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# Differences in the electrophysiological response to $I^-$ and the inhibitory anions $SCN^-$ and $ClO_4^-$, studied in FRTL-5 cells

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**Abstract**

The electrophysiological properties of the $Na^+/I^-$ symporter (NIS) were examined in a cloned rat thyroid cell line (FRTL-5) using the whole-cell patch-clamp technique. When the holding potential was between −40 mV and −80 mV, 1 mM NaI and NaSCN induced an immediate inward current which was greater with $SCN^-$ than with $I^-$. The reversal potential for $I^-$ and $SCN^-$ induced membrane currents was +50 mV. This is close to the value of +55 mV calculated by the Nernst equation for $Na^+$. These results are consistent with $I^-$ and $SCN^-$ translocation via the NIS that is energized by the electrochemical gradient of $Na^+$ and coupled to the transport of two or more $Na^+$. There was no change in the membrane current recording with $ClO_4^-$ indicating that $ClO_4^-$ was either not transported into the cell, or the translocation was electroneutral. $ClO_4^-$ addition, however, did reverse the inward currents induced by $I^-$ or $SCN^-$. These effects of $I^-$, $SCN^-$ and $ClO_4^-$ on membrane currents reflect endogenous NIS activity since the responses duplicated those seen in CHO cells transfected with NIS. There were additional currents elicited by $SCN^-$ in FRTL-5 cells under certain conditions. For example at holding potentials of 0 and +30 mV, 1 mM $SCN^-$ produced an increasingly greater outward current. This outward current was transient. In addition, when $SCN^-$ was washed off the cells a transient inward current was detected. Unlike $SCN^-$, 1–10 mM $I^-$ had no observable effect on the membrane current at holding potentials of 0 and +30 mV. The results indicate FRTL-5 cells may have a specific $SCN^-$ translocation system in addition to the $SCN^-$ translocation by the $I^-$ porter. Differences demonstrated in current response may explain some of the complicated influx and efflux properties of $I^-$, $SCN^-$ and $ClO_4^-$ in thyroid cells. 

*Keywords:* Sodium iodide symporter; $N^+/I^-$ symporter; Thyroid; FRTL-5; Electrophysiology

## 1. Introduction

The translocation of iodide across thyroidal membrane has long been the subject of extensive studies

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[1–7]. From these studies, iodide transport can be characterized as being saturable with iodide, competitively inhibited by related anions independent of hormone synthesis, and concentrated against both a chemical and an electrical gradient [1]. The related anions $SCN^-$ and $ClO_4^-$ competitively inhibit $I^-$ transport into the thyroid as well as discharge $I^-$ already present [8–11]. In rat thyroid, perchlorate is 10–100 times more effective than $SCN^-$ in preventing the accumulation of $I^-$ [12,13]. The basis for differences in the effects of $SCN^-$ and $ClO_4^-$ on the accumulation and retention of $I^-$ by rat thyroid, however, remains unclear. An important advance in the study of thyroidal iodide transport has been the cloning of the sodium iodide symporter (NIS) from rat thyroid cells [14]. In a prior study, we measured $^{125}I^-$ flux in Chinese hamster ovary (CHO) cells transfected with NIS [13]. In common with studies that used thyroid tissue, iodide transport in the transfected cells was (1) dependent on $Na^+$, (2) half-saturated with iodide near 30 μM, and (3) inhibited by anions, such as $SCN^-$ and $ClO_4^-$. We concluded from these studies on transfected CHO cells that NIS activity can be demonstrated that is independent of thyroidal influences.

Another advance in the study of anion translocation has been the refinement of electrophysiological techniques. Patch-clamp measurement on single cell currents is a powerful tool for understanding mechanisms of anion transport [15,16]. Electrophysiological studies, using CHO cells transfected with NIS, demonstrated for the first time the current associated with $I^-$ and $SCN^-$ translocation. A further finding in these studies was the absence of an electrophysiological response to $ClO_4^-$ addition. In the present report, we extend these studies to document the electrophysiological events that occur in thyroid cells after the addition of $I^-$, and further explore differences between responses to $SCN^-$ or $ClO_4^-$. While many of the responses duplicated those reported in our prior studies with cells transfected with the NIS, there were several specific responses seen only in thyroid cells.

For these studies we used FRTL-5 cells, a rat thyroid cell line that grows in continuous culture and has been a model for studies of thyroidal iodide transport. The cell line survives the removal of TSH, and has been particularly useful in defining the role of TSH and other hormonal influences on the expression of iodide transport [4,5,17–19]. These cells were utilized in the successful cloning of the thyroidal NIS [14]. By combining results on FRTL-5 cells and the NIS-transfected CHO cells, we now have a more comprehensive understanding of $I^-$ transport and its inhibition by related anions in thyroid.

## 2. Materials and methods

### 2.1. Chemicals

Materials were obtained from the following suppliers: Coon's Modified Ham's F-12 medium from Hazleton (Denver, CO); calf serum (CS) from Gibco (Chagrin Falls, OH); bTSH from Armour Pharmaceutical (Phoenix, AZ); trypsin from Difco Laboratories (Detroit, MI); all other chemicals were from Nakarai Chemicals (Japan) and of the highest quality available.

### 2.2. Continuous culture of FRTL-5 cells

The cloned normal rat thyroid cell line FRTL-5 was kindly provided by Dr. L.D. Kohn (NIH, Bethesda, MD). FRTL-5 cells were seeded in of 24-well plates (Corning) and grown in Coon's modified Ham's F-12 medium supplemented with 5% CS, bTSH (1 mU/ml), and a 5-hormone (5H) mixture consisting of insulin (10 μg/ml), hydrocortisone ($10^{-8}$ M), transferrin (5 μg/ml), glycyl histidyl-L-lysine acetate (10 ng/ml) and somatostatin (10 μg/ml). Cells were used for the experiments after culture at 37°C for 7 days in a humidified atmosphere of 95% air and 5% $CO_2$ without TSH.

### 2.3. Voltage-clamp and recording techniques

Monolayer cultures of FRTL-5 cells were perfused with a Tyrode solution consisting of 140 mM NaCl, 5.4 mM KCl 1.8 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (HEPES), and 5 mM glucose at room temperature (22°C) and at pH 7.4 with or without NaI, $NaClO_4$ and NaSCN. Individual FRTL-5 cells were voltage-clamped using the whole-cell configuration of

the patch-clamp technique. The glass suction pipette had a tip diameter of approx. 2 μm and a resistance of 3–5 MΩ when filled with the internal solution. The current and voltage signals were stored on a digital magnetic tape (RD101, TEAC) for later computer analysis (PC98, NEC). The liquid junction (−10 mV) was not corrected for all membrane potential recordings.

The pipette was filled with an internal solution with the following composition: 140 mM KOH, 140 mM aspartate, 5 mM $MgCl_2$, 5 mM EGTA, 5 mM $K_2ATP$ and 10 mM HEPES, at pH 7.2.

## 3. Results

### 3.1. $I^-$ and $SCN^-$ induce membrane currents that are blocked by $ClO_4^-$

Fig. 1 shows the effects of $I^-$, $SCN^-$, and $ClO_4^-$ on membrane current. The currents were recorded from a single FRTL-5 cell under voltage-clamp conditions as detailed in Section 2. With the membrane potential held at −40 mV, changing the bath solution to one containing 1 mM NaI led to an immediate inward current that is seen in the recording as a downward deflection from the baseline (middle tracing). Addition of 1 mM $SCN^-$ (lower tracing) also produced an inward current but the magnitude of the deflection was greater with $SCN^-$ than with $I^-$. Higher concentrations of $I^-$ and $SCN^-$ (50–1000 mEq) induced similar changes in inward currents and therefore 1 mM $I^-$ and $SCN^-$ were selected for this study and the studies described below.

The electrogenic response (inward current) induced by $I^-$ or $SCN^-$ is in agreement with $I^-$ and $SCN^-$ transport that is coupled to the transport of two or more $Na^+$. The electrogenic response induced by $I^-$ or $SCN^-$ contrasts with the response seen when the Tyrode bathing solution is changed to one containing 1 mEq $ClO_4^-$. Unlike when $I^-$ or $SCN^-$ was added, there was no change in the current recording with $ClO_4^-$ (Fig. 1, top tracing). This observation is consistent with either the lack of $ClO_4^-$ transport into the cell, or with electroneutral transport of $ClO_4^-$. $ClO_4^-$ addition, however, does reverse the inward currents induced by $I^-$ or $SCN^-$. The downward deflections induced by $I^-$ or $SCN^-$ (Fig. 1, middle and lower tracings) return to the baseline when $ClO_4^-$ is added.

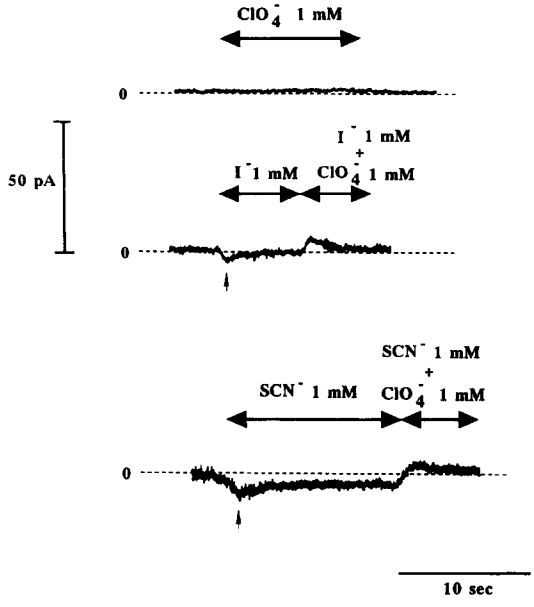


Fig. 1. Transporter current with $I^-$, $SCN^-$ and $ClO_4^-$ solution. Chart recording of the membrane current changes induced by switching the bathing solution as indicated above the current recording. The membrane potential was held at −40 mV. 0 indicates basal current. Changing the bath solution to one containing 1 mM NaI induced an immediate inward current (indicated by arrow in middle tracing). The addition of 1 mM $SCN^-$ also produced an inward current (arrow in lower tracing). Unlike when $I^-$ or $SCN^-$ was added, there was no change in the current recording with $ClO_4^-$ (top tracing). The downward deflections induced by $I^-$ or $SCN^-$ (middle and lower tracings) return to the baseline when $ClO_4^-$ is added.

The effect of $SCN^-$ on inward current duplicated the response seen with CHO cells transfected with NIS. In the transfected cells, the current response to $SCN^-$ was also greater than with $I^-$. Differences, however, became evident when $SCN^-$ was added to cells and the membrane potential was more than −40 mV, as shown in Fig. 2.

### 3.2. Membrane currents induced by $SCN^-$ are voltage dependent

The holding potential was varied between +30 and −80 mV, as indicated in Fig. 2. When the holding potential was between 0 mV and −80 mV, $SCN^-$ induced an inward current. However, when the holding potential was between −20 mV and +30 mV, 1 mM $SCN^-$ produced an increasingly greater out-

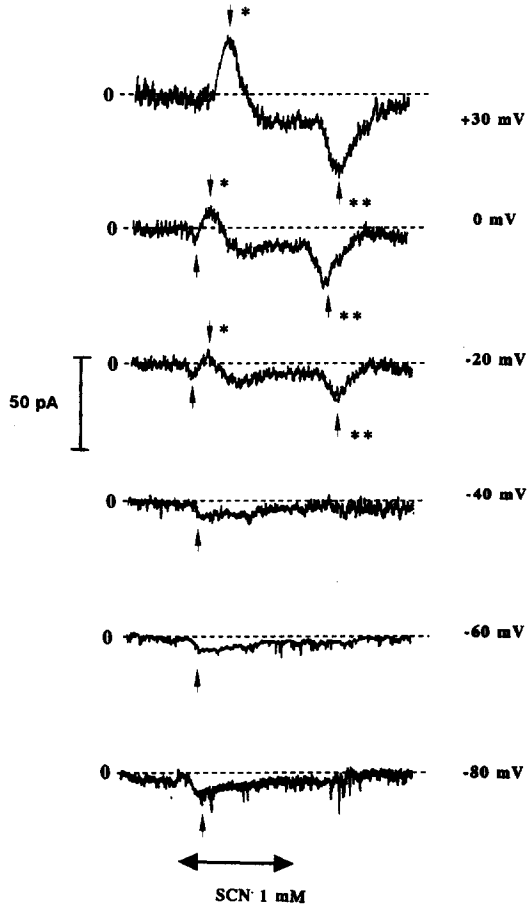


Fig. 2. Chart recording of the membrane current changes at each membrane potential induced by switching the bathing solution as indicated below the current recording. The holding potential was varied at levels ranging from +30 to −80 mV. 0 indicates basal current. When the holding potential was between 0 mV and −80 mV, $SCN^-$ induced an inward current (indicated by arrow). When the holding potential was between −20 mV and +30 mV, 1 mM $SCN^-$ produced an increasingly greater outward current (indicated by arrow with *). This outward current was transient and was not maintained. After washing $SCN^-$ off the cells, a transient inward current was also recorded (indicated by arrow with **).

ward current. This outward current was transient and not maintained. After washing $SCN^-$ off the cells, a transient inward current was also recorded. The outward current was found only with FRTL-5 cells, and was not seen in CHO cells transfected with NIS studied under similar conditions. This effect of $SCN^-$ (outward current at membrane potentials of −20 mV and below) in FRTL-5 cells is further dissociated from NIS transport in the study shown in Fig. 3.

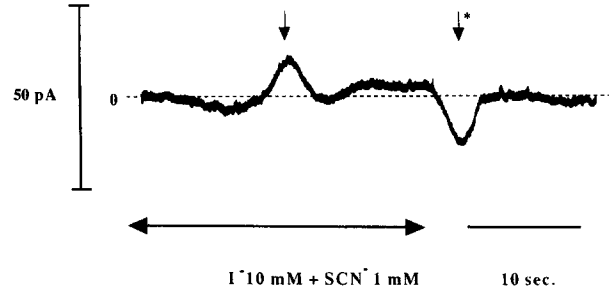


Fig. 3. Chart recording of the membrane current induced by switching the bathing solution as indicated below the current recording. The cells were bathed in 10 mM NaI before and also during the addition of 1 mM $SCN^-$. 0 indicates basal current. 1 mM $SCN^-$ led to an outward current (arrow) and a transient inward current following $SCN^-$ washout (arrow with *).

In this experiment 10 mM NaI was used to saturate the NIS. The cells were bathed in 10 mM NaI before and also during the addition of 1 mM $SCN^-$. As shown in Fig. 3, the effect of $SCN^-$ was preserved under these conditions, e.g. 1 mM $SCN^-$ led to an

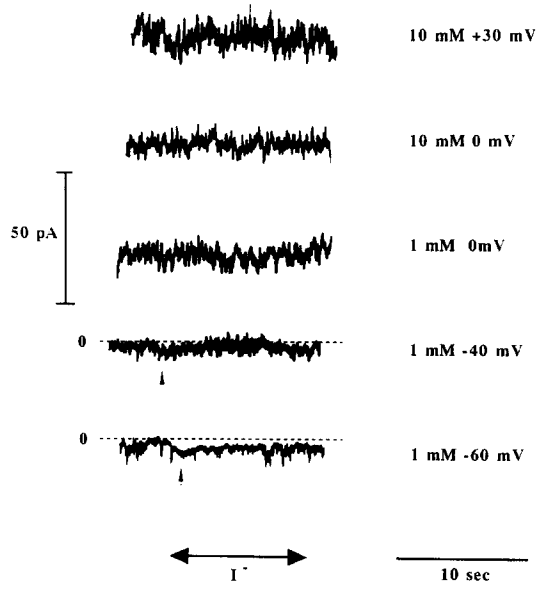


Fig. 4. Chart recording of the membrane current changes at each membrane potential induced by switching the bathing solution as indicated below the current recording. Concentration of iodide in bathing solution is indicated at the left of the membrane potential. 1 mM $I^-$ induced inward current at holding potentials of −40 mV and −60 mV (indicated by arrow). 1 mM $I^-$ had no observable effect on current and did not produce an outward current at the holding potential of 0 mV. Increasing the $I^-$ to 10 mM also failed to elicit a current at holding potentials of 0 and +30 mV. Fluttering of the baseline reflects background.

outward current and a transient inward current following $SCN^-$ washout. 10 mM NaI did not modify the transient inward current following $SCN^-$ washout.

### 3.3. $I^-$ induced current is voltage dependent

The voltage dependence of $I^-$ induced current was also examined (Fig. 4). 1 mM $I^-$ induced an inward current at holding potentials of −40 mV and −60 mV. Unlike $SCN^-$ at the holding potential of 0 and +30 mV, 1 mM $I^-$ had no observable effect on current and did not produce an outward current. Increasing the $[I^-]$ to 10 mM also failed to elicit a current at holding potentials of 0 and +30 mV. The results are consistent with a $SCN^-$ influx pathway that is not shared with $I^-$, a pathway found in FRTL-5 but not CHO cells transfected with NIS.

### 3.4. The reversal potential and current-voltage relationship demonstrated for $I^-$ and $SCN^-$ induced inward currents

Fig. 5 shows the current-voltage relationship of the inward current induced by $SCN^-$ and $I^-$ at different holding potentials. The inward current increased progressively as the membrane potential decreased. At all holding potentials, the current induced by $SCN^-$ was greater than that induced by $I^-$. The greater current is seen in Fig. 1, and also in the prior study with CHO cells transfected with NIS. The greater inward current elicited by $SCN^-$ when compared to $I^-$ thus appears to define a property of the NIS. If FRTL-5 cells are perfused with 10 mM NaI prior to 1 mM $SCN^-$, $SCN^-$ does not induce an additional inward current (not shown).

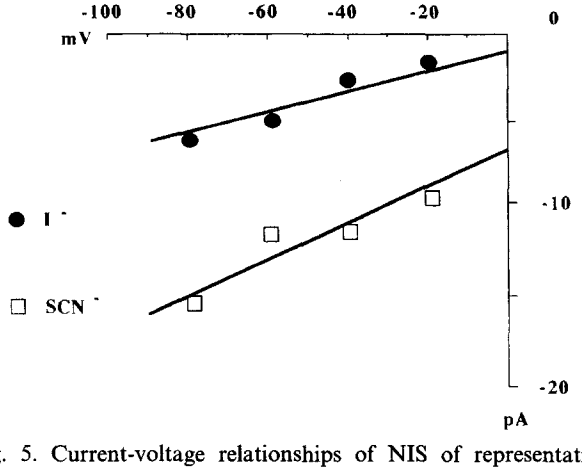


Fig. 5. Current-voltage relationships of NIS of representative current induced by iodide (closed circles) and by $SCN^-$ (open squares). The ordinate indicates current amplitude, the abscissa, membrane potential. The inward current increased progressively as the membrane potential decreased. At all holding potentials, the current induced by $SCN^-$ was greater than that induced by $I^-$. The reversal potential for $I^-$ and $SCN^-$ induced inward currents was +50 mV.

The reversal potential (when the current is 0) for $I^-$ and $SCN^-$ induced inward currents was +50 mV. This reversal potential is close to the value calculated using the Nernst equation for $Na^+$ of +55 mV. Also, in CHO transfected cells, the membrane potential was +50 mV when the membrane current was zero. These results are consistent with $I^-$ (and $SCN^-$) translocation via the NIS being energized by the electrochemical gradient of $Na^+$.

## 4. Discussion

In the present report, we described the electrophysiological properties of $I^-$, $SCN^-$ and $ClO_4^-$ transport in FRTL-5 cells, and compare the findings to similar studies in NIS-transfected CHO cells. In FRTL-5 cells, $I^-$ and $SCN^-$ induce an inward current similar to that found in NIS-transfected CHO cells. This finding suggests that the measured current reflects NIS presence in the membrane, independent of anion metabolism or thyroidal influences.

The inward current is ascribed to the transport of $I^-$, along with the translocation of two or more $Na^+$ ions as proposed. O'Neill et al. [18] and Nakamura et al. [20] using thyroid vesicles, showed that $Na^+$ dependent $I^-$ uptake was sigmoidal, with a Hill coefficient of 1.6–1.8. Dai et al. noted the high degree of homology in molecular structure of NIS when compared with the human $Na^+$/glucose cotransporter [14]. Translocation of at least two $Na^+$ proposed for $Na^+$/glucose cotransport is supported by the electrophysiological measurement of inward current in intact cells [21,22].

In electrophysiological studies of NIS using the *Xenopus* oocyte expression system, $I^-$ induced a greater inward current than did $SCN^-$ [23]. This was the converse of that found in FRTL-5 cells or in NIS-transfected CHO cells. In FRTL-5 cells and

transfected CHO cells, the inward current induced by $SCN^-$ was consistently higher than with $I^-$. The difference may reflect differences inherent in model systems used. For example, ion-transport properties of a carrier are known to be influenced by the lipid environment, and there is marked difference in the lipid composition of the *Xenopus* oocyte membrane compared to that found in mammalian cells. As noted below, there are additional differences between $SCN^-$ effects in FRTL-5 cells when compared to NIS-transfected cells. These differences draw attention to the fact that the functioning cell may be more complex than the transfected cell and that not all studies with transfected cells can be construed as the final answer.

As indicated above, some responses in FRTL-5 cells are not duplicated in NIS-transfected cells. For example, with a membrane potential greater than −20 mV, $SCN^-$ induced an outward current in FRTL-5 cells, a current that was not seen with NIS-transfected CHO cells. Further evidence for the limited role of $SCN^-$ metabolism in the current measurements is that sufficient $SCN^-$ would need to be present to account for the large outward current observed with $SCN^-$. It is unlikely that the basis for this current is the translocation of $SCN^-$ through a chloride or non-specific anion channel since the bathing solution contained 140 mEq $Cl^-$ and did not interfere with the observed response. The addition of 10 mM $I^-$ did not induce an outward current nor did it disturb the current induced by $SCN^-$.

The SCN induced outward current measured in FRTL-5 cells was followed by an inward current. This current may represent $SCN^-$ efflux as the inward current increased at higher membrane potentials, but did not depend on membrane potential. Since this current is seen only in FRTL-5 cells, and not in transfected cells, it suggests a pathway for $SCN^-$ translocation restricted to thyroid tissues and independent of $I^-$ transport.

The anion concentrations used in the present study were between 1 and 10 mM and therefore not 'physiological'. Within the time of observation and using these high concentrations of anions, there was no evidence that electrical properties of the membrane were not changed nor that exposure to salts at these concentrations had detrimental effects on membrane permeability.

There was no current response following $ClO_4^-$ addition in FRTL-5; NIS-transfected CHO cells also failed to respond to $ClO_4^-$. Evidence that $ClO_4^-$ is in fact concentrated in human and rat thyroid tissue was reviewed recently [24]. The absence of a measurable response in FRTL-5 suggests that $ClO_4^-$ translocation is electroneutral and therefore different from the easily demonstrated electrogenic translocation of NaI and NaSCN. Besides the possibility that $ClO_4^-$ enters with NIS electroneutrally, another possibility is that the binding activity is sufficiently high that any current change is below detection. Finally, it should be mentioned that although $ClO_4^-$ may inhibit competitively NaI entry by NIS, $ClO_4^-$ also may cross the membrane independently of NIS.

In summary, FRTL-5 cells and NIS-transfected CHO cells transport $I^-$ and $SCN^-$ but not $ClO_4^-$. Further, FRTL-5 cells have a specific $SCN^-$ translocation system in addition to $SCN^-$ transport by the $I^-$ symporter. These differences in current properties may contribute the complexity of $I^-$, $SCN^-$ and $ClO_4^-$ influx and efflux in the functioning thyroid.

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