# Pendrin Transporter Carries Out Iodide Uptake into MCF-7 Human Mammary Cancer Cells

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**Previous studies have shown that iodide is actively taken up into mammary alveolar epithelial cells and secreted into milk. In the present studies we demonstrate that $^{125}I$ also accumulates in MCF-7 cells against a concentration gradient; distribution ratios of greater than 30 were achieved. Iodide uptake into MCF-7 cells is transient, with peak accumulations occurring in about 5 min. The iodide is rapidly metabolized, probably to iodine, and it then exits the cells. The iodide transporter identified in MCF-7 cells is pendrin. DIDS, a nonspecific inhibitor of anion exchange, inhibits iodide uptake. Iodide uptake is impaired at reduced temperature, but is not dependent on sodium. Inhibitors of the sodium-iodide symporter (NIS) as well as ouabain did not affect the extent of iodide uptake. The pendrin transporter but not NIS was identified via western blotting techniques. Pendrin appears to be the primary iodide transporter in the MCF-7 cell line stocks that were employed for these studies.** Exp Biol Med 228:1078–1082, 2003

**Key words:** mammary; MCF-7 cells; iodide transport; pendrin

The levels of iodide in the milk of a variety of species are 10- to 30-fold higher than those present in the maternal plasma (1–5). The iodide in milk is of critical importance for the growth and development of the neonate, particularly because iodide is essential for the synthesis of the thyroid hormones. We as well as several other investigators have identified and characterized a sodium-iodide symporter (NIS) that actively transports iodide from the maternal plasma into the alveolar epithelial cells of the mammary gland (6–12). This transporter has also been reported to be expressed in MCF-7 breast cancer cells; retinoic acid stimulated the expression of NIS (13). More recently, Shennan (14), as well as our laboratory (15), have identified a DIDS-sensitive anion exchange transporter in the mammary gland; this transporter accepts iodide as a substrate and was identified via western blotting techniques as pendrin in thyroid cells (16–18). The present studies were designed to assess the possible contribution of the pendrin iodide transporter in human MCF-7 mammary cancer cells.

## Materials and Methods

Materials used in these studies were from the following sources: penicillin and streptomycin from Eli Lilly (Indianapolis, IN); $^{3}HOH$ from New England Nuclear Inc. (Boston, MA); $^{125}I$ (423 mCi/mMol) from Amersham Life Science Inc. (Arlington Heights, IL); all other culture supplies and chemicals were from Sigma-Aldrich, Inc. (St. Louis, MO). Stocks of the MCF-7 human mammary epithelial cell line were obtained from the Michigan Cancer Foundation (now Karmanos Cancer Institute, Detroit, MI) and maintained in continuous culture; passages 30 to 195 were employed in these studies. Pendrin antibody initially was a gift from Dr. Eric A. Green, NIH, Bethesda, MD; most experiments were carried out with rabbit antipeptide antibodies, raised against human pendrin sequence 630-643 (PTKEIEIQUDWNSE; GenBank AF 167412) as specified by Royaux *et al.* (17); the antibody was prepared by Zymed Laboratories, Inc., South San Francisco, CA. The human NIS antibody was provided by Dr. Sissy M. Jhiang of the Ohio State University.

The MCF-7 cells were cultured (19) in Eagle's minimal essential medium (EMEM) supplemented with 2× nonessential amino acids, L-glutamine (292 mg/l), 10% newborn bovine serum (inactivated), penicillin (100 U/ml), streptomycin (100 μg/ml), and insulin ($10^{-6}$ M). Cultures in log growth were harvested with 0.25% trypsin in phosphate-buffered saline (pH = 7.4). The cells were seeded at a density of 0.5-1 × $10^{6}$/60 mm plate in supplemented Eagle's minimal essential medium (Earle's salts) and incubated at 37°C in a humidified atmosphere of 5% $CO_2$/95% air. The medium was aspirated and replaced on day 2. On day 4, the medium was removed and the cells washed with 5 ml/plate Hanks' balanced salts solution; at this time the cells were at about 50% confluence. The cells were then cultured for specified times 0°C or 37°C with 5 ml HBSS containing 1 μCi/ml $^{125}I$ and drugs where specified. The cells were then

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rinsed with 5 ml HBSS and homogenized in 3 ml 5% trichloroacetic acid (TCA). After centrifugation for 10 min at 2,000 g, the radioactivity in 1 ml aliquots of the supernatants was determined by liquid scintillation. $^{125}I$ in the TCA-pellet fraction was determined after solubilization of the pellets in 2 ml 1N NaOH; only trace amounts of $^{125}I$ were found in the pellet fraction indicating a limited incorporation into cellular protein. In each experiment, intracellular water was quantitated by equilibrating 3 plates of cells at 37°C for 1 hr with $^{3}HOH$ (1 μCi/ml) contained in 5 ml HBSS; in preliminary studies the $^{3}HOH$ was found to equilibrate within 10 min of incubation. After the incubation with $^{3}HOH$, the cells were rinsed with HBSS and then homogenized in 3 ml 5% TCA. Afterwards centrifugation radioactive [$^{3}H$] in the TCA supernatant was determined and water content of the cells calculated. Using these data, the results of the iodide uptake studies are expressed as a distribution ratio, which represents the ratio of the intracellular specific activity divided by the extracellular specific activity of the radioactive iodide.

For western blotting studies with the pendrin antibody, plates of MCF-7 cells were homogenized in 1 ml lysis buffer in a ground glass homogenizer; the lysis buffer contained 2% NP40, 10 mM Tris, 50 mM NaCl, 30 mM sodium pyrophosphate, 2.5 mM EDTA, 1 mM orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, at pH 7.6. After 30 min on a rocking platform, lysates were centrifuged (100,000 g) for 30 min at 4°C. After protein determination by the method of Bradford, the resulting supernatants, containing greater than 95% of extractable protein, were separated by SDS-PAGE (8%–20% linear gradient) under reducing conditions and transferred to polyvinylidine fluoride (PVDF) membranes (Schleicher and Schuell). Membranes were probed with 1:2,500 human anti-pendrin for 2 hrs, followed by treatment with anti-rabbit IgG HRP conjugate (Amersham NA934; 25 ml at 1:3,000 dilution for 1.5 hrs). Detection was accomplished by incubation with enhanced chemiluminescence reagents (Amersham) and exposure to photographic film. Statistical comparisons were made with Student's *t* test for comparing two means, or with an analysis of variance followed by Scheffe's test for multiple comparisons.

## Results

Figure 1 shows the time course for $^{125}I$ accumulation in MCF-7 cells. Peak uptakes were observed after culture periods between 2.5 and 15 mins; thereafter the $^{125}I$ progressively exited the cells. Peak distribution ratios of greater than 30 were achieved during the early minutes of uptake determinations, indicating a concentrating mechanism for iodide accumulation. Radioactivity in the culture media remained constant during the time course studies (data not included). Although significant amounts of $^{125}I$ were not incorporated into cellular proteins (in the TCA-precipitable fraction), $^{125}I$ incorporation into serum proteins occurred when $^{125}I$ uptake was determined with the $^{125}I$ in serum-

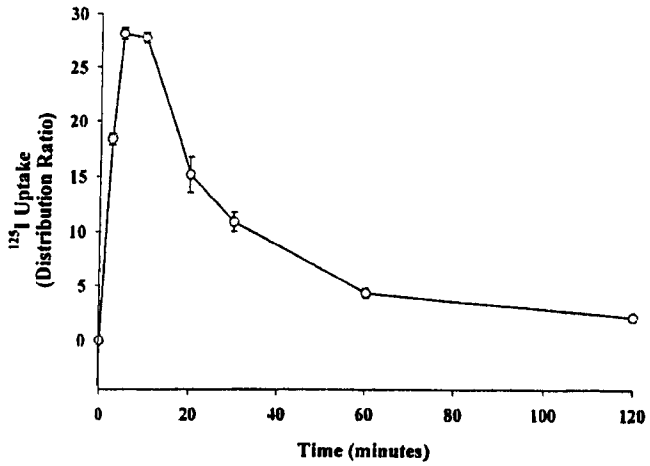


**Figure 1.** Time-course for $^{125}I$ uptake in MCF-7 cells. Cells were cultured for the times indicated with 1 μCi/ml $^{125}I$. Intracellular $^{125}I$ was then determined. Numbers represent the mean ± SE of distribution ratios from 4 plates.

containing medium rather than in HBSS; the conversion of radioactive iodide to iodine, which then effluxes from the cells is thus suggested (data not included). Figure 2 shows the effect of iodide concentration on $^{125}I$ accumulation after a 10-min uptake of $^{125}I$ in MCF-7 cells. The distribution ratio decreases progressively as the iodide concentration increases, but distribution ratios of greater than 25 were still achieved with iodide at a concentration of 25 mM. The $^{125}I$ concentrating mechanism in MCF-7 cells can thus handle a very high concentration of iodide. The temperature dependence of the iodide uptake mechanism in the MCF-7 cells is presented in Figure 3. When uptake of $^{125}I$ was determined with the cells at 0°C, it took 30 to 60 mins to achieve a maximum cellular accumulation, and an efflux from the cells was only observed after 60 mins (data not presented).

The experiment shown in Figure 4 was carried out to

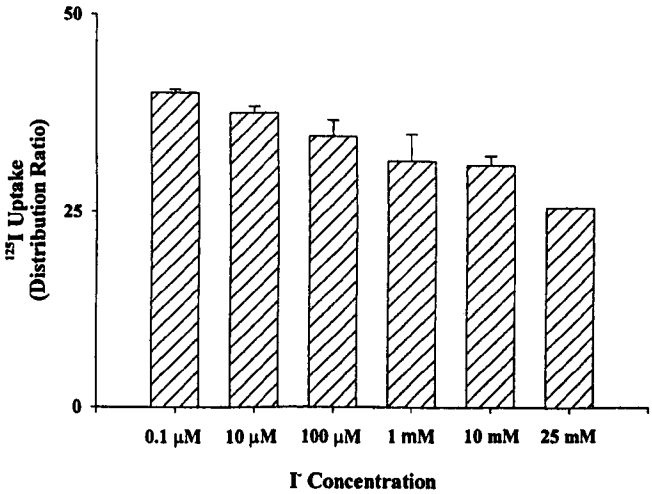


**Figure 2.** Effect of iodide concentration on $^{125}I$ uptake in MCF-7 cells. Cells were cultured for 10 mins with $^{125}I$ (1 μCi/ml) with iodide at the concentrations indicated. Intracellular $^{125}I$ was then determined. Numbers represent the mean ± SE of distribution ratios from 4 plates.

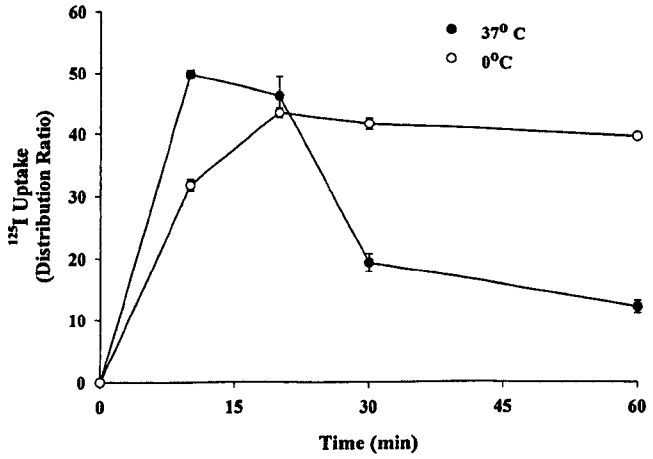


**Figure 3.** Effect of temperature on $^{125}I$ uptake in MCF-7 cells. Cells were cultured for 0 to 60 min at 0°C or 37°C with 1 μCi/ml $^{125}I$. Intracellular $^{125}I$ was then determined. Numbers represent the mean ± SE of distribution ratios from 4 plates.

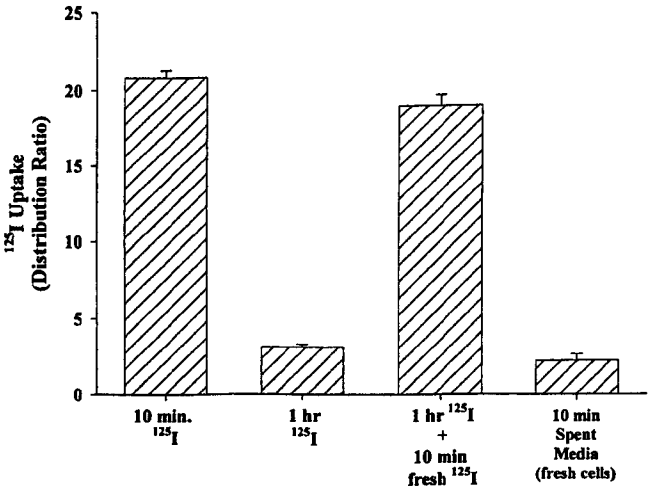


**Figure 4.** Metabolism of $^{125}I$ in MCF-7 cells. $^{125}I$ uptake was assessed for 10 mins (bar 1) or 1 hr (bar 2) as in Figure 1. In bar 3, the cells were incubated for 1 hr with $^{125}I$ as in Figure 1, and then an additional 10 mins with fresh media containing 1 μCi/ml $^{125}I$. In bar 4, fresh MCF-7 cells were incubated for 10 mins with the "spent" media from the cells in bar 2. Numbers represent the mean ± SE of distribution ratios from 4 plates.

assess the reason for the efflux of $^{125}I$ from MCF-7 cells employing culture times of greater than 15 minutes at 37°C. Control uptakes at 10 and 60 mins are shown in the first two bars respectively. The uptake in the cells of bar 3 was assessed after a 1-hr incubation with $^{125}I$ followed by a 10-min incubation with fresh $^{125}I$; these data show that the cells exposed to $^{125}I$ for 1 hr are as capable of accumulating $^{125}I$ during a 10-min incubation as are the cells in bar one. The fourth bar contains fresh MCF-7 cells that were cultured for 10 min with the "spent" media of the cells in bar 2 that were cultured with $^{125}I$ for 1 hr. The fact that the fresh cells in bar 4 did not accumulate $^{125}I$ from the "spent" media of bar 2 indicates that the $^{125}I$ in this media has been chemically altered, probably to iodine via an oxidation-reduction reaction.

To determine the mechanism by which $^{125}I$ is taken up into MCF-7 cells the following experiments were carried out. Figure 5 shows an experiment in which iodide uptake was assessed in the absence of sodium. Clearly, after a 10 or 60 min incubation with $^{125}I$, the absence of sodium had no effect on the distribution ratio, indicating that the $^{125}I$ uptake is not sodium dependent. The data in Figure 6 show that 3 inhibitors of the NIS, as well as an inhibitor of sodium-potassium ATPase (ouabain), had no effect on $^{125}I$ accumulation during a 10-min uptake period. Even in experiments where we precultured tissues for 1 hr with these drugs prior to the 10-min $^{125}I$ uptake determination in the presence of these drugs, inhibitory effects were not observed (data not included). In the MCF-7 cells that we employed for these studies, the NIS transporter appears to be of little importance.

In western blotting studies, the only iodide transporter that we could identify was the pendrin iodide transporter with a molecular weight of about 90 kDa (Fig. 7). Using an NIS antibody that we used in earlier studies to identify the NIS transporter in mouse mammary tissues (8), we could not identify the NIS transporter in extracts of the MCF-7 cells. In further studies we determined the dose-response effect of an anion exchange inhibitor, DIDS, on $^{125}I$ accumulation in MCF-7 cells; DIDS has earlier been shown to inhibit the pendrin iodide transporter. Figure 8 shows that DIDS inhibited iodide accumulation in a concentration-dependent fashion.

Figure 9 shows the data from a time course experiment in which RNA and protein synthesis were inhibited by actinomycin D and cycloheximide respectively. The turnover of the transporter mechanism, probably the pendrin protein, appears to be quite slow since even after 8 hrs in the presence of cycloheximide there is only about a 25% reduction in $^{125}I$ accumulation.

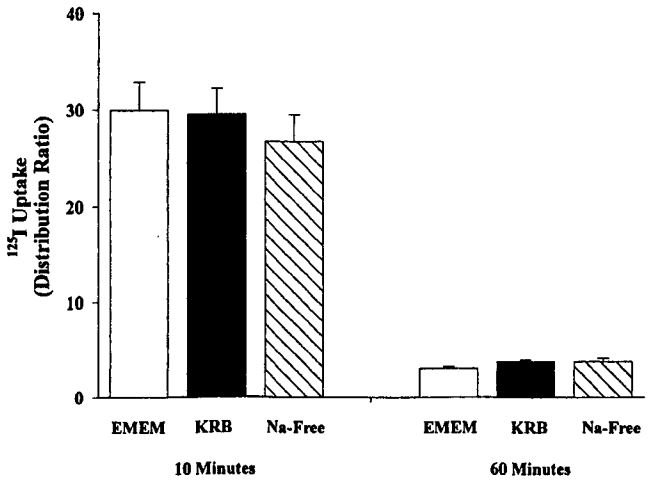


**Figure 5.** Effect of sodium on $^{125}I$ accumulation in MCF-7 cells. Cells were cultured for 10 mins or 60 mins with 1 μCi/ml $^{125}I$ contained in EMEM, KRB, or sodium-free KRB. Intracellular $^{125}I$ was then determined. Numbers represent the mean ± SE of distribution ratios from 4 plates.

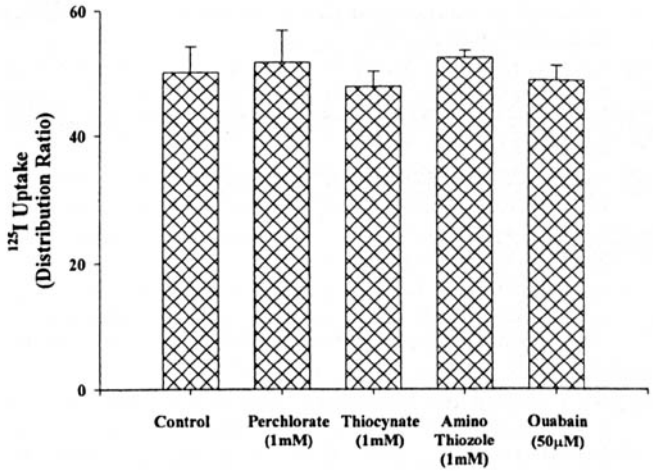


**Figure 6.** Effect of NIS inhibitors and ouabain on $^{125}$I uptake in MCF-7 cells. Cells were incubated for 10 mins with 1 μCi/ml $^{125}$I plus the inhibitors specified in the figure. Intracellular $^{125}$I was then determined. Numbers represent the mean ± SE of distribution ratios from 4 plates.

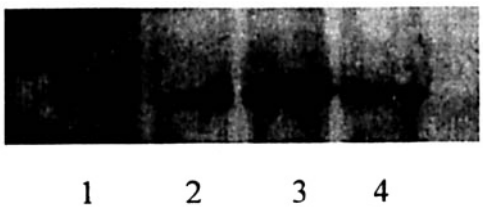


**Figure 7.** Western blot of pendrin in proteins from MCF-7 cells. Proteins from MCF-7 cells were subjected to western blot analysis as described in the Materials and Methods section. Lanes 1 and 2 (15 μg protein); lanes 3 and 4 (45 μg protein). The band appeared in the gels indicating a MW of about 91 kDa.

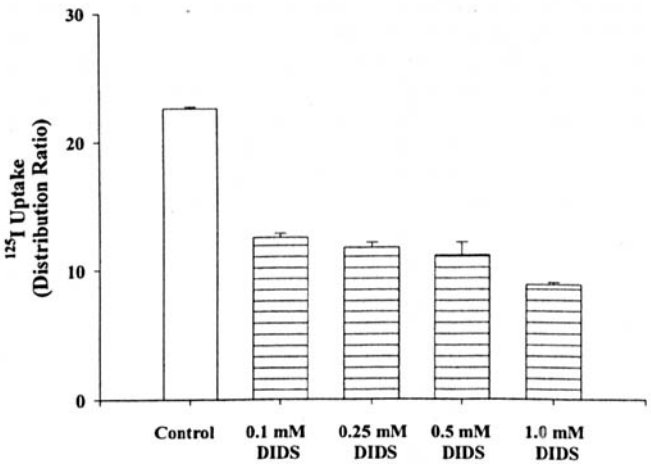


**Figure 8.** Dose-response effect of DIDS on $^{125}$I uptake in MCF-7 cells. Cells were cultured for 10 mins with 1 μCi/ml $^{125}$I plus DIDS at the concentrations indicated in the figure. Intracellular $^{125}$I was then determined. Numbers represent the mean ± SE of distribution ratios from 4 plates.

## Discussion

These studies clearly show that the pendrin iodide transporter is expressed in human MCF-7 mammary cancer cells. The pendrin transporter was identified via western

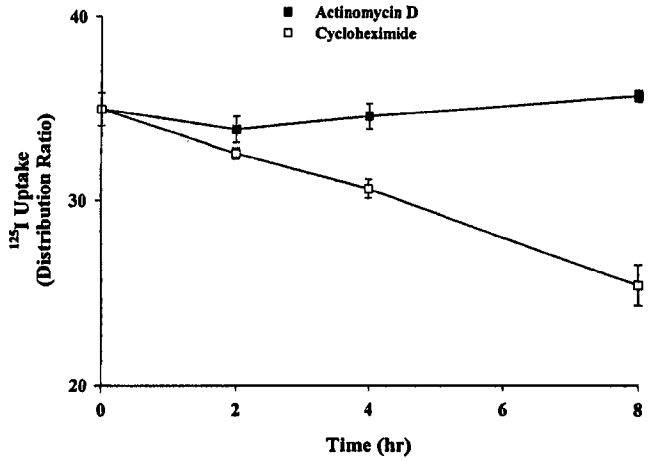


**Figure 9.** Effect of actinomycin D and cycloheximide on $^{125}$I uptake in MCF-7 cells. Cells were cultured for the times indicated with 2 μg/ml actinomycin D or 5 μg/ml cycloheximide. Intracellular $^{125}$I wsa then determined. Numbers represent the mean ± SE of distribution ratios from 4 plates.

blotting analysis as a 90 kDa protein. In addition, DIDS, which is known to inhibit the pendrin transporter as well as other anion exchange transporters (14–18), inhibited iodide uptake in the MCF-7 cells. Iodide uptake was temperature dependent, but sodium independent; these are additional characteristics of the pendrin transporter. Since the MCF-7 cell line was originally developed at the Michigan Cancer Foundation, which is affiliated with our institution, more than 5 stocks of the MCF-7 cells were employed in these studies; each of these took up iodide in a similar fashion. In further studies, we were unable to identify the NIS transporter in proteins from the MCF-7 cells. Via western blotting techniques employing a human NIS antibody that we had used in earlier studies (8), the NIS protein was not identified. Nor did several NIS transporter inhibitors impair iodide uptake. Further, the NIS transporter is sodium dependent, but iodide uptake was not impaired in the absence of sodium. In view of the results of Kogai *et al.* (13) in which the NIS transporter, but not the pendrin transporter, was clearly identified and characterized in MCF-7 cells, it is quite apparent that there may be variants of the MCF-7 cell line that express different iodide transporters or the specific culturing conditions employed may allow the expression of the genes for the NIS or pendrin transporters. Kogai *et al.* further reported a retinoic acid stimulation of iodide uptake and NIS expression in their studies.

$^{125}$I uptake in the MCF-7 cells is transient with a peak accumulation occurring between 2.5 and 15 mins. The transient nature of the $^{125}$I accumulation is likely due to the conversion of the iodide to iodine, as cells in culture are known to have high levels of peroxidase activity. The efflux of $^{125}$I-iodine would then explain the efflux of radioactivity from the cells during extended culture times. The total radioactivity in the culture media was not altered in the time course studies, but when the "spent" media was added to fresh cells, $^{125}$I uptake was impaired. All the data therefore

seem to indicate that iodide is rapidly converted to iodine in the MCF-7 cells. The fact that iodide-uptake distribution ratios of greater than 50 were observed in the MCF-7 cells is suggestive of an active uptake mechanism via the pendrin transporter. However, in view of the rapid metabolism of $^{125}I$, after being taken up into the MCF-7 cells, it is not possible to conclude that iodide is transported into the cells against a concentration gradient.


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