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Inhibition of Iodide Transport in Thyroid Cells by Dysidenin, a Marine Toxin, and Some of Its Analogs

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SUMMARY

Dysidenin, a hexachlorinated tripeptide-like molecule extracted from the sponge *Dysidea herbacea*, has lethal effects on fishes and some marine organisms. In an *in vitro* screening study, this molecule appeared to be a strong inhibitor of iodide transport in dog thyroid slices. Ouabain blocks iodide transport by inhibiting the Na^+/K^+ ATPase, which sustains the Na^+ gradient needed to drive iodide transport. Dysidenin and ouabain block iodide transport with the same kinetics but not by the same mechanism; dysidenin, unlike ouabain, did not inhibit $^{86}Rb^+$ uptake or increase its efflux. Inhibitors of chloride channels or carriers did not reduce the *T/M* value of $^{131}I^-$, with the exception of phloretin, a relatively nonspecific anion transport blocker. Monensin (or Na^+ ionophores) but not dysidenin clearly increased $^{22}Na^+$ efflux in tracer-preloaded thyroid slices treated with ouabain. This suggests that dysidenin does not act as a chloride channel inhibitor or a Na^+ ionophore. Increasing the iodide concentration in the medium decreased the inhibition by dysidenin, suggesting a pseudocompetitive type of effect. To study the structure-activity relationship of dysidenin, several hydrolytic products and synthetic derivatives have been prepared. The data obtained showed that the inhibition is sensitive to stereochemical effects and that the trichloromethyl terminus of the molecule is recognized by the binding site. The presence of only one trichloromethyl terminus is sufficient to exert the inhibitory effect.

Many sponges have the remarkable property of synthesizing secondary metabolites that prevent invasion by algae, fungi, and other epizoite organisms and deter mobile predators such as fish, molluscs, and echinoderms. Dysidenin (Fig. 1, compound **1**) and its C-5 epimer isodysidenin (Fig. 1, compound **2**) are two hexachlorinated metabolites isolated from the sponge *Dysidea herbacea*. The structure and absolute configuration of these epimers have been established (1–3). They are lethal to the fish *Lebistes reticulatus* at between 10 and 15 ppm and induce local necrosis in a variety of corals and sponges. Their mechanism of action is unknown. While screening the effects of toxins on the metabolism of dog thyroid slices, we observed a potent inhibition of iodide transport by dysidenin and isodysidenin.

Materials and Methods

Dog thyroid glands were sliced at room temperature and incubated at 37°, under an atmosphere of O_2/CO_2 (95:5, v/v), in 2 ml of KRB containing 8 mM glucose. We used approximately 50 to 60 mg of wet weight tissue/flask (one slice of equal weight from each of three or four dog thyroids in each flask). The thickness of the slices, cut with a Staddie Riggs microtome (Thomas Scientific, Swedesboro, NJ) was around 0.3 mm. For cyclic AMP measurements, the slices were preincubated for 1 hr or more and then transferred for 30 min to fresh medium containing a phosphodiesterase inhibitor (0.1 mM Ro 20-1724). At the end of the incubation, the slices were dropped in boiling water (1.5 ml) for 5 min, homogenized, and centrifuged; the supernatant was lyophilized, and the tissue extract was resuspended in water (10–20 μl/mg of wet weight tissue) for cyclic AMP measurements (4).

To measure iodide transport, the slices were incubated for various periods of time (see text) with $^{131}I^-$ (0.5 μCi/ml), KI (0.1 μM), and methimazole (100 μM) to block iodide organification. Transport was evaluated by the ratio (*T/M*) of cpm ^{131}I in 100 mg of tissue (*T*) to cpm of ^{131}I in 100 μl of incubation medium (*M*) at the end of the incubation.

To estimate Na^+/K^+ ATPase activity in the intact cell, ^{86}Rb was used (rubidium chloride at a specific activity of 1–8 mCi/mg of rubidium) as a tracer at a concentration of 0.5 μCi/ml of incubation medium, for measuring K^+ uptake (5, 6) without the addition of nonradioactive rubidium. The concentration of K^+ in the medium used in these experiments was 0.5 mM. In some experiments, the transport of $^{131}I^-$ and $^{86}Rb^+$ was measured in the same slices. For this purpose, the slices were incubated in 0.5 mM K^+ KRB supplemented with 0.5 μCi/ml $^{86}Rb^+$, 0.1 μM KI, 0.1 mM methimazole, and 0.5 μCi/ml $^{131}I^-$. The preincubation medium was regular KRB. To measure $^{131}I^-$ and $^{86}Rb^+$ efflux, the slices were loaded for 3 hr in the 0.5 mM K^+ KRB. The

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ABBREVIATIONS: KRB, Krebs Ringer bicarbonate buffer; DITS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid; SITS, 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

slices were then rinsed in 0.5 mM K^+ KRB and reincubated in 0.5 mM K^+ KRB containing the test substances. The efflux was measured by determining the rate of loss of tracer from the slices into the tracer-free external medium. Two hundred-microliter aliquots of medium were removed at time intervals, and at the end of the incubation both isotopes in the slices and the aliquots were counted at the same time in a γ-counter. The $^{131}I^-$ value was corrected to account for the contribution of $^{86}Rb^+$ and vice versa. The results were calculated as release of $^{131}I^-$ or $^{86}Rb^+$, expressed as percentage of total radioactivity in the slices at the beginning of the test incubation.

To measure putative Na^+ ionophore-like behavior of dysidenin, dog thyroid slices were loaded for 1 hr in KRB containing 5 μCi/ml $^{22}Na^+$ (NaCl in the medium was 130 mM) and 10 μM ouabain to inhibit the Na^+/K^+ ATPase (7) and thus block the exit of Na^+ from the slices. They were washed for 5 min in Krebs HEPES buffer without NaCl and KCl, which was supplemented with sucrose to maintain isotonicity. They were then transferred (four to seven slices) into 3 ml of regular Krebs HEPES buffer to measure efflux by removing 200-μl aliquots of medium at time intervals. Ouabain (10 μM) was present throughout. Monensin, as Na^+ ionophore (8), or dysidenin was added in the third incubation. The data are presented as radioactivity accumulating in the medium for 3 to 60 min, expressed as a percentage of the calculated radioactivity in the slices at the beginning of the release.

For K and Na content determinations, thyroid slices (±70 mg) were incubated 45 min in the presence of the test agent; after blotting on paper, the slices were dried overnight in an oven at 90° and their dry weights were measured. They were then agitated for 4 hr at room temperature in 2 ml of 0.3 M HNO_3. K and Na contents were measured in the supernatant by flame spectrophotometry.

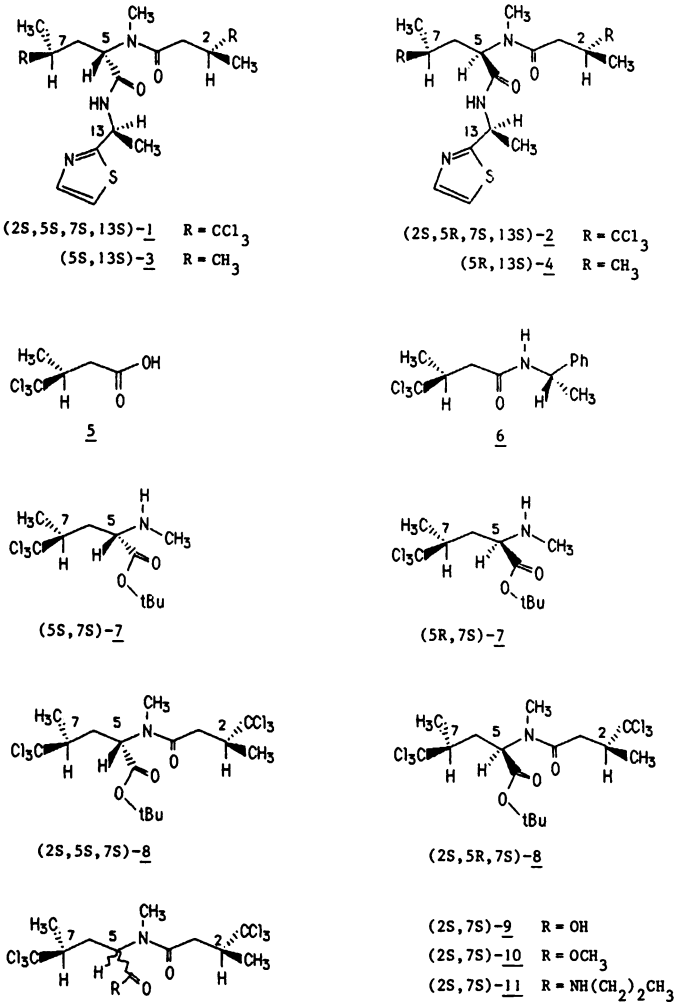

Fig. 1. Structure of compounds **1** to **11** (for convenience, the carbon atoms of all compounds have been numbered according to the numbering of dysidenin **1**. *tBu*, *t*-butyl- *Ph*, phenyl.

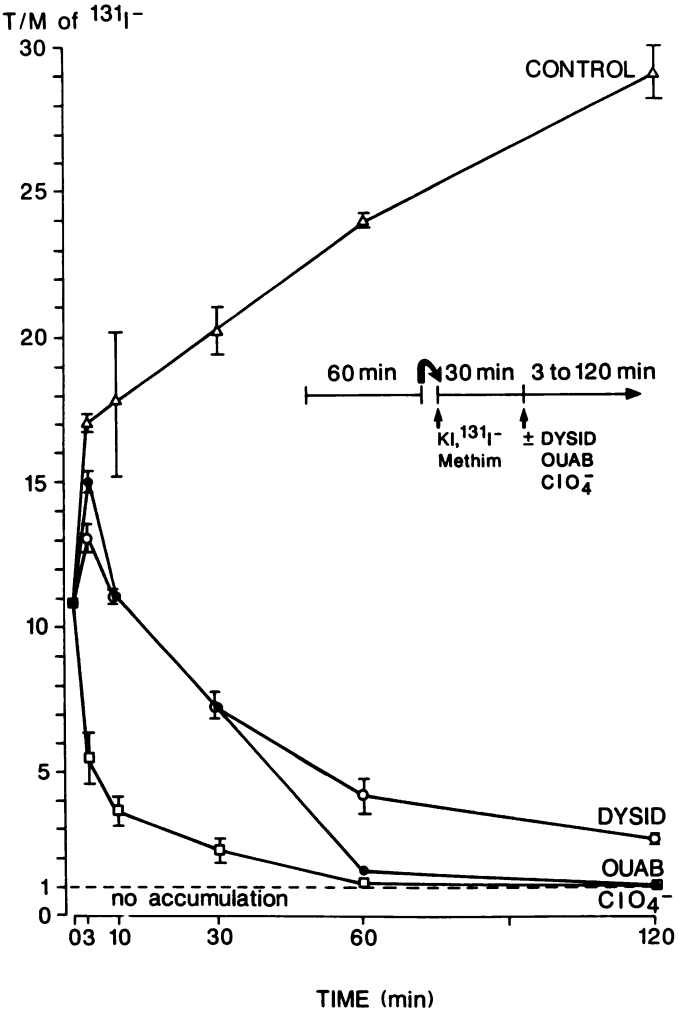

Fig. 2. Comparative kinetics of the effect of dysidenin (*DYSID*) (20 μM), ouabain (*OUAB*) (10 μM), and perchlorate (ClO_4^-) (1 mM) on iodide transport in dog thyroid slices. After a preincubation of 60 min in KRB, the slices were transferred to other flasks containing $^{131}I^-$ (0.5 μCi/ml), 0.1 μM KI, and 0.1 mM methimazole (*Methim*) for iodide uptake measurement. After 30 min, drugs were added to the medium (zero time) and the *T/M* of $^{131}I^-$ was measured in different flasks at various times (0 to 120 min) after drug addition. Results are expressed as means ± standard errors of duplicates vials containing the same set of equally distributed slices from four thyroids. The results presented are for one experiment of three. △, Control; □, ClO_4^-; ●, ouabain; ○, dysidenin; – – –, no accumulation.

Dysidenin and its derivatives were dissolved in dimethyl sulfoxide as stock solutions and the final concentration of dimethyl sulfoxide in test as well as control vials never exceeded 0.5%. The results were expressed as means ± standard errors of results of triplicates flasks of slices in one typical experiment. When the results of several experiments were pooled, mean, standard deviation, and statistical significance of the observed effect were calculated on the logarithm of the individual data. This procedure has been shown to normalize the distribution of metabolic variables in the thyroid (9). Results are expressed as antilogarithms of the mean ± the standard deviation of the mean and are presented in text or legends. Dysidenin (Fig. 1, **1**) and isodysidenin (Fig. 1, **2**) were isolated from the dichloromethane extract of a Papuan specimen of the sponge *D. herbacea* (1). A fraction

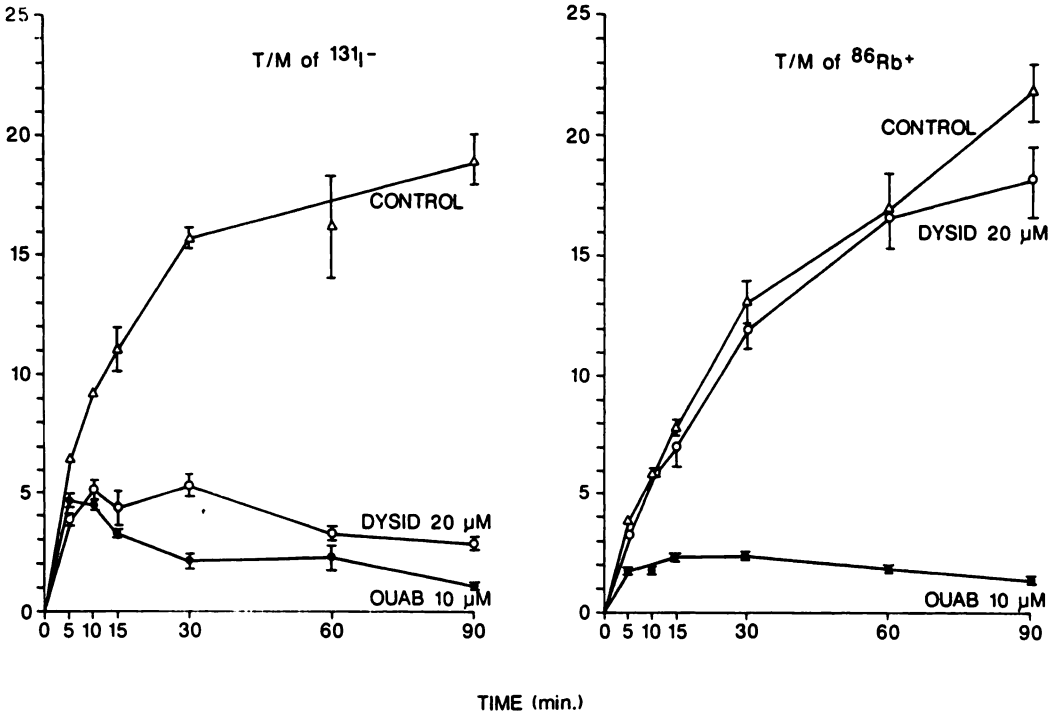

Fig. 3. Kinetics of the effect of dysidenin (*DYSID*) (20 μM) and ouabain (*OUAB*) (10 μM) on iodide trapping (*left*) and rubidium uptake (*right*) in the same dog thyroid slices. After a preincubation of 60 min in normal KRB, the slices were incubated for various times in the presence of $^{131}I^-$, $^{86}Rb^+$, and the test drug, in an incubation medium containing 0.5 mM K^+. Results are expressed as means ± standard errors of three different flasks with slices of three equally distributed dog thyroids. The results presented are from one experiment of three. △, Control; ○, dysidenin; ●, ouabain.

containing the two epimers was obtained after two successive chromatography steps on silica gel (eluant, hexane/acetone, 8:2, and hexane/ether, 65:35). Pure dysidenin and isodysidenin were obtained following further chromatography of this fraction on a Lobar column (LiChroprep Si 60; Merck) using chloroform containing 0.1% methanol. A mixture of dechlorodysidenin (Fig. 1, **3**) and dechloroisodysidenin (Fig. 1, **4**) was prepared by reduction of a mixture of dysidenin and isodysidenin, following the procedure reported by Charles *et al.* (1). (*S*)-3-Trichloromethylbutanoic acid (Fig. 1, **5**) was obtained by hydrolysis of either compound **1** or **2** (Fig. 1) with a 2:1 mixture of acetic acid and concentrated hydrochloric acid (2). The derivatives **6** to **11** have been synthesized starting from crotonic acid, using a protocol that will be published elsewhere. All these compounds show spectral properties entirely compatible with the proposed structures and their purity was checked by thin layer chromatography and by proton NMR. Ro20-1724 was a generous gift of Hoffman-La Roche (Nutley, NJ). Carrier free $^{131}I^-$ was purchased from IRE (Fleurus, Belgium); carrier-free $^{86}Rb^+$, as rubidium chloride (1–8 mCi/mg of Rb), and $^{22}Na^+$ (615 μCi/μg of Na) were obtained from Amersham (Buckinghamshire, England). DITS, SITS, and phloretin were purchased from Sigma (St. Louis, MO). Furosemide was a gift from Hoechst (Belgium) and amiloride from Merck Sharp and Dohme (Rahway, NJ). Anthracene-9-carboxylic acid was a gift from Janssens Pharmaceutica (Beerse, Belgium) and acetazolemide from Lederle (Belgium); diphenylamino-2-carboxylate was a gift of Dr. R. Greyer from the Max Planck Institut für Biophysics (Freiburg, West Germany).

Results

Dysidenin (20 μM) strikingly inhibited iodide transport in all the experiments. In 30 experiments, the T/M of $^{131}I^-$ was reduced to 12.8% of the controls (from 11.5 to 14.28% $2p < 0.005$) after 1-hr treatment.

In a typical experiment, 0.1 and 1 μM dysidenin had no effect on iodide trapping, whereas 5, 10, and 20 μM exerted an inhibitory effect of 50, 55, and 82%, respectively. Preincubation with the toxin for up to 5 hr showed that the effect was already pronounced without toxin during preincubation (50% inhibition) and maximal with only 1-hr preincubation (data not shown). The kinetics of action of dysidenin on iodide transport were compared with those of two well known inhibitors, a fully competitive inhibitor, perchlorate (ClO_4^-) (10, 11), and an indirect inhibitor, ouabain (12). As shown in Fig. 2, the effect of dysidenin (20 μM) paralleled the effect of ouabain (10 μM) initially but was not as complete. In four experiments, the T/M of ^{131}I was reduced to 10.3% (from 5.6 to 16.9%) of the controls in dysidenin-treated slices versus 2.6% (from 1 to 6%) of the controls in the ouabain-treated slices after 1-hr treatment. The difference was highly significant ($2p \ll 0{,}001$). ClO_4^- action was more rapid than those of dysidenin and ouabain and was as pronounced as that of ouabain.

The kinetics of inhibition of dysidenin were similar to those for ouabain; thus, we wondered whether its effect could be secondary to an inhibition of the Na^+/K^+ ATPase, as is the case for ouabain (12). To determine whether dysidenin is an inhibitor of the Na^+/K^+ ATPase, we checked, as a preliminary step, whether $^{86}Rb^+$ uptake could be taken as an index of K^+ uptake in dog thyroid slices as in other tissues. Kinetics of $^{86}Rb^+$ uptake from 30 to 240 min showed that half-maximum uptake was reached in 40 to 50 min and that the uptake of the tracer was 3 times higher in the presence of 0.5 mM K^+ than in the presence of 5 mM K^+ (data not shown). We elected to use 0.5 mM K^+ medium to study the effect of dysidenin on $^{86}Rb^+$ uptake, measuring the effect of the toxin on iodide transport in the same slices as a control of its action in these experiments. As shown in Fig. 3, both dysidenin (20 μM) and ouabain (10 μM) strongly and rapidly inhibited the iodide uptake but, in contrast to ouabain, dysidenin did not change the $^{86}Rb^+$ uptake. A slight inhibition was observed at 90 min in this experiment and at 60 min in others but it was never observed at 30 min, i.e., at a time when iodide transport was already strongly inhibited by dysidenin.

The effect of dysidenin on the efflux of $^{131}I^-$ and $^{86}Rb^+$ was

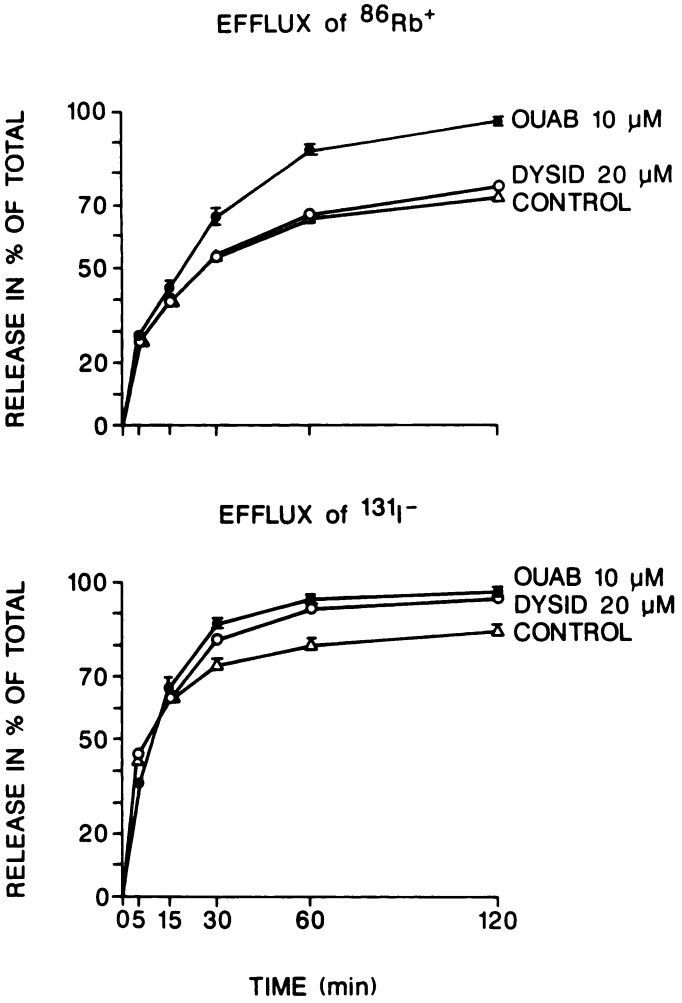

Fig. 4. Comparison of the effects of dysidenin (*DYSID*) (20 μM) and ouabain (*OUAB*) (10 μM) on efflux of iodide and rubidium in the same preloaded dog thyroid slices. After a preincubation of 3 hr with $^{131}I^-$ (0.5 μCi/ml), 0.1 μM unlabeled KI, $^{86}Rb^+$ (0.5 μCi/ml), and 0.5 mM K^+ for loading, the slices were quickly rinsed in KRB (0.5 mM K^+) and transferred at time 0, for various times, into fresh medium containing the drug under study. Release is expressed in percentage of total radioactivity of the slices at time 0. The results are expressed as means ± standard errors of triplicate vials incubated with the same set of thyroids in one experiment of 3. △, Control; ○, dysidenin; ●, ouabain.

studied and compared with that of ouabain. After 3 hr of loading with the tracers in a 0.5 mM K^+ medium, the slices were quickly rinsed and incubated in tracer-free medium. The accumulation of tracer in the medium was measured. As illustrated in Fig. 4, dysidenin had no effect on $^{86}Rb^+$ efflux, unlike ouabain. Dysidenin (20 μM) and ouabain (10 μM) increased $^{131}I^-$ efflux.

The effect of dysidenin is poorly reversible. Indeed, thyroid slices incubated with the toxin for 90 min, transferred to fresh medium, and prepared for the measurement of iodide uptake were still strongly inhibited 6 hr later (Fig. 5).

Increasing the iodide concentration in the incubation medium decreased the percentage of inhibition obtained with dysidenin. This "competitive effect" of iodide was compared with that obtained with ClO_4^-, a known competitive inhibitor of iodide for the iodide pump (10). In the experiment presented in Table 1, iodide (0.1 or 50 μM), $^{131}I^-$ (0.5 μCi/ml), and dysidenin (1, 5, or 20 μM) were added together and the *T/M* values for ^{131}I were measured at the end of 1 hr of incubation.

Dysidenin could interfere with the activity of a nonselective anion transport pathway located on the apical cell border, upon which iodide transport would depend. The effect of a series of inhibitors of Cl^- channels or Cl^- carriers in other tissues on the *T/M* of ^{131}I value were studied separately. DITS (100 μM), SITS (100 and 500 μM), and phloretin (500 μM), three blockers of the "band 3" protein that physiologically exchanges HCO_3^- for Cl^- in the red blood cell (13, 14), anthracene-9-carboxylic acid (100 μM), an inhibitor of the Cl^- channel (15, 16), diphenylamine-2-carboxylate (100 μM), another inhibitor of the Cl^- channel (17), furosemide (100 μM), an inhibitor of the Na-K-Cl co-transport (18), and, lastly, the omission of Cl^- in the medium (replaced by gluconate, as Na^+, K^+, Ca^{2+} gluconate) were used as Cl^- transport antagonists. With the exception of 500 μM phloretin, none of these agents known to inhibit Cl^- transport in other systems significantly inhibited the *T/M* of ^{131}I, whether the drug was added at the beginning of the 1-hr incubation or 1 hr in advance. As a positive control, the inhibitory effect of dysidenin (>80%) was always observed in these experiments. Similarly, neither 1 mM amiloride, an inhibitor of Na^+-H^+ exchange (19), nor 1 mM acetazolamide, an inhibitor of carbonic anhydrase (20), had any effect on I^- trapping. Phloretin (500 μM), which also inhibits other co-transporters (14), was strongly inhibitory, lowering the *T/M* value of $^{131}I^-$ close to 1. Sodium fluoride (0.3 and 10 mM) did not compete with 0.1 μM I^- in a 20-min incubation, in which ClO_4^- (0.1 mM) completely suppressed iodide accumulation in the tissue (not shown).

If dysidenin acts as a Na^+ ionophore, one may expect that it would increase $^{22}Na^+$ efflux from preloaded tissue. This was tested in the presence of ouabain (which suppresses the active Na^+/K^+ transport system), i.e., under conditions best able to measure Na^+ channel activity. In three independent experiments, a clear effect of monensin (10 μM), used as a positive control, was observed, but $^{22}Na^+$ efflux in slices treated with 20 μM dysidenin was not significantly different from control slices, even at 60 min (Fig. 6). We also measured the effect of dysidenin on Na and K content in toxin-related slices. The effect of dysidenin (20 μM) was compared to that of ouabain (10 μM). One can see from Table 2 that ouabain, as expected, increases Na and decreases K in the tissue. Dysidenin had no effect on Na and K content in these conditions.

In order to study the structure-activity relationship of dysidenin and isodysidenin, several degradation products (Fig. 1, compounds **3** to **5**) and some synthetic derivatives (Fig. 1, compounds **6** to **11**) were prepared and tested for their iodide transport-inhibitory effect. Dysidenin and isodysidenin, which are epimeric at C-5, have comparable activities. In contrast, a mixture of dechlorodysidenin (Fig. 1, **3**) and dechloroisodysidenin (Fig. 1, **4**), in which the six chlorine atoms have been replaced by hydrogen atoms, was inactive at 20 μM, after 1 and 2 hr of incubation. After 4 hr, a 15% inhibition was observed, compared with 91% inhibition with dysidenin. The compound (*S*)-3-trichloromethylbutanoic acid (Fig. 1, **5**), obtained by acid cleavage of dysidenin or isodysidenin (2), was tested at 20, 50, and 200 μM. Compound **5** was ineffective at the two lower concentrations at 5, 20, and 60 min and slightly active (20%) at 120 min. About 20% inhibition was observed at 5, 20, 60, and 120 min at 200 μM. The inhibitory activity of the synthetic amide (Fig. 1, **6**) at 20 μM was weaker than that of dysidenin but after 120 and 180 min it reduced to 50% the *T/M* values

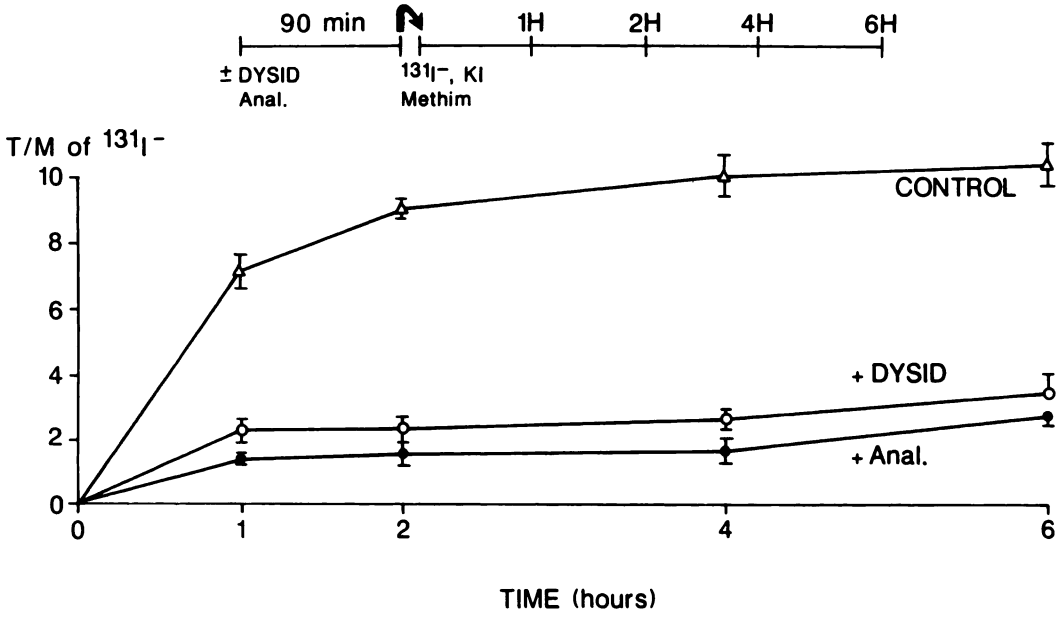

Fig. 5. Kinetics of the poor reversibility of the dysidenin (*DYSID*) (20 μM) effect, once the toxin is withdrawn from the medium. The effects of a synthetic analog, (5*S*,7*S*)-7 (*Anal.*), used at the same concentration (20 μM) are compared to dysidenin action. After a preincubation of 90 min with or without the test drug, the slices were then transferred at time 0 to fresh medium containing $^{131}I^-$, 0.1 μM KI, and 0.1 mM methimazole (*Methim*). The *T/M* for ^{131}I was measured at various times. Results are expressed as means ± standard errors of triplicates vials containing the same set of thyroids, in one experiment of three. △, Control; ○, dysidenin; ●, analog of dysidenin.

TABLE 1

Competition between iodide and dysidenin on *T/M* of ^{131}I

The results are expressed as the percentage of *T/M* values obtained in control slices incubated without any drug. The 100% value with KI = 0.1 μM was 43 ± 0.2; the 100% value with KI = 50 μM was 10.6 ± 0.4; the incubation lasted 1 hr. Results of one experiment of three are shown. In the three experiments, the *T/M* values in the presence of 20 μM dysidenin were 19.8% of the control (18.3 to 21.3%) in the presence of 0.1 μM KI and 35.75% of the control (32.34 to 39.53%) in the presence of 50 μM KI. The difference was highly significant ($2p < 0.001$).

KI	*T/M*					
	0 μM[a]	1 μM[a]	5 μM[a]	20 μM[a]	1 μM[b]	10 μM[b]
	% of control					
0.1 μM	100	51 ± 2	28 ± 4	19 ± 2	83 ± 2.5	6 ± 1.6
50 μM	100	73 ± 2	53 ± 2	39 ± 0.5	98 ± 2	23 ± 3

[a] Dysidenin concentration.
[b] ClO_4^- concentration.

for $^{131}I^-$ in three experiments. Under these conditions, dysidenin (20 μM) had an inhibitory effect of 90%. Adding the amide (Fig. 1, **6**) for 1 hr of preincubation did not improve the results obtained at 2 or 3 hr but slightly increased the inhibition after 10 and 60 min (data not shown).

Several other synthetic analogs of dysidenin (Fig. 1, compounds **7** to **11**) have also been tested for their action on iodide transport. As shown in Table 3, all but compound (5*R*,7*S*)-**7** were very active inhibitors. At equal concentrations, compounds (2*S*,5*S*,7*S*)-**8** and (5*S*,7*S*)-**7** were even more potent than dysidenin itself. This indicates that the affinity of these compounds for the target molecule is at least of the same order of magnitude as that of dysidenin. The recovery of $^{131}I^-$ *T/M* values after 1 hr of preincubation with dysidenin or compound (5*S*,7*S*)-**7** followed by a 1- to 6-hr incubation without any drug was similar (Fig. 5).

The effect of dysidenin (20 μM) on cyclic AMP accumulation was studied in control and thyroid-stimulating hormone (1 milliunits/ml)-stimulated dog thyroid slices. Even after 5 hr of preincubation with the toxin, no significant effect was observed. The cyclic AMP concentrations in control and thyroid-stimulating hormone-stimulated slices were 71 ± 10 and 424 ± 50 without and 84 ± 9 and 398 ± 5 pmol of cyclic AMP/100 mg of tissue with dysidenin, respectively. The incubation lasted 30 min.

Discussion

The "iodide pump," an extremely efficient mechanism developed by the thyroid gland to concentrate the rare element iodine, has not yet been isolated. There is good evidence in favor of a model in which Na^+ influx into the thyroid cell might be coupled to I^- influx by a mobile carrier protein (11), which would explain why inhibition of Na^+/K^+ ATPase leading to abolition of the Na^+ gradient abolishes iodide accumulation (21).

In this work, we have shown that dysidenin, a marine toxin, strongly inhibits the transport of iodide into dog thyroid slices. The kinetics and magnitude of the effect of dysidenin on iodide influx and efflux were very similar to those of ouabain and suggested, at first view, an ouabain-like effect. The present results clearly show that this is not the case. Dysidenin (20 μM) had an inhibitory effect on $^{86}Rb^+$ uptake that was much smaller than that of ouabain, but it started long after the dysidenin effect on iodide uptake was already fully expressed. It cannot, therefore, explain the iodide transport inhibition. We may also deduce from these experiments on $^{86}Rb^+$ fluxes that dysidenin is not a general metabolic poison, inasmuch as any strong reduction in ATP level would have impaired the Na^+/K^+ ATPase activity.

In the system tested here, iodide uptake is expected to be modified not only by inhibitors of the iodide pump but also by agents that inhibit the exit of iodide out of the cell and into the colloid at the apical border. The latter mechanism has not yet been identified. However, because iodide is concentrated into the thyroid cell, this exit into the colloid, where iodide is oxidized and organified, is expected to occur down its concentration gradient, i.e., iodide transport at this site could be passive, relying either on a channel or on a carrier. The specificity for Cl^- in passive chloride transport mechanisms is limited between halides, with Cl^- being transported mainly because it is the major halide under physiological conditions (22, 23).

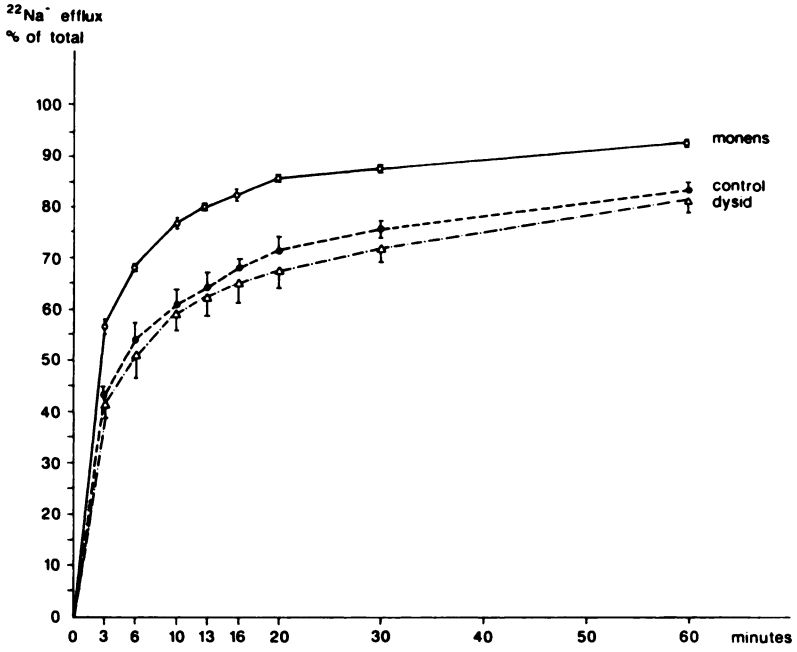

Fig. 6. Effect of monensin (*monens*) (10 μM) and dysidenin (*dysid*) (20 μM) on $^{22}Na^+$ efflux from tracer-preloaded dog thyroid slices. ●, Control; △, dysidenin; ○, monensin. The slices were loaded with $^{22}Na^+$ (5 μCi/ml) for 1 hr, in the presence of 10 μM ouabain to block Na^+ active outward transport by Na^+/K^+ ATPase (8). Then they were transferred for 5 min of washing in Krebs Ringer HEPES buffer without NaCl and KCl but supplemented with sucrose for isotonicity. They were then transferred (four to seven slices) into 3 ml of regular KRB (time 0) to follow tracer appearance in the medium. Ouabain was present in the three successive media. Monensin or dysidenin was added at time 0 of the figure (third medium). Results are expressed as mean $^{22}Na^+$ efflux, in percentage of the $^{22}Na^+$ at the beginning of the test incubation. The means ± standard errors are calculated from triplicates vials of the same set of thyroids in one typical experiment of three.

TABLE 2

Effect of dysidenin on Na and K tissue content

For experimental protocol, see Materials and Methods. The results are expressed as means of triplicates vials ± standard errors, in μmol/mg of dry weight. Results of one experiment of three are shown.

Ion	Tissue content		
	Control	Ouabain (10 μM)	Dysidenin (20 μM)
		μmol/mg	
K	0.234 ± 0.03	0.104 ± 0.007	0.226 ± 0.05
Na	0.507 ± 0.009	0.677 ± 0.050	0.486 ± 0.015

TABLE 3

Effects of 1, 2, and some of their synthetic analogs on iodide uptake in dog thyroid slices (concentration, 20 μM) after 2-hr incubation with the test drug.

All tested compounds were added at the beginning of the 2-hr incubation period.

	Compound	T/M of ^{131}I
Expt. 1	**1**	1.9 ± 0.1
	3 + 4	18 ± 0.5
	5	14.5 ± 1.5
	6	9 ± 2
	(5*S*,7*S*)−7	1.4 ± 0.2
	(5*R*,7*S*)−7	15.2 ± 2
	(2*S*,5*S*,7*S*)−8	1.2 ± 0.1
	(2*S*,5*R*,7*S*)−8	3.7 ± 0.2
	9*	3.4 ± 0
	10*	3.4 ± 0.3
	11*	3.2 ± 0.1
	Control	18 ± 4
Expt. 2	**1**	1.5 ± 0.1
	2	2.1 ± 0.2
	Control	21.8 ± 0.9

* These compounds have been tested as a 1/2 mixture of 5R and 5S epimers.

Band 3 protein is also able to transport iodide (24), and the Na^+-K^+-$2Cl^-$ co-transporter can carry pertechnetate and most probably it can also be loaded with I^- (25). Therefore, several "more or less" specific inhibitors of these transport mechanisms were tested for potential inhibition of iodide exit at the apical cell border, in order to compare them with dysidenin. However, with the exception of phloretin, they failed to decrease the value of T/M for $^{131}I^-$. If inhibition of chloride channels depressed iodide accumulation, such a mechanism could account for the action of dysidenin. The negative results with Cl^- channel inhibitors, therefore, do not support but obviously cannot exclude an action of dysidenin on iodide transport through such a mechanism. An inhibitory effect of phloretin, used at high concentrations, on the T/M value of iodide has already been reported (10, 26). It may be due to direct inhibition of the Na-I co-transport mechanism or of the Na^+/K^+ ATPase.

A trivial action of dysidenin as a Na^+ ionophore was also ruled out. Indeed, dysidenin-treated slices behaved like control slices as regards $^{22}Na^+$ efflux, whereas monensin, a known Na^+ ionophore, clearly enhanced it. If, instead of measuring dysidenin action on $^{22}Na^+$ efflux from tissue to medium, we measure its effect on tissue Na content, the data obtained were similar to controls, although ouabain clearly enhanced it. These data are in agreement with the absence of an effect on $^{22}Na^+$ efflux. The results obtained with the synthetic analogs of dysidenin show that the inhibition is sensitive to stereochemical effects (see Table 3). This suggests an effect on a structurally well defined site, presumably a protein. The effect of dysidenin and (5*S*,7*S*)-**7** (Fig. 1) is slowly reversible; moreover, high iodide levels partially relieve the effect of dysidenin, suggesting that this effect is pseudocompetitive for iodide.

The number of molecules that have been tested until now is too small to permit the proposal of a straightforward structure-activity relationship. Nevertheless, some preliminary structural factors necessary for the appearance of the inhibitory activity can be deduced from the gathered data. At least one trichloromethyl group must be present in the molecule. The 5*S*-epimers are always more active than the corresponding 5*R*-derivatives, suggesting that the configuration at this center is important for the activity. Interestingly, compound (5*R*,7*S*)-**7** has no inhibitory effect but, when the secondary amino group of (5*R*,7*S*)-**7** is acylated by (*S*)-3-trichloromethylbutanoic acid to give compound (2*S*,5*R*,7*S*)-**8**, some activity is recovered. This suggests that it is the trichloromethyl terminus of the molecule that is recognized by the binding site. The recovery of the

activity for (2*R*,5*R*,7*S*)-**8** could be attributed to the fact that the interaction with the binding site can now take place through the "right terminus" of the molecule. In this case, the configuration at C-5 has no influence on the activity. Finally, the available data suggest that the influence of the structure of the C-5 acylating group is not crucial. The synthesis of more analogues of dysidenin is currently in progress in our laboratory, in order to refine the structure-activity relationship. Moreover, experiments are underway to evaluate the use of compound (5*S*,7*S*)-**7** as a tool for the isolation of the iodide carrier from the thyroid gland by affinity chromatography.

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