Effects of ketoconazole on the iodide uptake by FRTL-5 cells

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Ketoconazole is an imidazole derivative used as an antifungal agent with reported effects on the endocrine system, but very little is known about its possible actions on thyroid function. Our purpose was to study the influence of this substance on the basal and TSH-stimulated iodide uptake in the rat thyroid cell strain FRTL-5. Ketoconazole (1–50 μmol/l) was shown to slightly increase the basal iodide uptake but, at higher concentrations (75–100 μmol/l), it sharply decreased iodide uptake below the basal levels. When the cells were cultured under bTSH stimulation (30 UI/l), the inhibitory effect of ketoconazole was exerted at concentrations as low as 25 μmol/l. This inhibition was observed even if it was added to the culture medium immediately before the Na125I addition. Forskolin, a stimulator of adenylate cyclase activity, was unable to prevent the iodide uptake inhibition. Low doses of ketoconazole increased cAMP concentrations. In the presence of TSH this effect was more evident in an inverse dose-dependent way. Because of its dual action, it can be assumed that ketoconazole could influence the iodide uptake in the FRTL-5 cells through more than one mechanism.

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Ketoconazole (KTZ) is an imidazole derivative used in the oral treatment of mycotic infections (1); furthermore, it has a well-known blocking effect on steroid biosynthesis by inhibiting the cytochrome P-450 enzyme system. This has led to its use in clinical endocrinology in cases of hyperandrogenism and hypercortisolism, with favorable reports in the treatment of precocious puberty, hirsutism, breast or prostate cancer and Cushing's syndrome (2–8). KTZ exerts its antifungal action by inhibiting the conversion of lanosterol into ergosterol, since it also interferes with the 14α-demethylase of the microsomal cytochrome P-450 (9) and other enzyme systems as well. It results in an increase of membrane permeability, in the uptake inhibition of RNA and DNA precursors and in the inhibition of oxidative and peroxidative enzymes synthesis (10).

In a short paper (11), Namer et al. reported that some patients treated with high doses of KTZ for breast cancer presented a "biological hypothyroidism" that required hormone replacement. Recent studies have shown that this imidazole derivative is able to stimulate PRL release from rat anterior pituitary cells in vitro and to decrease its TRH-induced response in the GH3 cell line. The mechanism by which KTZ acts in these situations is still unclear and the arachidonic metabolism, the cAMP and the phosphatidic pathways were postulated to be involved (12).

Since these systems actively participate in thyroid cell function (13, 14), together with the fact that other imidazole-derivatives affect thyroid metabolism (15), we studied the effects of KTZ on the FRTL-5 cell line, a rat normal thyroid cell strain. Our purpose was to assess the possible influence of KTZ on thyroid function, which would eventually have important clinical implications.

Material and methods

Reagents

The reagents and materials were purchased from the following sources: non-essential aminoacids solution (NEAA), calf serum (CS) and Trypan Blue solution from Gibco; Nutrient HAM-F12 medium; Coon modified (HAM-F12) from KCBiologicals, USA; culture flasks and 96-well plates from Corning; HEPES, L-glutamine, bovine albumin fraction V, bovine insulin, human transferrin, somatostatin, glycyl-l-histidyl-l-lysine (GHL), hydrocortisone, Hank's balanced salts (modified) without Ca++, Mg++ and NaHCO3 (HBSS), 5'-Guanylylimidodiphosphate [Gpp(NH)pp], and 3-isobutylmethylxantine (IMX) and dimethyl sulfoxide (DMSO) from Sigma; bTSH from Armour Laboratories, Na125I from NEN; forskolin from Calbiochem; Methyl-mercaptopimidazol (MI) from Gador.

Ketoconazole (Janssen Laboratories) was dissolved in ethanol/dimethyl sulfoxide (50–50) and, in all the cases, the final concentration of the solvent never exceeded 0.1% showing no interferences with the results. KTZ was used at different final concentrations ranging from 1 to 100 μmol/l.

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Roberto J Soto died on June 24th, 1991
Iodide uptake assay

FRTL-5 cells were cultured as previously described (16) and are a generous gift of Dr L Kohn and the Interthyry Research Foundation (Baltimore). The cells were seeded (4 × 10^4 cells/0.2 ml) in 96 well-plates and grown up to confluence with culture medium (HAM-F12 supplemented with 5% of CS) and six hormone mixture (bTSH, b-insulin, cortisol, h-transferrin, somatostatin and GHL) (6H), at 37°C in 95% air-5% CO₂. When confluence was achieved, the cells were washed twice with HBSS and incubated for five days with culture medium and 5H (without bTSH). Then, the cells were changed to medium-3H (without bTSH, b-insulin and cortisol) for an additional period of three days.

The different substances studied were added to each well in 0.2 ml of culture medium containing 3H and IMX (5 µmol/l) for 72 h. The iodide uptake (¹²⁵I-U) was performed removing the medium, rinsing the cells in HBSS containing HEPES (10 mmol/l) and covering them with 0.2 ml of HBSS-HEPES containing carrier-free Na¹²⁵I and NaI (10 µmol/l) to obtain a specific activity of 10–20 Ci/mol. After 30 min the reaction was stopped by aspiration, washed in 0.2 ml of ice-cold HBSS-HEPES followed immediately by an extraction with 0.2 ml of ice-cold ethanol for 1 h in the freezer. The ethanol extracts were quantitatively transferred to 12 × 75 mm tubes for radioactivity counting. Data (as a percentage of variation with respect to the basal uptake) were expressed as mean ± SD of values obtained from two separate experiments performed in triplicate.

To study the effect of pre-incubation with KTZ on the cell response, it was sequentially added to different sets of wells 72, 48, 24, 12, 6 and 0 h before the addition of Na¹²⁵I. These experiments were performed in the presence and absence of bTSH for 72 h.

The influence of KTZ (100 µmol/l) over the action of different substances such as forskolin (FK) (10 µmol/l), Gpp(NH)p (10 µmol/l) and MMF (100 µmol/l) was investigated in the presence and absence of bTSH (30 UI/l).

The recovery of the ¹²⁵I-U activity by the FRTL-5 cells after KTZ withdrawal was studied at two different times. The cells were treated for 24 or 48 h with KTZ (100 µmol/l) after which, they were rinsed twice in HBSS. Fresh medium (without KTZ) was added and the incubation was continued until a total period of 72 h had passed.

Other assays

To study the effect of KTZ over the cAMP production, the cells were cultured following the same procedure described above but incubating for 3 h in hypotonic HBSS (1/10 folds) with different concentrations of KTZ. The cAMP concentration was measured in the incubation medium by RIA (17). This experiment was performed with and without bTSH.

The effect of KTZ on the binding of [¹²⁵I]bTSH to its receptor was studied using the method of Southgate et al. (18). KTZ (100 µmol/l) was dissolved in DMSO and diluted with TSH-receptor antibodies negative serum. Results were expressed as a percentage of inhibition of the binding. DNA was measured by the method of Labarca and Paigen (19). The cellular viability was assayed in parallel experiments with Trypan Blue.

Statistic

Data were analyzed for statistical significance using Student's t-test.

Results

As seen in Fig. 1, KTZ had a bimodal effect on [¹²⁵I-U], which depended on its concentration. At low concentra-

![Graph](image.png)
tions (≤ 50 μmol/l), there was a significant increase (up to 57.4 ± 9.6%) but, at higher concentrations (≥ 75 μmol/l), there was a strong inhibition of the 125I-U (up to −73.5 ± 8.4%). In the presence of bTSH (30 UI/l), the low-doses stimulatory effect was not observed; with bTSH, the inhibitory effect was exerted at a lower concentration of KTZ (25 μmol/l).

The effect of KTZ, when incubated together with forskolin, Gpp(NH)p or MMI in the absence and presence of bTSH, is shown in Fig. 2. FK, which stimulates the basal 125I-U in the absence of KTZ, could not revert the inhibition produced by KTZ alone, even in the presence of TSH. Similar inhibitions were observed when incubations were performed with Gpp(NH)p and MMI.

The influence of different pre-incubation times with KTZ is shown in Fig. 3. A significant inhibitory effect was
Table 1. Effect of ketoconazole (KTZ) on cAMP production in FRTL-5 cells. The cells were cultured for five days in 5H and three days in 3H by the addition of KTZ, with (1-A) or without bTSH (1-B) for 3 h in hypotonic HBSS (1:10 fold). cAMP was measured in the incubation medium by RIA. Results on 1-A and 1-B are the mean of triplicate determinations and were performed on different experiments.

<table>
<thead>
<tr>
<th>KTZ (µmol/l)</th>
<th>bTSH (UI/l)</th>
<th>cAMP (pmol/well)</th>
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<tbody>
<tr>
<td>(A) with bTSH (30 UI/l)</td>
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<tr>
<td>0</td>
<td>30</td>
<td>4.9</td>
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<td>10</td>
<td>30</td>
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<td>14.7</td>
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<td>100</td>
<td>30</td>
<td>7.2</td>
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<tr>
<td>(B) without bTSH</td>
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<tr>
<td>0</td>
<td>—</td>
<td>0.8</td>
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<tr>
<td>10</td>
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observed with pre-incubations of 72 to 12 h before the addition of Na125I (Fig. 3a). When this experiment was performed in the presence of bTSH, the inhibition was exerted even if KTZ was added immediately before the Na125I addition (Fig. 3b).

The binding of bTSH to its solubilized receptor was not affected by KTZ (100 µmol/l), since the inhibition of [125I]bTSH binding was 1% compared with the binding without KTZ (5940 cpm vs 6100 cpm, not significant).

Low doses of KTZ induced a moderate increment in cAMP production but this effect is less evident at higher doses. In the presence of bTSH, the cAMP concentration in the incubation medium was also strongly increased at low KTZ doses but, with increasing doses, cAMP synthesis was proportionally decreased (Table 1).

The effect of KTZ on the 125I-U persisted 48 h after its removal from the medium and a clear inhibition was observed even in the presence of bTSH (data not shown).

The results shown in this study could not be attributed to a decrease in the number of cells, since their viability as well as DNA cell content were studied in parallel experiments without significant changes.

Discussion
The effects of KTZ over different endocrine systems were widely studied in vitro and in vivo. However, the information available about its actions on thyroid cells is extremely rare. In 1986, Namer et al. (11) described a "biological hypothyroidism" in patients with breast cancer treated with high doses of KTZ (1200 mg/day). They eventually required hormonotherapy, suggesting a decrease in thyroid hormone levels.

Other imidazole derivatives were reported as having an effect on thyroid metabolism. The most frequently used in relation to thyroid function is MMI, which exerts its effect on the thyroid peroxidase-catalyzed iodination of tyrosine or tyrosyl residues of thyroglobulin (20). Aminogluthethimide, a drug used in the past as an anticonvulsivant agent and in Cushing’s syndrome treatment was reported as goitrogenic (21). Omeprazole, an inhibitor of gastric acid secretion that binds to the H+ /K+ ATPase, could also have an effect on thyroid tissue and it was reported that in vivo it potentially inhibits iodide organification. Other proton pump inhibitors were tested in porcine thyroid cell cultures showing that, like KTZ, they exert no effect on H+ /K+ ATPase at low doses (20 µmol/l) but, at higher concentrations (200 µmol/l) most of them significantly inhibit iodide uptake without an effect on cell viability (22).

In our study, we observed that KTZ exerts a bimodal action on the basal iodide uptake in the FRTL-5 cells, a normal rat thyroid line. At low doses of KTZ, there was a significant stimulation of the uptake in a dose-dependent mode. This effect was sharply reverted at concentrations of 75 µmol/l or higher and a strong reduction of the intracellular 125I was found. In the presence of TSH, the inhibitory effect was also observed, but with lower KTZ concentrations (≥25 µmol/l). In these experiments, no stimulatory effect could be demonstrated at the KTZ concentration range used (1–100 µmol/l). KTZ exerts bimodal effects over other systems. Gupta et al. (23) described that KTZ caused a concentration-dependent inhibition and stimulation of 3-hydroxy-3-methylglutaryl-CoA-reductase in cultured rat intestinal cells. This effect is not due to a direct action over the enzyme but through intermediates.

As it was reported (12, 13), iodide uptake is regulated by different mechanisms such as the cAMP pathway and the ATPase H+ /K+. KTZ seems to affect both, depending on its concentration. From our studies some evidences were obtained: (a) the effect of KTZ would not be exerted by affecting the binding of bTSH to its receptor, as it was shown using solubilized TSH receptor; (b) since FK – a well-known stimulator of adenylatecyclase activity (24) – could not prevent the 125I-U inhibition by KTZ at the concentration used in this experiment, it can be postulated that this inhibition is exerted in a posterior step. (c) KTZ stimulated the cAMP synthesis with and without bTSH. Since this effect was more evident at low doses of KTZ we postulate that the cAMP pathway could be implicated in the 125I-U increment found at those concentrations. (d) The inhibition of the 125I-U observed at high doses of KTZ could not be explained by this mechanism and could be attributed to a direct effect over the ATPase H+ /K+ pump.

In conclusion, KTZ exerts a complex effect on FRTL-5 cells. At low concentrations it stimulates the 125I-U via the cAMP pathway. In accordance with experience with other imidazole derivatives, the cause of the marked inhibition of the 125I-U at higher concentrations could be explained by an inhibiting effect over the ATPase H+ /K+ antiport. Furthermore, its effects on other proteins that
are actively involved in the iodide uptake have to be explored. This is the first report of KTZ action in a normal rat thyroid cell line. More investigations have to be performed in order to evaluate the mechanisms and the possible implications in human thyroid physiopathology.

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