Relative Potencies and Additivity of Perchlorate, Thiocyanate, Nitrate, and Iodide on the Inhibition of Radioactive Iodide Uptake by the Human Sodium Iodide Symporter

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The presence of perchlorate (ClO_4^-) in some U.S. drinking water supplies has raised concern about potential adverse thyroidal health effects, because ClO_4^- is known to competitively inhibit iodide uptake at the sodium iodide symporter (NIS). Humans are nutritionally and environmentally exposed to other competitive inhibitors of iodide uptake, including thiocyanate (SCN^-) and nitrate (NO_3^-). The joint inhibiting effects of these three anions was studied by exposing Chinese hamster ovary cells stably expressing human NIS to varying concentrations of each anion separately, and in combination, and conducting measurements of $^{125}I^-$ uptake. The entire data set was fit to a single Hill equation using maximum likelihood. The relative potency of ClO_4^- to inhibit $^{125}I^-$ uptake at the NIS was found to be 15, 30 and 240 times that of SCN^-, I^-, and NO_3^- respectively on a molar concentration basis, with no evidence of synergism. These results are consistent with a common mode of action by these anions of simple competitive interaction, in which a concentration of any one of ClO_4^- SCN^-, and NO_3^-, occurring either individually or as part of a mixture of the three anions, is indistinguishable from a concentration or dilution of either one of the remaining two ions in inhibiting iodine uptake at the NIS.

Introduction

A NUMBER OF different inorganic anions can block iodide (I^-) uptake at the thyroidal sodium iodide symporter (NIS) in a competitive manner. Of these, nitrate (NO_3^-), thiocyanate (SCN^-), and perchlorate (ClO_4^-) are of particular dietary and/or environmental importance. Sufficient inhibition of iodide uptake can lead to decreased thyroid hormone production and, ultimately, result in adverse health effects secondary to hypothyroxinemia.

NO_3^- is ubiquitous in foods, either occurring naturally, as in green leafy vegetables, or added as a preservative, as in processed meats. NO_3^- is also very common in both surface and ground sources of drinking water, primarily due to the agricultural use of nitrate fertilizers. The average daily intake of NO_3^- is estimated to be about 40–50 mg/d for adults, based on studies in Denmark and the United Kingdom (1–3). Typical serum levels in the Western world range from 10–140 μmol/L with a mean of 30–50 μmol/L (3–9), and the serum half life is 5–8 hours (10–12).

Foods that are particularly rich in thiocyanate include cabbage, broccoli, brussels sprouts, turnips, rapeseed and mustard seed (13–16). Cauliflower, cabbage, radishes, spinach, and tomatoes have been reported to contain on average 88, 86, 7, 5, and 2 mg/kg (wet weight) respectively of SCN^- (17). Milk contains 2–10 mg/L SCN^- derived from dairy cattle grazing on plants from the cruciferous family (17–22). In some areas of the world, SCN^- is added to milk as a preservative (23). Cassava, which can contain the equivalent of as much as 3400 mg/kg SCN^- on a dry weight basis, is the most important dietary source of SCN^- in some areas of the world (24). In areas that are not dependent upon cassava as a staple food, cyanide from cigarette smoking is the most important source of SCN^- in the body. Nonsmokers' serum levels are typically 10–70 μmol/L, compared to a typical range of 80–120 μmol/L among smokers (13,25–31). SCN^- has a reported "kidney threshold" at a serum level of 200–300 μmol/l (24). Serum half-life at levels over 200–300 μmol/L has been determined to be about 3 days among healthy volunteers (32), while serum half-life among cigarette smokers who quit (initial SCN^- concentrations of 130–186 μmol/L) has been estimated to be approximately 6 days (33).

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Drinking water supplies in some parts of northern Chile contain naturally occurring ClO_4^- at average levels ranging up to more than 100 μg/L (34). Since 1997, ClO_4^- has been detected in U.S. underground and surface water supplies, primarily in the southwest, in concentrations generally below 50 μg/L (35). These levels are thought to be mainly the result of military and industrial production and use of ammonium perchlorate. ClO_4^- is a natural contaminant in NO_3^- fertilizers mined in Chile that are widely used in the United States in tobacco and organic farming. The half-life of ClO_4^- in human serum has been estimated to be approximately 8 hours (36).

The relative effects of ClO_4^-, SCN^-, and NO_3^- on radioactive iodide uptake (RAIU) inhibition and upon RAI discharge were evaluated in rats. It was shown that, on a serum molar concentration basis, ClO_4^- was approximately 10 times as potent as SCN^- and about 300 times as potent as NO_3^- in inhibiting RAIU in the thyroid and that SCN^- was slightly more potent than I^-. All three treatments resulted in increased thyroid weight, decreased thyroid iodide, and follicular hyperplasia (37, 38). In another study in rats, the relative molar doses of ClO_4^-, SCN^-, and NO_3^- for causing 50% increase in thyroid weight were 1:20:550. The molar concentrations of ClO_4^-, SCN^-, and NO_3^- required to produce a 50% inhibition in the concentration of radiolabeled iodide in thyroid slices *in vitro* were determined to be in a ratio of 1:20:400 (39). The concentrations of ClO_4^-, SCN^-, and NO_3^- required to decrease the uptake of radiolabeled iodide in isolated rat thyroids by 50% was determined to be in a molar ration of 1:20:240 (40).

Two separate studies with Chinese hamster ovary (CHO) cell lines stably transfected with human NIS demonstrated that ClO_4^- and SCN^- inhibited RAIU in a dose-dependent manner, reaching nearly complete inhibition at 20 μmol/L and 500 μmol/L, respectively. ClO_4^- was nearly 10 times as potent as SCN^- in inhibiting RAIU throughout the range of concentrations tested (41). In these two studies, 5-fold to 10-fold dilutions of normal serum was demonstrated to inhibit RAIU up to 20%–25% (41,42).

Thus *in vivo* and *in vitro* studies are in substantial agreement, indicating that on a molar concentration basis, ClO_4^- is 10 to 20 times more potent than SCN^- and several hundred times more potent than NO_3^- in inhibiting RAIU, with unlabeled iodide having roughly the same potency as SCN^-. However the combined effect of the simultaneous administration of these inhibitors has not been investigated in any detail in these studies.

In the present study, we use data from an *in vitro* test system in which human NIS was stably transfected into a CHO cell line to investigate the simultaneous joint effects of ClO_4^-, SCN^-, NO_3^-, and (nonradioactive) I^- in inhibiting RAIU. In particular, we investigated (1) whether the concentration-RAIU curves for these anions are the same or different (after adjusting for differences in relative potency) and (2) whether there is evidence for nonadditive interactions (synergistic or antagonistic) among these RAIU inhibitors.

Methods

Experimental protocol

The experimental protocol was similar to that used previously as part of a study to investigate effects of autoanti-

bodies on the NIS (41) and performed in the same laboratory. One hundred thousand cells from a CHO cell line into which human NIS had been stably transfected were seeded in 24-well plates and cultured in Dulbecco modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS). When cells reached 100% confluence, the medium was removed, and cells were washed with physiologic solution and combined with 500 mL buffer A (Hanks' balanced salt solution [HBSS] containing 0.5% bovine serum albumin [BSA] and 10 mmol N-2-hydroxy-ethylpiperazine-N'-2-ethanesulfonic acid [HEPES], pH 7.4) containing 100,000 counts per minute (cpm) of carrier-free $Na^{125}I$ (0.1 mCi, 0.4 nmol/L). This mixture was separated into wells, and various concentrations of one of three RAIU inhibitors (SCN^-, NO_3^-, or ClO_4^-) were added to individual wells to provide information on concentration-uptake response for that anion. Each experimental concentration was duplicated in two separate wells. In a number of cases, fixed molar concentrations of other RAIU inhibitors (including non-radioactive iodide) were added prior to separating the mixture into wells. After incubation at 37°C for 45 minutes, cells were quickly washed twice with 2 mL of ice-cold buffer A, and then solubilized with 1 mL of 0.1 mol/L sodium hydroxide. $^{125}I^-$ uptake was determined by measuring the radioactivity counts per minute from each well using a γ-counter.

Data collected at the same time starting with the same basic mixture of incubated cells and $^{125}I^-$ are identified as comprising the data from a single "experiment." The designs of separate experiments included in the present study are shown in Table 1. Except for the three range finding experiments (experiments 1–3), concentrations in each concentration-uptake response consisted of consecutive doubles of the lowest positive concentration.

Data analysis

A common method of evaluating data from RAIU studies is to plot the relative radioactive uptake (uptake at a given dose divided by the uptake at zero concentration) and to use this plot to estimate the concentration that produces 50% inhibition of iodide uptake. However, a somewhat more detailed approach is required here. First, the goals of the present analysis include determining whether the different anions inhibit uptake via a common concentration response (once differences in potencies are accounted for) and whether the presence of one anion (e.g., SCN^-) potentiates or inhibits the effect of another anion (e.g., ClO_4^-). This requires comparing the shapes of uptake response curves in addition to estimating 50% uptake inhibition concentrations. Second, not all of the experiments included a true background point (i.e., a well in which none of the four RAIU inhibitors were added), in which case there is no denominator readily available for direct computation of percent RAIU. All of these issues can be addressed by application of a statistical model of uptake inhibition. The statistical model used in the present analysis is described below.

A form of the Hill model, which is often used to model drug action (43), is used to model the competitive inhibition of thyroidal iodide uptake. Let X_T, X_N, X_P, X_I, (where T = SCN^-, N = NO_3^-, P = ClO_4^-, and I = nonradioactive I^-) denote the concentrations (μmol/L) of the respective anions applied to a single well in an experiment, and let C be the

TABLE 1. DESIGN OF EXPERIMENTS MEASURING RADIOACTIVE IODIDE UPTAKE IN CHINESE HAMSTER
OVARY CELLS TRANSFECTED WITH HUMAN SODIUM IODIDE SYMPORTER

	Concentrations (μmol/l)			
Exp. No.	SCN	NaI	NO_3	ClO_4
1				CUR10: 0.1–100
2	CUR10: 0.1–100			
3			CUR7: 0.1–10000	
4				CUR9: 0.1–12.8
5	CUR9: 1–128			
6			CUR9: 10–1280	
7		1		CUR9: 0.1–12.8
8	CUR9: 1–128	1		
9		1	CUR9: 10–1280	
10		10		CUR9: 0.1–12.8
11	CUR9: 1–128	10		
12		10	CUR9: 10–1280	
13A				CUR9: 0.1–12.8
13B			10	CUR9: 0.1–12.8
14A				CUR9: 0.1–12.8
14B			40	CUR9: 0.1–12.8
15A				CUR9: 0.1–12.8
15B			160	CUR9: 0.1–12.8
16A	CUR9: 1–128			
16B	CUR9: 1–128		10	
17A	CUR9:1–128			
17B	CUR9: 1–128		40	
18A	CUR9: 1–128			
18B	CUR9: 1–128		160	
19A		10		CUR9:0.1–12.8
19B		10	10	CUR9: 0.1–12.8
20A		10		CUR9: 0.1–12.8
20B		10	40	CUR9: 0.1–12.8
21A		10		CUR9: 0.1–12.8
21B		10	160	CUR9: 0.1–12.8
22A	CUR9: 1–128	10		
22B	CUR9: 1–128	10	10	
23A	CUR9: 1–128	10		
23B	CUR9: 1–128	10	40	
24A	CUR9: 1–128	10		
24B	CUR9: 1–128	10	160	
25A		1		CUR9: 0.1–12.8
25B	2	1		CUR9: 0.1–12.8
25C	8	1		CUR9: 0.1–12.8[a]
25D	18.5	1		CUR9: 0.1–12.8[a]
26A	2	1		CUR9: 0.1–12.8
26B	8	1		CUR9: 0.1–12.8
26C	18.5	1		CUR9: 0.1–12.8

CUR9: 0.1–12.8 signifies a concentration-uptake response investigation involving 9 distinct concentrations, including 0, with the 8 positive concentrations being distributed between 0.1 and 12.8 μmol/L.
[a]Group exposed to zero concentration of ClO_4 was not exposed to SCN.
SCN, thiocyanate; NaI, sodium iodide; NO_3 nitrate; ClO_4, perchlorate.

corresponding counts per minute recorded on the γ-counter. The expected counts per minute, $C_{expected}$, is assumed to be related to the anion concentrations according to the following model:

$$C_{expected} = \frac{C_0}{1 + \left(\frac{X_T}{T_{50}} + \frac{X_I}{I_{50}} + \frac{X_N}{N_{50}} + \frac{X_P}{P_{50}}\right)^r} \quad \text{(Eq. 1A)}$$

where T_{50}, I_{50}, N_{50}, P_{50} are the concentrations of SCN$^-$, I$^-$, NO_3^-, and ClO_4^-, respectively, that, when applied alone, re-

sult in 50% inhibition of RAIU. To see this, note that if, for example, SCN$^-$ is present at the level, $X_T = T_{50}$, and no other anion is present ($X_N = X_P = X_I = 0$), then Equation 1 becomes

$$C_{expected} = \frac{C_0}{1 + \left(\frac{T_{50}}{T_{50}} + \frac{0}{I_{50}} + \frac{0}{N_{50}} + \frac{0}{P_{50}}\right)^r} = \frac{C_0}{1 + (1)^r} = 0.5C_0$$

The parameter C_0 represents the expected background count in the absence of any RAIU inhibitor, and r is a shape pa-

rameter that influences the curvature of the concentration response curve. In the implementation of the model, C_0 is fixed for a given experiment but allowed to vary from experiment to experiment to reflect differences in experimental conditions, which might include such factors as changes over time in radioactivity of $^{125}I^-$, different initial numbers of cells, and differences in environmental conditions such as temperature, etc. All other parameters are assumed to be the same for all experiments.

The model (Equation 1A) can be written in the equivalent form

$$C_{expected}$$

$$= \frac{C_0 \times P_{50}^r}{P_{50}^r + (\beta_T \times X_T + \beta_I \times X_I + \beta_N \times X_N + X_P)^r} \quad \text{(Eq. 1B)}$$

where the β's represent the relative potencies of the respective anions (relative to ClO_4^-) for inhibiting iodide uptake, that is, $\beta_T = P_{50}/T_{50}$, $\beta_N = P_{50}/N_{50}$, and $\beta_I = P_{50}/I_{50}$. The expected relative uptake can be written as

$$RU_{expected} = \frac{C_{expected}}{C_0}$$

$$= \frac{P_{50}^r}{P_{50}^r + (\beta_T \times X_T + \beta_I \times X_I + \beta_N \times X_N + X_P)^r} \quad \text{(Eq. 2)}$$

$$= \frac{P_{50}^r}{(P_{50}^r + PEC^r)}$$

where the perchlorate equivalent concentration (PEC) is given by

$$PEC = \beta_T \times X_T + \beta_I \times X_I + \beta_N \times X_N + X_P \quad \text{(Eq. 3)}$$

This model assumes that each of the four anions has exactly the same effect as a dilution or concentrate of any of the other three anions, namely, the effect of X_T $\mu mol/L$ of SCN^- is exactly the same as $\beta_T \times X_P$ $\mu mol/L$ of ClO_4^-. The PEC of a mixture is the equivalent concentration of ClO_4^- that would cause the same inhibition of iodide uptake as the mixture.

In order to test for possible interaction (e.g., synergy or antagonism) between two anions in inhibiting RAIU, the model was augmented by adding a term to the PEC equal to the product of the molar concentrations of the two anions times an interaction parameter, β_{INT}. If β_{INT} is different from zero, the model no longer predicts that an RAIU inhibitor acts exactly as a concentrate or dilution of another inhibitor. Positive values of β_{INT} indicate a synergistic interaction in which the presence of one inhibitor potentiates the inhibitory effect of the other inhibitor, whereas negative values of β_{INT} indicate an antagonistic (opposite to synergistic) effect.

The model was fit by maximum likelihood to the data from each of the experiments using the statistical model, $C = C_{expected} + \epsilon$, where ϵ for different wells are independent and normally distributed, with a constant standard deviation, σ. Likelihood ratio tests were used to test hypotheses and statistical confidence limits were computed by the profile likelihood method (44,45). Graphical representations of the fit of the model to the relative uptake data were developed by plotting the raw counts divided by the model-derived expected count in the absence of inhibitory anions (C/C_0), and comparing these ratios to curves of the model prediction of relative uptake (Equation 2).

Results

Allowing the background counts per minute (C_0) to vary with experiment produced a highly significant improvement in fit over assuming a fixed background applicable to all experiments ($\chi^2 = 1639$, 25 df, $p < 0.00001$), which indicates, as expected, the presence of significant heterogeneity in absolute cpm from different experiments. Table 2 indicates that fitting the model with the shape parameter, r, estimated (best estimate $r = 1.04$) or with r fixed at $r = 1$ produce similar results. These two model fits produce very similar estimates of the 50% RAIU inhibition concentrations, and the variations in the data around the two model fits are almost identical ($\sigma = 1716$ cpm with $r = 1.04$ and $\sigma = 1722$ cpm with $r = 1$). Furthermore, it can be shown that the difference in RAIU predicted by the two models is never larger than 1%

TABLE 2. RESULTS FROM APPLYING MODEL FOR RADIOACTIVE IODIDE UPTAKE INHIBITION (EQUATION 1) TO DATA FROM ALL EXPERIMENTS

	r estimated		r = 1	
		90% CI		90% CI
Concentrations (μmol/L) corresponding to 50% inhibition				
P_{50}	1.27	(1.23, 1.32)	1.22	(1.19, 1.26)
T_{50}	19.3	(18.4, 20)	18.7	(17.9, 19.3)
I_{50}	33.9	(29.8, 35.8)	36.6	(29.3, 38.6)
N_{50}	297	(283, 312)	293	(279, 309)
Potencies relative to ClO_4				
β_P	1[a]		1[a]	
β_T ($= P_{50}/T_{50}$)	0.0660	(0.063, 0.0692)	0.0656	(0.0625, 0.0688)
β_I ($= P_{50}/I_{50}$)	0.0376	(0.035, 0.0404)	0.0335	(0.0313, 0.0358)
β_N ($= P_{50}/N_{50}$)	0.00429	(0.00403, 0.00455)	0.00417	(0.00392, 0.00444)
r	1.04	(1.02, 1.08)	1	
σ (cpm)	1716		1722	

P, ClO_4; T, SCN; I, NaI; N, NO_3.
[a]By definition, because potencies are computed relative to ClO_4.

for any PEC. Thus, although allowing r to be different from 1.0 provides a statistically significant improvement in the fit ($p = 0.02$), this improvement is inconsequential biologically. Consequently, we henceforth consider only the reduced model with r fixed at $r = 1.0$. According to this model, on a molar basis, ClO_4^- was 15 ($1/\beta_T$), 30 ($1/\beta_I$) and 240 ($1/\beta_N$) times more potent than SCN^-, I^-, and NO_3^-, respectively, in inhibiting RAIU.

Figure 1 shows the relative RAIU (observed counts per minute divided by the estimated mean background count, Co) as a function of the molar concentration, using only concentration response data involving a single inhibitor (Table 1: experiments 1–6, and "A" portions of experiments 13–18). This figure shows clearly that ClO_4^- was the most potent inhibitor on a molar basis, followed by SCN^-, and then NO_3^-. The three curves shown in the figure were based on the single model fit to the entire database reported in Table 2 ($r = 1$). This single model appears to describe well the concentration-responses of the respective anions. The molar concentrations in Table 2 corresponding to 50% inhibition of iodide uptake can be visualized in this graph by dropping vertically from where a curve crosses the 50% uptake line to the (molar concentration) x-axis.

Figure 2 shows the same data as in Figure 1, but plotted as a function of the ClO_4^- equivalent concentration (PEC). Intuitively, Figure 2 is derived from Figure 1 by sliding the data for SCN^- and NO_3^- to the left until they match up with those for ClO_4^-. The amount (factor) by which data points for SCN^- must be shifted to match those for ClO_4^- is a measure of the relative potency (β_T) of SCN^-. Although the β's could be estimated in this intuitive manner, they were in fact estimated by the more precise statistical method of maximum likelihood. Figure 2 confirms that the data, when plotted as a function of the PEC, are well described by a single curve.

The data points involving concentrations of multiple RAIU inhibitors can be added to the graph in Figure 2 by computing the PEC for each concentration combination. Figure 3 shows the resulting graph when all data points involving concentration of any inhibitor(s) are plotted as a function of the PEC. This figure demonstrates that the total-
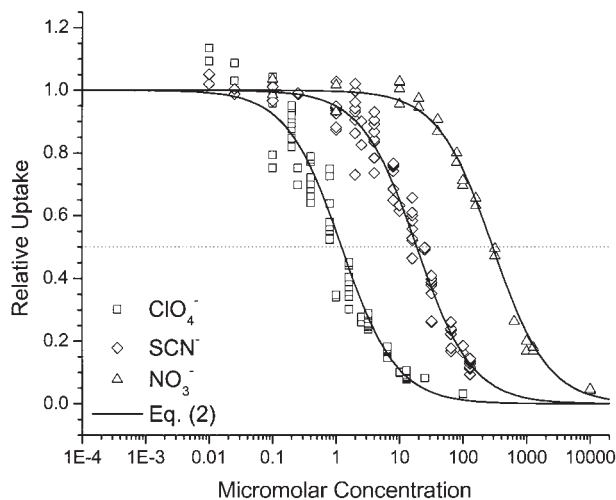
ity of the data can be adequately described by a single curve when concentrations are expressed in terms of the PEC.

Statistical tests for interactions (synergistic or antagonistic) between anion pairs (SCN^-, ClO_4^-), (NO_3^-, ClO_4^-), and (SCN^-, NO_3^-) indicated that in each case the estimate of the interaction parameter was slightly negative (indicative of antagonism rather than synergism), but in no case significantly different from zero ($p \geq 0.18$ in all cases). Figures 4–6 show data from experiments that generated the concentration response for a single RAIU inhibitor in the presence of fixed concentrations of other RAIU inhibitors. Figure 4 is based on data from experiments 25 and 26 (Table 1) in which concentration-response data for ClO_4^- were generated at four concentrations of SCN^-, (0, 2, 8, and 18.5 $\mu mol/L$), all in the presence of 1 $\mu mol/L$ sodium iodide. Figure 4A shows the experimental relative RAIU values plotted against the ClO_4^- molar concentration, whereas Figure 4B shows the same

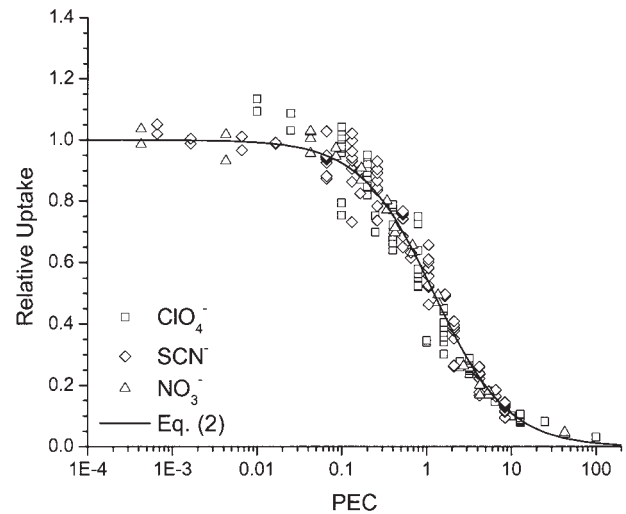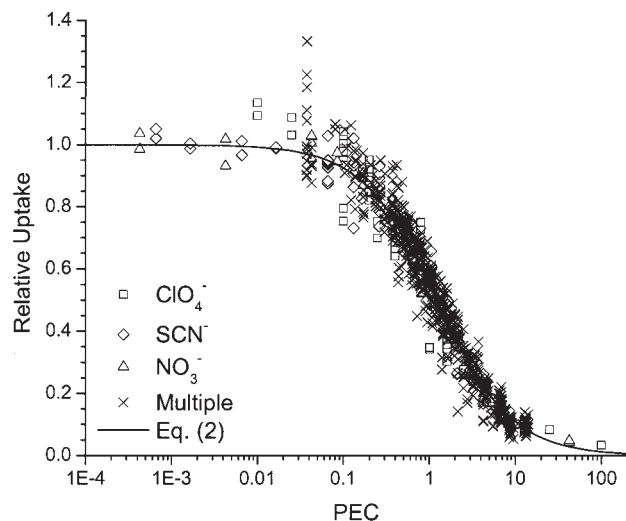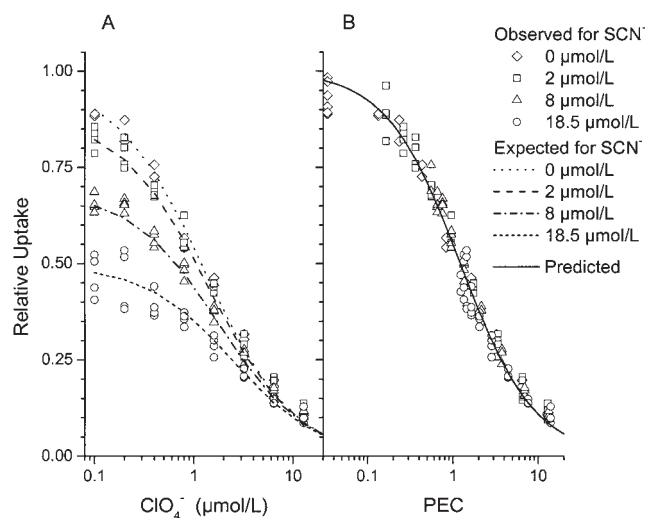**FIG. 2.** Relative iodine uptake as a function of perchlorate equivalent concentration (PEC) for exposures to a single anion.

FIG. 1. Relative iodide uptake as a function of molar concentration of SCN^-, ClO_4^- and NO_3^-.

FIG. 3. Relative iodine uptake as a function of perchlorate equivalent concentration (PEC) for all data.

FIG. 4. Relative iodine uptake as a function of the molar concentration of ClO_4^- (**A**) or perchlorate equivalent concentration (PEC) (**B**), for four background concentrations of SCN^- (all in presence of 1 μmol NaI).

FIG. 6. Relative iodine uptake as a function of the molar concentration of SCN^- (**A**) or the perchlorate equivalent concentration (PEC) (**B**), for four background concentrations of NO_3^-.

RAIU data plotted against the PEC. In both figures, the curves are the expected values for relative RAIU, based upon the fit of the model (equation 1) derived from all 26 studies (Table 2, $r = 1$). Figure 5 shows similar plots of concentration-response data for ClO_4^- generated in the presence of different concentrations of NO_3^- (experiments 13, 14 and 15), and Figure 6 shows similar plots of concentration-response data for SCN^- generated in the presence of different concentrations of NO_3^- (experiments 16, 17, 18). A synergistic effect between two anions would manifest itself in these graphs by a tendency for the relative RAIU concentration-response values to fall below the model predictions at the higher concentrations of other anion, and above the model predictions at the lower concentrations. No such tendency is
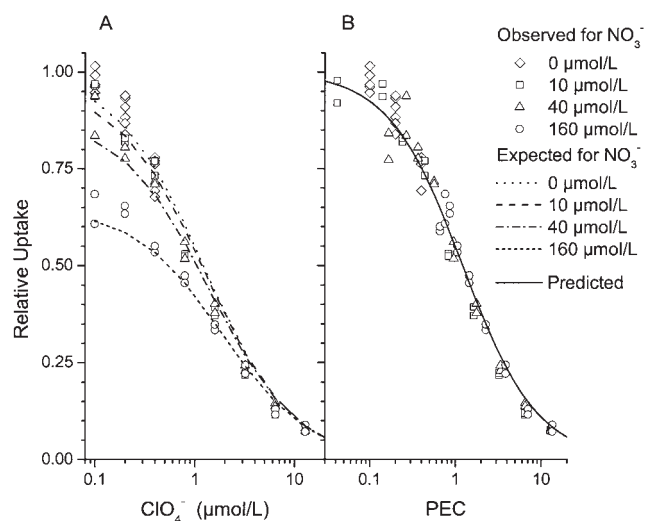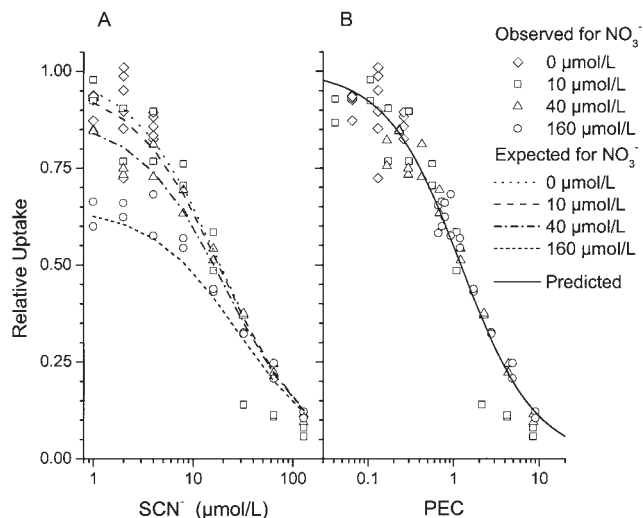
apparent in any of these graphs, which is consistent with lack of evidence for synergism among ClO_4^-, SCN^-, and NO_3^- from the statistical analysis..

Discussion

The present study investigated the joint effects of simultaneous exposure to multiple RAIU inhibitors in some detail by developing numerous concentration-response curves for specific RAIU inhibitors (ClO_4^-, SCN^-, and NO_3^-) generated in the presence of fixed concentrations of other inhibitors (Table 1). These data were examined for nonadditive interactions (either synergism or antagonism) using both formal statistical tests and graphical methods (Figures 4–6). Neither of these approaches found evidence of either synergism or antagonism. Rather, the data indicated that these RAIU inhibitors interact in a simple additive fashion. The concentration-response for each inhibitor was indistinguishable from that of each of the others, once adjusted for differences in inhibition potencies, that is, the effect of each of the RAIU inhibitors was indistinguishable from either a concentrate or dilution of any one of the other inhibitors. Correspondingly, the inhibitory effect of a mixture was shown to be a function of the "perchlorate equivalent concentration," or PEC, defined as the sum of the molar concentrations (μmol/L) of each individual inhibitor, each multiplied by the inhibition potency (β) of the inhibitor relative to ClO_4^- (Equation 3 and Table 2). This can be more simply rewritten as

$$RAIU = 1.22 \ / \ (1.22 + PEC)$$

where

$$PEC = [ClO_4^-] + [SCN^-] \ / \ 15 + [I^-] \ / \ 30 + [NO_3^-] \ / \ 240$$

Thus, the PEC for a mixture of RAIU inhibitors represents the molar concentration of ClO_4^- alone that would result in the same amount of inhibition. Responses to mixtures of inhibitors exhibit a coherent concentration-response when plotted as a function of the PEC of each mixture, and that

FIG. 5. Relative iodine uptake as a function of the molar concentration of ClO_4^- (**A**) or the perchlorate equivalent concentration (PEC) (**B**), for four background concentrations of NO_3^-.

concentration-response curve has the same shape as the concentration-response curve for ClO_4^- alone (Figs. 1–3).

These results are consistent with a mechanism of simple competition by the four different monovalent anions with similar size for access to the NIS sites on the cell membrane. Estimates from this study of the concentrations corresponding to 50% RAIU inhibition were in the ratios of 1:15:30:240 for ClO_4^-, SCN^-, I^-, and NO_3^-, respectively. These values are in good agreement with the values reported in the literature (as summarized in the introduction) based upon other test systems, including both *in vitro* and whole animal systems.

A central question in environmental exposure to NIS inhibitors is the modulation of inhibition by dietary iodine. The total iodide uptake is proportional to the amount of I^- present, times the relative uptake, or, based on our model for relative uptake, (Total Iodide Uptake) = (constant) $\times$ (I^-)/ (1.22 + PEC). This equation predicts that for all levels of iodide nutrition no greater than 1 μmol/L serum, and all possible background levels of the three competing anions, doubling the level of serum iodide will cause an increase in total iodide uptake of between 1.95 and 2.0. Thus, the model of competitive inhibition developed herein predicts that thyroidal I^- uptake is approximately proportional to iodide nutrition for any fixed underlying goitrogen load.

The model does not predict the levels of dietary iodine and goitrogen load at which effects on thyroid economy will occur. We did find one relevant study that looked at the interaction of iodide nutrition and goitrogen (SCN^-) load. In an endemic goiter area in central Africa, 393 neonates, 347 children aged 0 to 7 years and 776 subjects aged 10 to 35 were studied and compared to Belgian controls (46). In approximately half of the neonates, maternal iodide deficiency had been corrected during the fifth month of pregnancy with slowly resorbable iodized oil. With the exception of breast fed infants, cassava was the dietary staple for the population. The authors observed a decrease in goiter prevalence and serum SCN^- among breast-fed infants relative to neonates or weaned infants. The authors reported a significant and progressive increase in goiter prevalence, serum SCN^- and hypothyroidism in the months and years after weaning. The multiple correlation coefficient of urinary I^- and urinary SCN^- (which should correlate with serum concentrations) was highly significant for thyroxine (T_4), free T_4, and thyrotropin (TSH). Clearly, in this study, additional iodine did ameliorate the effect of high goitrogen load.

The model predictions developed in this study can also be utilized to place perspective on health risks associated with environmental exposure to trace amounts of ClO_4^-, SCN^-, and NO_3^- from drinking water sources. Environmental exposure recommendations and standards for water are generally based on a dose metric (mg/kg-day) and then translated into drinking water concentrations assuming that a 70-kg adult drinks 2 liters of water daily. After accounting for differences in the molecular weights of the different anions, in terms of serum concentration on a weight basis, the relative IUI potencies of ClO_4^- relative to SCN^- and NO_3^- are 9 and 150, respectively. To consider relative potencies on an ingested-weight basis, the serum half lives (approximately 8 hours, 6 days, and 5 hours for ClO_4^-, SCN^-, and NO_3^-, respectively) become important because SCN^- stays in the serum 18–29 times longer than the other anions. In terms of ingested weight basis, the IUI potencies of ClO_4^- relative to SCN^- and NO_3^- are thus calculated to be 0.5 and 240, respectively.

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