken up into the heart is not known. In this report, the human erythrocyte's ability to exchange Na^+ for K^+ was used as a model for the $(Na^+ + K^+)$-ATPase of the heart. The data demonstrate that the influx of monovalent cations into erythrocytes is mediated by different mechanisms. Most clearly, the influx of $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ is not mediated by a mechanism similar to the utilized by K^+ or Tl^+.

Materials and Methods

Preparation of erythrocytes

100 ml of heparinized human blood was drawn weekly from different volunteers, and the erthrocytes were washed twice in a 'maintenance buffer' (10 mM NaCl, 1.0 mM $MgSO_4 \cdot 7\ H_2O$, 1.0 mM $CaSO_4 \cdot 2\ H_2O$, 1.5 mM KNO_3, 0.5 mM KH_2PO_4, 2.5 mM dextrose, 2.0 mM adenosine, 8.0 mM Trizma base, 3.0 mM $NaHCO_3$; pH adjusted to 7.4 with 1 M HCl) and resuspended in a volume of this buffer equal to the volume of plasma originally removed. The cells were stored in the maintenance buffer for 1–5 days at 4°C, and were used for experiments within 5 days after drawing.

Prior to experimental use, erythrocytes were washed three times with 'incubation buffer' (2.5 mM Na_2SO_4, 1.0 mM $MgSO_4 \cdot 7\ H_2O$, 0.5 mM $CaSO_4 \cdot 2\ H_2O$, 20 mM $NaHCO_3$, 14.0 mM dextrose, 220 mM sucrose, 20 mM choline chloride, pH adjusted to 7.4 with 1 M HCl). The cells were resuspended in this buffer, and a hematocrit of 25 was estabiished by diluting the cells with the 'incubation buffer'. The hematocrit was measured in microhematocrit tubes following 1 min of spinning in a microhematocrit centrifuge.

Efflux studies

Erythrocytes were loaded with ^{22}Na during an overnight incubation at 4°C. As indicated in the text the loading buffers containing $^{22}Na^+$ were either the 'incubation buffer' or 'Ringers buffer' (100 mM NaCl, 1 mM $MgSO_4 \cdot 7\ H_2O$, 1 mM $CaSO_4 \cdot 2\ H_2O$, 2 mM dextrose, 2 mM adenosine, 8 mM Tris, and 3 mM $NaHCO_3$). After the overnight loading, the erythrocytes were washed three times with the same loading buffer. Efflux of $^{22}Na^+$ was determined by a method identical to that described for the influx of $^{42}K^+$, except that the data are reported as percent efflux from the erythrocyte.

Influx studies

Two to four ml of prepared cells were pipetted into 20 ml glass vials which were placed in a 37°C shaker water bath. All vials were preincubated with or without 0.15 mM ouabain for 15–25 min before the addition of one of the labeled monovalent cations ($^{42}K^+$, $^{201}Tl^+$, or $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$). In addition the competing cations at the desired concentrations (K^+, Tl^+, or $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$) were added simultaneously. Influx studies were carried out in the incubation buffer. The influx of monovalent cations was followed for 60–120 min. Duplicate samples at each time point were drawn into hematocrit tubes from the incubation vials using a modified 1 ml tuberculin syringe. Following sealing of the tubes and one minute centrifugation in the microhematocrit centrifuge, the tubes were cut at the buffer/erythrocyte interface. The buffer and erythrocyte portions were counted separately in a gamma counter. The percent of isotopic uptake by the erthrocytes was based on the total counts of each hematocrit tube, and the counts found in the buffer and erythrocyte portions.

Statistical analysis

The statistical analysis used was the double tailed Student's *t*-test. p values 0.05 or less were considered to be statistically significant. Data are reported as the mean alue $\pm$S.E. of the mean. The number of experiments are indicated as 'n'.

Materials

$^{22}Na^+$ (no carrier added), $^{42}K^+$ (0.13 mCi/mg), $^{201}Tl^+$ (no carrier added), $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$

and $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ (no carrier added) were obtained from New England Nuclear Corporation, (North Billerica, MA). Ouabain and other biochemical were obtained from the Sigma Co., St. Louis, MO.

Results

$^{22}Na^+$ efflux

The validity of the cationic efflux system was demonstrated in Fig. 1. Erythrocytes were preloaded in Ringers Buffer with $^{22}Na^+$. Efflux of $^{22}Na^+$ was stimulated by 20 mM K^+ and inhibited by 0.15 mM ouabain. 5 mM $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$ had no affect on the efflux of $^{22}Na^+$. $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$ is a very low specific activity beta emitting compound, while the $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ is a high specific activity, no carrier added, gamma emitting analogue. The $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$ was, under these conditions, used as a non-radioactive analogue of ^{99m}Tc. The lack of an effect of 5 mM $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$ on $^{22}Na^+$ efflux, taken with the stimulation of $^{22}Na^+$ efflux by K^+ indicated the two monovalent cations are not physiologically interchangeable.

Since TlCl has a poor solubility, the experiments designed to study the interaction of Na^+ and Tl^+ were done in 20 mM choline chloride

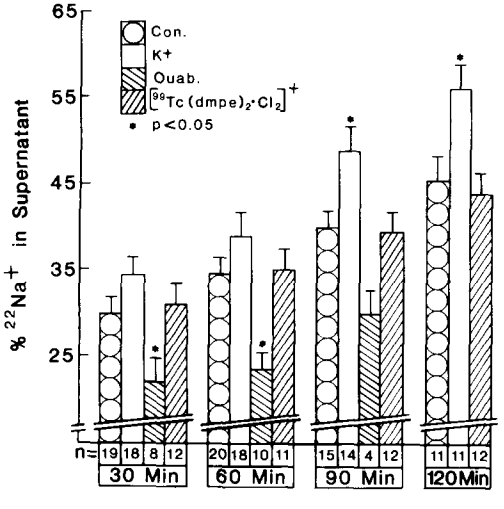

Fig. 1. The efflux of $^{22}Na^+$ out of human erythrocytes incubated in Ringers buffer. Conditions of incubation were as described under Methods.

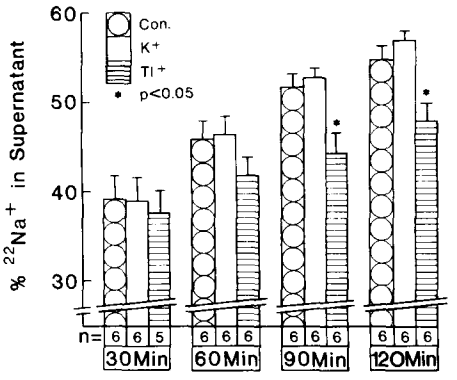

Fig. 2. The efflux of $^{22}Na^+$ out of human erthyrocytes incubated in choline chloride buffer. Conditions of incubation were as described under Methods.

using Tl acetate. No precipitation of TlCl at 20 mM Cl^- was seen and this agreed with Skulskii et al. [5]. Under these incubation conditions, K^+ had no effect on the efflux of $^{22}Na^+$ from the erythrocytes (Fig. 2). After 90 min of incubation in buffer containing 10 mM Tl^+ the efflux of $^{22}Na^+$ was inhibited. Similar inhibition of $^{22}Na^+$ efflux by high Tl^+ concentrations has been reported by Skulskii et al. [5].

$^{42}K^+$ influx

As shown in Fig. 3 the validity of the cationic influx system was demonstrated in that $^{42}K^+$ influx was inhibited by 0.15 mM ouabain and unlabeled K^+ or Tl^+ at 10 mM competed with $^{42}K^+$ influx into the erythrocyte. However, 5 mM $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$ had no effect on $^{42}K^+$ influx. This latter result strongly suggests that K^+

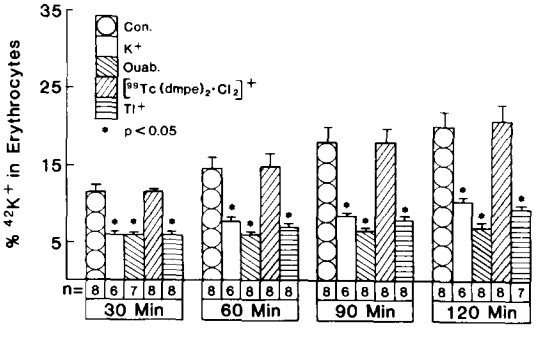

Fig. 3. The influx of ^{42}K into human erthrocytes. Conditions of incubation were as described under Methods.

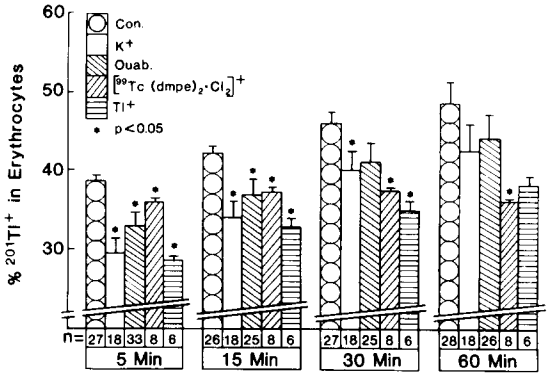

Fig. 4. The influx of $^{201}Tl^+$ into human erthrocytes. Conditions of incubation were as described under Methods.

and $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$ were not transported into the erythrocyte by the same mechanism.

^{201}Tl influx

The data on the influx of $^{201}Tl^+$ into erythrocytes are presented in Fig. 4. The rate of $^{201}Tl^+$ influx was considerably faster than that measured for $^{42}K^+$ and $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ (compare Figs. 4, 5, 6–note change in the ordinate scale). $^{201}Tl^+$ influx was inhibited by unlabeled Tl^+ or K^+ (both 10 mM) as well as by 0.15 mM ouabain. $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$ at 5 mM also inhibited $^{201}Tl^+$ influx. At this concentration $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$ had no effect on the transport of Na^+ or K^+, suggesting that its effect on $^{201}Tl^+$ uptake was specific and not due to compound-related toxicity.

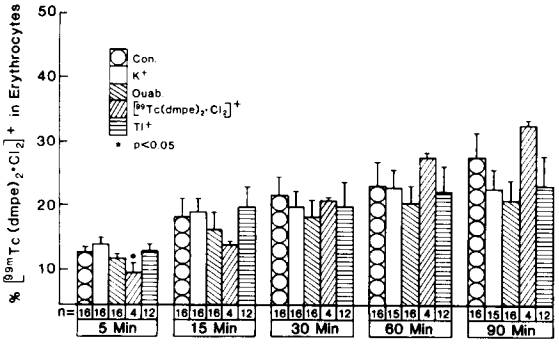

Fig. 5. The influx of $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ into human erthrocytes. Conditions of incubation were as described under Methods.

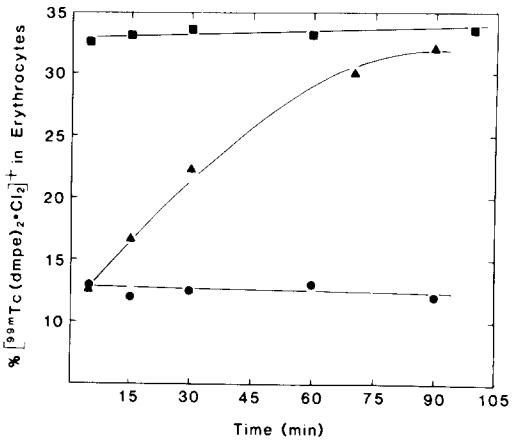

Fig. 6. The effect of pre-incubation of human erthrocytes with 5 mM $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$ on the influx of $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$. Conditions of incubation were as described under Methods. ■———■, 90 min preincubation with 5 mM $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$; ▲———▲, 5 mM $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$ added after a 90-min preincubation in 'incubation buffer'. ●———●, 90-min preincubation and incubation in 'incubation buffer'.

^{99m}Tc dmpe influx

Influx of the $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ was of a magnitude similar to that of K^+ (compare Fig. 3 with Fig. 5). Influx of $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ differed from that of K^+ in not being inhibited by unlabeled K^+ or Tl^+ (both 10 mM) or by 0.15 mM ouabain (Fig. 5). The addition of 5 mM $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$ had no statistically significant effect on the influx of $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$; however, at 60 and 90 min a slight increase in $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ uptake was noted. As can be seen in Fig. 5, the influx of $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ was increased above control. If the erythrocytes were pre-incubated for 90 min with 5 mM $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$ the 5 min time point for the uptake of $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ was found to almost equal that of the 90 min time point without prior incubation (Fig. 6). Clearly, the presence of 5 mM $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$ did not yield the expected effect of isotopic dilution but resulted in a stimulation of the influx of the $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ by an unknown mechanism.

Hemolysis of erythrocytes

When the erythrocytes loaded with $^{201}Tl^+$, $^{42}K^+$ or $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ were lysed by hypotonic

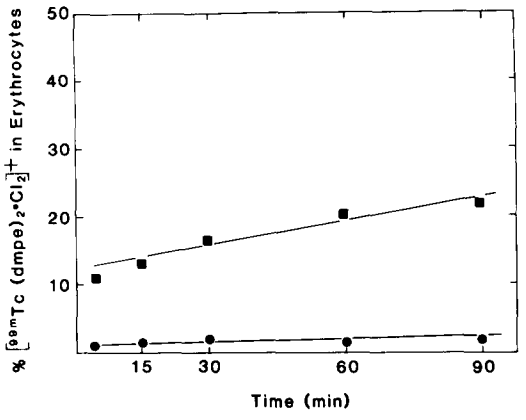

Fig. 7. $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ of human erythrocytes before (■———■) and after (●———●) lysis of the cells. Incubation conditions were as described under Methods in order to load erythrocytes with $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$. Cells were then lysed by being placed in distilled water.

shock, less than 1% of each labeled cation was found associated with the cell ghosts. A typical experiment using $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$ is shown in Fig. 7. Essentially similar results were obtained using $^{42}K^+$ and $^{201}Tl^+$. This indicates that the monovalent cations were transported inside the cells and not bound to the outside membranes of the erythrocytes. These results were confirmed by the use of broken cell membranes in addition to erythrocyte ghosts (data not shown).

Studies using erythrocytes from other species

In addition to human erythrocytes, similar data were obtained using erythrocytes obtained from dog, rabbit, pigs, guinea pigs, and baboons. No species difference in the efflux of $^{22}Na^+$, the influx of $^{42}K^+$, $^{201}Tl^+$ or $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ or in their interactions with each other, was observed (data not shown).

Discussion

Interest in the mechanism of transport of monovalent cations has recently increased due to the widespread use of $^{201}Tl^+$ for myocardial perfusion imaging. Previous reports [2–6] suggested that Tl^+ is taken up by the myocardium by an $(Na^+ + K^+)$-ATPase active transport system, and that Tl^+ is being handled by the $(Na^+ + K^+)$-ATPase as an analogue of K^+. The enzyme appears to have a greater affinity for Tl^+ than for K^+ [6]. We have adapted monovalent cation ion transport in erythrocytes as a model by which to compare the transport of Na^+, K^+, Tl^+ and of a potential new myocardial imaging agent, $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$. This method may be of value for the detection of other potential new, and unique myocardial imaging agents.

The $^{42}K^+$ influx and $^{22}Na^+$ efflux of the erythrocytes observed were in accord with other reports in the literature [3–5] (see Table I). Transport was reduced by the addition of unlabeled cation and inhibited by ouabain. However, high Tl^+ concentrations resulted in a decrease in $^{22}Na^+$ efflux. Similar results have been reported by Skulskii, et al. [5]. $^{201}Tl^+$ demonstrated uptake into the erythrocyte which was faster and of greater magnitude than $^{42}K^+$. This agrees with the reports of Skulskii and co-workers [3–5] using erythrocytes and Sessler et al. [13] using tumor cells.

TABLE I

SUMMARY OF ERYTHROCYTE MONOVALENT CATION TRANSPORT STUDIES

n.e., no statistically significant effect; n.d., not done.

Unlabeled ion / Labeled ion	Influx			Efflux	
	$^{42}K^+$	$^{201}Tl^+$	$[^{99m}Tc(dmpe)_2Cl_2]^+$	$^{22}Na^+$ Ringers buffer	$^{22}Na^+$ choline chloride buffer
K^+	↓	↓	n.e.	↑	n.e.
Tl^+	↓	↓	n.e.	n.d.	↓
$[^{99}Tc(dmpe)_2Cl_2]^+$	n.e.	↓	n.e.	n.e.	n.e.
Ouabain	↓	↓	n.e.	n.e.	n.e.

Of great interest are the data on the transport of $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$. This agent is a monovalent cation with a radius similar to that of the hydrated K^+. The diameter of the $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ is approx. 6 Å [9] compared to that of a hydrated K^+ of approx. 5 Å [2]. It has been suggested in several reports [7–12] that $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ can act as a K^+ analogue and thus replace $^{201}Tl^+$ as a myocardial imaging agent. While efficacy as a myocardial imaging agent in experimental animals has been shown, the suggested mode of uptake of $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ is not supported by the present data (see Table I). The rate and magnitude of uptake of $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ closely parallels that of $^{42}K^+$; however, its lack of competition with other monovalent cations and failure to show ouabain sensitivity suggest an uptake mechanism which differs from that of either K^+ or Tl^+. In addition, the paradoxical stimulation of $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ uptake by $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$ was unique to this cation. Preincubation with $[^{99}Tc(dmpe)_2 \cdot Cl_2]^+$ had no affect on the uptake of $^{42}K^+$ or $^{201}Tl^+$ (data not shown). We propose that an alternative mechanism independent of the $(Na^+ + K^+)$-ATPase is involved in $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ transport. A similar study using the $(Na^+ + K^+)$-ATPase of neonatal rat myocytes confirmed the results obtained using human erthrocytes (Delano, M.L., Sands, H., Camin, L.L. and Gallagher, B.M., unpublished data). The results reported here do not support the hypothesis that $[^{99m}Tc(dmpe)_2 \cdot Cl_2]^+$ represents a K^+ analogue which is taken up the myocardium by a mechanism similar to that of $^{201}Tl^+$.

Acknowledgements

We would like to thank Paula Shaw for her technical assistance and Dr. V. Subramanyam and K. Linder for preparing the dmpe.

References

1 Wackers, F.J.T. (1980) Semin. Nucl. Med. 10, 127–145
2 Gallagher, B.M. and Lebowitz, E. (1981) in Radiopharmaceuticals Structure and Activity Relationship (Spencer, R.P., ed.), pp. 619–644, Grune and Stratton, New York
3 Skulskii, I.A., Manninen, V. and Järnefelt, J. (1978) Biochim. Biophys. Acta 506, 233–241
4 Skulskii, I.A., Manninen, V. and Järnefelt, J. (1973) Biochim. Biophys. Acta 298, 702–709
5 Skulskii, I.A., Manninen, V. and Järnefelt, J. (1975) Biochim. Biophys. Acta 394, 569–576
6 Britten, J.S. and Blank, M. (1968) Biochim. Biophys. Acta 159, 160–166
7 Straus, H.W. and Pitt, B. (1977) Semin. Nucl. Med. 7, 49–58
8 Deutsch, E., Glavan, K.A., Sodd, V.J., Nishyama, H., Ferguson, D.L. and Lukes, S.J. (1981) J. Nucl. Med. 22, 897–907
9 Deutsch, E., Bushong, N., Glavan, K.A., Elder, R.C., Sodd, V.J., Fortman, D.L. and Lukes, S.J. (1981) Science 214, 85–86
10 Thakur, M.L. (1982) J. Nucl. Med. 23, p. 11
11 Nishiyama, H., Deutsch, E.A., Adolph, R.J., Sodd, V.J., Libson, K., Lukes, S.J., Gabel, M., Gerson, M.C., Vanderheyden, J.L. and Saenger, E.L. (1982) J. Nucl. Med. 23, p. 12
12 Nishiyama, H., Sodd, V.J., Deutsch, E.A., Adolph, R.J., Libson, K., Gerson, M.C., Vanderheyden, J.L., Gabel, M., Lukes, S.J. and Saenger, E.L. (1982) J. Nucl. Med. 23, p. 12
13 Sessler, M., Maul, F.D., Geck, P., Munz, D. and Hör, G. (1982) Biology: Proceedings of the Third World Congress of Nuclear Medicine and Biology, Paris, 1982, pp. 2281–2284, Pergamon Press, New York