^{99m}Tc-Pertechnetate Uptake in Parotid Acinar Cells by the $Na^+/K^+/Cl^-$ Co-transport System

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Abstract

^{99m}Tc-Pertechnetate ($^{99m}TcO_4^-$) has widespread clinical use in the diagnosis and evaluation of dysfunctions in many different tissues. However, despite the broad clinical application of this radionuclide, very little is known about the mechanism by which $^{99m}TcO_4^-$ enters a cell. We report evidence here that $^{99m}TcO_4^-$ shares the $Na^+/K^+/Cl^-$ co-transport system localized to the basolateral membrane of rat parotid acinar cells. $^{99m}TcO_4^-$ uptake by these cells was quite rapid ($t_{1/2} \sim 30$ s), was completely inhibited by the loop diuretics furosemide and bumetanide, and was markedly dependent on the presence of Na^+, K^+, and Cl^- in the extracellular medium. Relative to uptake measured in the presence of physiological extracellular salt concentrations (Hanks' salts), $^{99m}TcO_4^-$ uptake was inhibited 80% by sodium replacement and 50% by potassium replacement. When Cl^- was replaced with the physiologically inert anion gluconate a threefold stimulation in $^{99m}TcO_4^-$ uptake resulted. These observations provide strong evidence that $^{99m}TcO_4^-$ can substitute for Cl^- as a substrate for the $Na^+/K^+/Cl^-$ co-transporter and indicate that $^{99m}TcO_4^-$ uptake by salivary glands (e.g., as seen with salivary scintiscans), and possibly by a variety of other tissues, reflects the functional activity of this co-transport mechanism.

Introduction

^{99m}Technetium is the most commonly used radionuclide in diagnostic nuclear medicine (1). In the anionic form as pertechnetate ($^{99m}TcO_4^-$)[1] it has been utilized for over 20 years to visualize a number of tissues. For example, this radionuclide will concentrate in salivary gland, gastric mucosa, respiratory epithelium, synovial fluid, and areas of inflammation, and is selectively excluded from cerebrospinal fluid and normal brain tissue (2, 3). $^{99m}TcO_4^-$ is also particularly useful for evaluating thyroid function, since it is not metabolized by the thyroid and can be discharged from the gland by perchlorate (4, 5).

Considering the widespread clinical use of $^{99m}TcO_4^-$, it is surprising how little is known about the mechanism(s) by which this radionuclide enters a target cell. $^{99m}TcO_4^-$ is thought to act as a pseudohalide (6). Physiological studies with thyroid and salivary glands (4, 7, 8) indicate that $^{99m}TcO_4^-$ is handled in a manner analogous to radioiodide, and in some tissues iodide is known to prevent the uptake of $^{99m}TcO_4^-$. It also has recently been shown that $^{99m}TcO_4^-$ and radiolabeled Cl^- have similar bidirectional permeability coefficients in tracheal epithelium (3). Yet without a clear understanding of cellular mechanisms for $^{99m}TcO_4^-$ handling, a precise interpretation of clinical $^{99m}TcO_4^-$ scintiscans is impossible.

We have been particularly interested in the use of $^{99m}TcO_4^-$ as a clinical index of the fluid transporting ability of salivary glands. Using the rat parotid gland, a tissue considered to be a good model of the human parotid, we have recently shown that $^{99m}TcO_4^-$ output mimics salivary flow rate (9), suggesting that $^{99m}TcO_4^-$ transport may occur by an anion transport mechanism associated with transepithelial water movement. In the past few years, considerable data have pointed to the important role of a loop diuretic-sensitive, $Na^+/K^+/Cl^-$ co-transport system in this process. More specifically, it has been suggested that fluid secretion in the salivary gland (10, 11) and in a number of other secretory epithelia (10, 12) is the result of transepithelial chloride movements that are driven by a $Na^+/K^+/Cl^-$ co-transporter localized to the basolateral membrane of the acinar (fluid secreting) cell. In consideration of the many earlier reports indicating similar handling of Cl^-, I^-, and $^{99m}TcO_4^-$ by a number of epithelial tissues (7, 8), we hypothesized that $^{99m}TcO_4^-$ may enter these cells via the loop diuretic-sensitive $Na^+/K^+/Cl^-$ co-transporter. In the present report we present data supporting this hypothesis.

Methods

Materials. The animals used in these studies were male, Wistar strain rats (300–350 g) purchased from Harlan-Sprague-Dawley, Madison, WI. Rats were fed ad lib until exsanguination. Na $^{99m}TcO_4$ was produced by the National Institutes of Health Radiopharmacy using a Medi-Physics $^{99}Mo/^{99}Tc$ generating column (Richmond, CA). Furosemide, hyaluronidase (type 1-S), choline chloride, and all gluconate and iodide salts were from Sigma Chemical Co. (St. Louis, MO). Collagenase (type CLSPA) was from Worthington Biochemical Corp. (Freehold, NJ). Bumetanide was a gift from Hoffman-La Roche, Inc. (Nutley, NJ).

Preparation of parotid cell aggregates. Animals were killed between 10 and 11 a.m. by cardiac puncture after diethyl ether anesthesia. A suspension of parotid cell aggregates was prepared using the methods of Ito et al. (13). Briefly, after excision and mincing of parotid glands, the tissue was incubated with continuous agitation at 37°C for 1 h in Hanks' balanced salt solution with 30 mM Hepes, pH 7.4 (HBSS-H, see Table I) containing collagenase (96 U/ml) and hyaluronidase (0.19 mg/ml). Every 20 min this suspension was gassed with 95% O_2/5% CO_2 and subjected to gentle pipetting. The resulting cell aggregates were washed three times (40 g, 15 s) with HBSS-H containing 0.033% bovine serum albumin, resuspended in HBSS-H without albumin, and divided into aliquots. These cells were maintained at 37°C and gassed with O_2/CO_2 every 20 min until used in the uptake studies.

Uptake of $^{99m}TcO_4^-$. The uptake of $^{99m}TcO_4^-$ was measured as follows. An aliquot of rat parotid cell aggregates was spun down and resuspended in 2 ml of prewarmed (37°C) uptake medium (see below) containing 150 μCi of $^{99m}TcO_4^-$. After the incubation times indicated the cells were

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1. *Abbreviations used in this paper:* HBSS-H, Hanks' balanced salt solution with 30 mM Hepes, pH 7.4; $^{99m}TcO_4^-$, ^{99m}Tc in the anion form as pertechnetate.

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centrifuged and washed once in 2 ml of ice-cold uptake medium without $^{99m}TcO_4^-$. This pellet was resuspended in 2 ml of phosphate-buffered saline and homogenized using a Brinkmann Polytron (setting 5, 10 s). Aliquots of this homogenate were assayed for radioactivity in a Beckman Gamma 4000 gamma counter and for DNA by the method of Richards (14). Uptake (counts per minute retained by the cells) was normalized to total cellular DNA.

The uptake medium used in these studies was HBSS-H (control) or the modifications of HBSS-H listed in Table I. The loop diuretics furosemide and bumetanide, when present, were added at concentrations of 10^{-3} M.

Owing to variations in the specific activity of the $^{99m}TcO_4^-$ supplied to us, the results presented here are expressed as percentages of the uptake observed under an appropriate control condition (typically when the uptake medium was HBSS-H alone). The variability stated is the SEM. All results were corrected for nonspecific trapping of $^{99m}TcO_4^-$ by the cells. This trapping was determined by the same method as described above for uptake studies except that both the uptake medium and the cells were chilled to 4°C. Trapping measured in this way was independent of the composition of the uptake medium (all media studied in the paper were tested) and of the time the cells were left in the presence of $^{99m}TcO_4^-$ (up to 1 min). Nonspecific trapping averaged 60±3% of the uptake measured after 1 min of incubation at 37°C in the presence of HBSS-H.

Results

Time course of uptake. The time course of uptake of $^{99m}TcO_4^-$ into rat parotid acinar aggregates is shown in Fig. 1. $^{99m}TcO_4^-$ uptake appears to occur in two stages, a larger, more rapid component of uptake that occurs over the 1st minute of incubation ($t_{1/2} \sim 30$ s) and a smaller, slower component of uptake that may represent the slow diffusion of $^{99m}TcO_4^-$ into the less accessible interior of the acinar aggregates. In the experiments that follow we have concentrated on the larger, more rapid phase of $^{99m}TcO_4^-$ uptake by measuring fluxes after 1 min of incubation.

Effects of loop diuretics. The effects of the loop diuretics furosemide and bumetanide on $^{99m}TcO_4^-$ uptake into rat parotid cell aggregates are illustrated in Fig. 2. The data presented are the average of two independent experiments. Both of these known inhibitors of the $Na^+/K^+/Cl^-$ co-transport system completely block the uptake of $^{99m}TcO_4^-$ into the cells.

Table I. Composition of Incubation Media

	HBSS-H	Na^+ free	K^+ free	Cl^- free (gluconate)
	mM	*mM*	*mM*	*mM*
NaCl	137	—	142	—
NaI	—	—	—	—
Na gluconate	—	—	—	137
Choline chloride	—	137	—	—
Atropine	—	0.02	—	—
KCl	5.4	5.4	—	—
K gluconate	—	—	—	5.4
KH_2PO_4	0.4	0.8	—	0.4
NaH_2PO_4	0.33	—	0.66	0.33
$MgSO_4$	0.81	0.81	0.81	0.81
$CaCl_2$	1.27	1.27	1.27	—
Ca gluconate	—	—	—	1.27
Glucose	5.5	5.5	5.5	5.5
Hepes (pH 7.4)	30	30	30	30

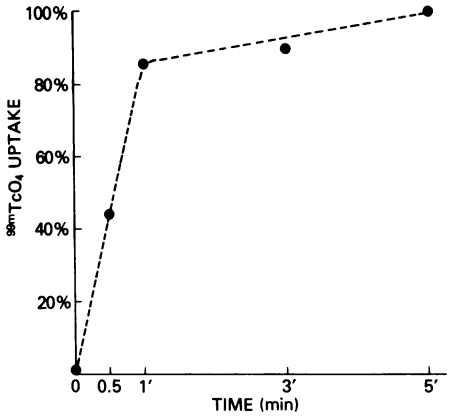

Figure 1. Time course of $^{99m}TcO_4^-$ uptake into rat parotid acinar cell aggregates. Uptake was measured as described in Methods. The incubation medium was HBSS-H. The data are expressed as a percentage of the uptake observed at 5 min. The results of a representative experiment are shown.

Effects of Na^+ and K^+ replacement. Since the uptake of Cl^- into rat parotid cell aggregates is known to be dependent on the presence of both Na^+ and K^+, presumably owing to its transport via the basolateral $Na^+/K^+/Cl^-$ co-transporter (15), we next investigated the effects of these ions on $^{99m}TcO_4^-$ uptake. The results are shown in Fig. 3. The data presented are the average of two independent experiments. When Na^+ was replaced in the uptake medium (with choline) $^{99m}TcO_4^-$ uptake was inhibited by 80%, and when K^+ was replaced (with Na^+) $^{99m}TcO_4^-$ uptake was inhibited 50%. Since some loss of intracellular K^+ into the uptake medium might be expected when the cells are resuspended in a K^+-free solution, this latter value should be regarded as a lower limit on the inhibitory effect of extracellular K^+ removal.

Effects of Cl^- replacement. To further test our hypothesis that $^{99m}TcO_4^-$ shares the $Na^+/K^+/Cl^-$ co-transport system we

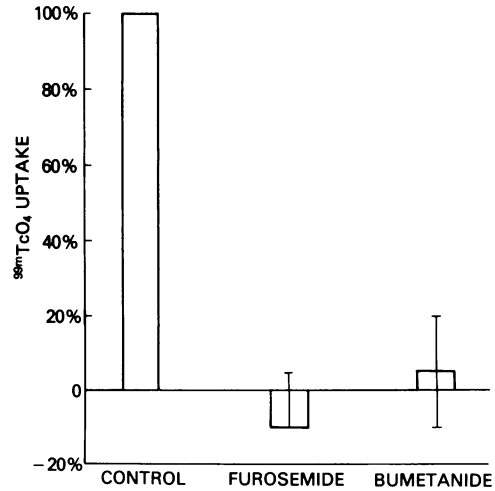

Figure 2. Effects of loop diuretics on the uptake of $^{99m}TcO_4^-$ into rat parotid acinar cell aggregates. Uptake was measured after 1 min of incubation at 37°C in HBSS-H (control, 100%) and in HBSS-H plus 1 mM furosemide (−10±15% of control) or 1 mM bumetanide (5±15% of control). The data presented are the average of two independent experiments.

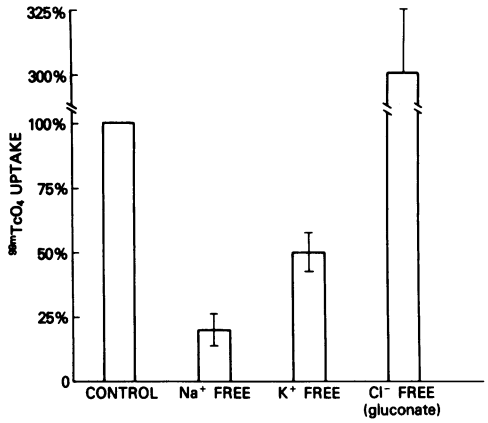

Figure 3. Effects of Na^+, K^+, or Cl^- replacement on the uptake of $^{99m}TcO_4^-$ into rat parotid acinar cell aggregates. Uptake was measured after 1 min of incubation at 37°C in HBSS-H (control, 100%) and in sodium-free (20±7% of control), potassium-free (50±8% of control), or chloride-free (300±24% of control) media. In these experiments sodium was replaced by choline (plus atropine), potassium was replaced by sodium, and chloride was replaced by gluconate (see Table I). The data presented are the average of two (sodium and potassium free) or three independent experiments.

investigated the effects of replacing Cl^- in the uptake medium with the physiologically inert anion gluconate. As illustrated in Fig. 3 (the average of three independent experiments), when Cl^- is replaced with gluconate, $^{99m}TcO_4^-$ uptake into rat parotid cell aggregates is stimulated over threefold. Since physiological studies with salivary glands have indicated that $^{99m}TcO_4^-$ is handled in a manner analogous to radioiodide (7, 8), we also attempted to study the effect of replacing Cl^- in HBSS-H with I^-. Although we observed a significant inhibition of $^{99m}TcO_4^-$ uptake (75%) when Cl^- was replaced with I^- in HBSS-H, we found that nonspecific trapping of $^{99m}TcO_4^-$ by the acinar aggregates was also significantly reduced (~ 40%) by I^- replacement (data not shown). Since we had previously established that this nonspecific trapping was not affected by the presence of furosemide or bumetanide, by Na^+ or K^+ replacement, or by replacement of Cl^- with gluconate$^-$, we can not convincingly exclude the possibility that I^- has some nonspecific effect on the parotid aggregates unrelated to an effect on $^{99m}TcO_4^-$ uptake.

Discussion

The studies reported here were carried out to clarify the mechanism whereby the widely used radionuclide $^{99m}TcO_4^-$ enters epithelial cells. We chose as a model the rat parotid acinar cell. A number of observations described earlier in this paper lead us to hypothesize that the handling of $^{99m}TcO_4^-$ by parotid acinar cells may mimic the handling of Cl^- and in particular, that $^{99m}TcO_4^-$ may share the basolateral $Na^+/K^+/Cl^-$ co-transporter thought to be responsible for transepithelial Cl^- (and thereby water) movements. We have recently demonstrated that this co-transporter is present in the basolateral membrane of parotid gland acinar cells (16) and is quite similar to comparably characterized Cl^- co-transport mechanisms found in the shark rectal gland (17, 18) and the rabbit thick ascending limb of Henle's loop (19). We show here that $^{99m}TcO_4^-$ uptake by rat parotid cell aggregates is very rapid, mimicking closely the time course of $^{36}Cl^-$ uptake in the same preparation (15). This $^{99m}TcO_4^-$ uptake is completely inhibited by the loop diuretics furosemide and bumetanide (Fig. 2), which are well-known inhibitors of $Na^+/K^+/Cl^-$ co-transport systems. In addition, we demonstrate that $^{99m}TcO_4^-$ uptake is markedly dependent on the presence of both K^+ and Na^+ in the extracellular medium, with uptake being inhibited 50% by K^+ removal and 80% by Na^+ removal (Fig. 3). We also demonstrate that when Cl^- in the extracellular medium is replaced by the physiologically inert anion gluconate, $^{99m}TcO_4^-$ uptake is stimulated over threefold (Fig. 3). Taken together, these observations provide strong evidence that $^{99m}TcO_4^-$ can replace Cl^- as a substrate on the $Na^+/K^+/Cl^-$ co-transporter.

While it is possible that $^{99m}TcO_4^-$ may share transporters other than the $Na^+/K^+/Cl^-$ co-transporter, and that these systems may play important roles in the uptake of $^{99m}TcO_4^-$ from the circulation in some tissues, it is unlikely that this is the case in the rat parotid. Our data show that almost all of the $^{99m}TcO_4^-$ uptake into rat parotid acinar cells is sodium dependent and inhibitable by the loop diuretics furosemide and bumetanide. Thus, this uptake is virtually completely attributable to the co-transporter. Consequently, the present findings suggest that the uptake of $^{99m}TcO_4^-$ from the circulation observed clinically during salivary gland scintiscan reflects the functional status of the basolateral $Na^+/K^+/Cl^-$ co-transporter.

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