Influence of Imipramine on the Hypothalamic/Pituitary/Thyroid Axis of the Rat

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The effects of the tricyclic antidepressant drug imipramine at different levels of the hypothalamic/pituitary/thyroid axis were investigated in the rat. Intraperitoneal (IP) treatment for 14 days with imipramine at 10 mg/kg, but not 2 mg/kg, reduced serum total thyroxine (T_4) and triiodothyronine (T_3). A similar decrease in serum total T_4 was observed in thyroidectomized T_4-treated rats, suggesting that imipramine treatment enhances T_4 clearance instead of reducing T_4 secretion. There were no parallel decreases in serum free T_4 and T_3 concentrations, due to the simultaneous increase in the free fractions of both T_4 and T_3 following imipramine treatment. In vitro experiments using equilibrium dialysis indicated that neither imipramine nor its metabolite desipramine directly influenced the binding of T_4 or T_3 to their transport proteins following addition to normal serum, suggesting an indirect effect of imipramine or desipramine on free hormone concentrations in vivo. Concentrations of T_4 and T_3 in the brain, liver, and heart were unaffected by imipramine treatment, suggesting that the drug did not affect cellular uptake and metabolism of T_4 and T_3. Serum concentrations of thyrotropin (TSH) were unaffected by imipramine pretreatment at either dose level, compatible with the fact that serum free T_4 and T_3 concentrations were not reduced. Moreover, there was no difference in thyrotrope responsiveness to stimulation by TSH-releasing hormone (TRH) and to inhibition by T_4 and T_3 in rat anterior pituitary cells cultured ex vivo for 18 hours from control and imipramine-treated rats. Furthermore, in vitro exposure of cultured rat anterior pituitary cells to imipramine and desipramine indicated that both agents decreased TSH secretion only at concentrations greater than 10^{-6} mol/L. These concentrations of imipramine and desipramine in the culture medium would exceed the free concentrations of these drugs seen in vivo therapeutically. In addition, no direct effects of 10^{-6} mol/L imipramine or desipramine on the TSH response to TRH or to T_3 were observed in vitro in cultured pituitary cells. A potential indirect effect of imipramine or desipramine on TSH secretion via altered hypothalamic control of thyrotropes does not seem likely, due to the lack of effect of imipramine treatment on serum TSH concentrations in imipramine-treated rats. In conclusion, imipramine treatment reduces serum total T_4 and T_3 in the rat, with enhanced clearance being the most likely explanation for the effect on T_4. There was no evidence for altered tissue T_4 or T_3 concentrations or for altered thyrotrope function. The enhanced T_4 clearance may explain the reduction in total T_4 reported for imipramine-treated depressed patients. However, the effects of imipramine treatment on the transport of thyroid hormones in plasma need to be examined in more detail in patients, since interspecies differences in the nature of the transport proteins preclude extrapolation of the present results from the rat.

THERE IS ACCUMULATING evidence for three main types of interactions between antidepressant drugs such as the tricyclic agent imipramine and the thyroid hormones thyroxine (T_4) and triiodothyronine (T_3). T_3 has been used to enhance antidepressant treatment in patients showing resistance to therapy.[1] The mechanism of this enhanced effect is obscure, but there is the suggestion that treated depression may already represent a state of subclinical hypothyroidism.[2] However, in some cases, the beneficial effects of T_3 treatment have not been confirmed in tricyclic-resistant patients.[3] Secondly, there is evidence from animal experiments that the metabolism of imipramine may be influenced by thyroid hormone status.[4,5] Thirdly, there is some evidence from both patients[6] and laboratory animals[7-10] that treatment with imipramine or its active metabolite desipramine reduces circulating T_3 and T_4 concentrations. However, some investigators[11] have failed to corroborate a significant effect of imipramine on peripheral thyroid indices in patients. We have therefore undertaken an extensive study of the effects of imipramine on the hypothalamic/pituitary/thyroid axis in the rat using in vitro, in vivo, and ex vivo measurements in an attempt to clarify interactions with thyroid hormones at different levels of the axis. Accordingly, in vitro and ex vivo effects of imipramine treatment on thyrotropin (TSH) secretion by anterior pituitary cells were examined under basal conditions and in response to both positive and negative stimulation with TSH-releasing hormone (TRH) and thyroid hormones, respectively. Concurrent effects on basal and growth hormone (GH)-releasing hormone (GHRH)-stimulated GH secretion were assessed in the same cell preparations. The effect of in vivo treatment of rats with imipramine was assessed in terms of total circulating thyroid hormones and TSH. Plasma protein binding of thyroid hormones was assessed by measuring the free fraction of T_3 and T_4 from rats treated with imipramine, as well as by assessing any direct effect of the drug on plasma protein binding in vitro. In addition, the effects of the drug on total thyroid hormone concentrations were assessed in thyroidectomized T_4-replaced animals to distinguish between the effects of imipramine on the clearance versus the secretion of T_4 from the thyroid gland.

The true thyroid status of a tissue is determined by its nuclear T_3 concentration. Since the proportion of nuclear T_3 derived from T_3 uptake from the circulation and intratissue T_4 to T_3 conversion varies for different organs,[12] tissue handling of thyroid hormones represents a potential source of influence of imipramine on thyroid hormone action that cannot be determined from circulating hormone concentrations. Therefore, tissue concentrations of T_3 and T_4 were also assessed in a number of target organs including the liver, heart, and brain.

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MATERIALS AND METHODS

Treatment of Animals

Male Sprague-Dawley rats with a body weight of 300 to 400 g were treated intraperitoneally (IP) with imipramine 2 mg/kg or 10 mg/kg for 14 days. Control rats received the same volume (1 ml/kg) of saline vehicle. In one series of experiments, the rats were thyroidectomized under ketamine/xylazine anesthesia using aseptic technique and administered replacement doses of T_4 50 μg/kg/d subcutaneously for 14 days before imipramine or control treatment, to determine whether the effects of imipramine were manifest via an action on the thyroid gland or on the clearance of thyroid hormones. At the end of the treatment period, the rats were bled from the vena cava under ether anesthesia between 9:00 and 10:00 AM, and the serum was used to measure total T_3, total T_4, TSH, and the free fractions of T_3 and T_4. The anterior pituitary glands were removed and cultured overnight to determine the ex vivo responsiveness of thyrotropes to TRH, T_3, and T_4. The brain, heart, and liver were also removed from each animal, snap-frozen in liquid nitrogen, and stored at −70°C until assayed for T_3 and T_4 content.

Pituitary Cell Cultures

Euthyroid male Sprague-Dawley rats with a body weight of 300 g or rats treated with imipramine as already described were lightly anesthetized with ether and decapitated. The pituitary glands were removed under aseptic conditions and placed in sterile Dulbecco's phosphate-buffered saline (PBS). The anterior pituitaries were dissected away from the posterior and intermediate lobes and cut into 1-mm sections in two directions at right angles to one another. The sliced pituitaries were washed three times with 3 mL sterile Dulbecco's PBS containing 1% bovine serum albumin (BSA). They were then incubated in a shaking water bath at 37°C in 10 mL trypsin solution (330 U/mL in PBS) containing DNase type 1 (80 U/mL) for 25 minutes. The reaction was stopped by removing the trypsin solution and incubating the pituitary sections with 10 mL trypsin inhibitor (0.5 mg/mL) at 37°C for 10 minutes. The trypsin inhibitor was then removed and replaced with 10 mL calcium-free Dulbecco's PBS containing EDTA (2×10^{-3} mol/L), and the sections were incubated for another 3 minutes. After incubation, the pituitary sections were washed three times with 4 mL calcium-free PBS and dispersed by gentle pipetting in 3 mL calcium-free PBS. The dispersed cells were centrifuged at 300 x*g* for 3 minutes and resuspended in Dulbecco's modified Eagle's medium (DMEM). The washing procedure was repeated twice. The dispersed cells were plated at 0.3×10^6/mL per well in 24-well plates (Nunc, Roskilde, Denmark) in DMEM containing 2.5% fetal calf serum and 5% horse serum supplemented with nonessential amino acids, sodium bicarbonate (3.7 g/L), and penicillin (100,000 U/L). In these experiments, the serum was depleted of thyroid hormones as described by Samuels et al.[13] The cells were incubated at 37°C in an atmosphere of 95% air/5% CO_2 with a relative humidity of 95%. For in vitro experiments, the cells were cultured for 4 days before exposure to test agents. For ex vivo experiments in which pituitaries were compared from control and imipramine-treated animals, pituitary cells were cultured overnight before exposure to test agents.

The cells were washed with PBS and then exposed at 37°C to test agents dissolved in DMEM containing 0.1% BSA. All treatments were performed at least in duplicate in each experiment. The effects of test agents (imipramine, desipramine, T_3, and T_4) were examined under the basal condition and during simultaneous exposure to TRH (10^{-8} mol/L) or GHRH (10^{-9} mol/L). Preliminary experiments indicated that these concentrations of releasing hormones provided stimulation of anterior pituitary cells at approximately 70% of maximum (ED_{70}), allowing sufficient sensitivity to demonstrate any inhibitory effects of the agents on pituitary hormone secretion. Effects of T_3 and T_4 were assessed over an incubation period of 18 hours, since preliminary experiments indicated that a shorter incubation time of 4 hours was insufficient to demonstrate inhibitory effects on TSH. Pituitary cells were exposed to 10^{-9} mol/L T_3 and 10^{-8} mol/L T_4, since preliminary experiments indicated that these concentrations were submaximal but in the upper portion of the dose-response curves. At the end of incubation, the media were collected and centrifuged at 1,000 × *g* for 10 minutes, and the supernatants were stored at −20°C until assayed for pituitary hormone content within 3 weeks of collection. The cells were washed with PBS, and 400 μL calcium-free PBS containing 2×10^{-3} mol/L EDTA was added to each well. The plates were stored at −20°C until assay for DNA content by the method of Labarca and Paigen.[14] Viability of the cells at the end of incubation was routinely checked by trypan blue exclusion. Secretion of GH and TSH was expressed relative to the DNA content of the wells.

Extraction of Tissue Thyroid Hormones

Tissues were subject to chloroform/methanol extraction before assay of tissue thyroid hormones essentially as described by Morreale de Escobar et al.[15] Briefly, the tissues were homogenized in methanol containing 10^{-3}-mol/L propothiouracil 4 mL/g on ice in a Teflon glass homogenizer. Trace amounts of high–specific activity ^{125}I-T_4 or ^{125}I-T_3 were added to separate aliquots from the same tissue homogenate to correct for recovery in the extraction process. In this regard, it differed from the method of Morreale de Escobar et al, in which simultaneous recoveries of ^{131}I-T_4 and ^{125}I-T_3 are estimated. The homogenates were transferred to stoppered glass tubes, and chloroform was added at a rate of two parts chloroform to 1 part methanol. The final volume of extract was approximately 20 times the tissue weight. T_3 and T_4 were then back-extracted from the chloroform:methanol extracts with 0.05% $CaCl_2$, followed by two extractions with pure upper phase.[16] These pooled aqueous phases were then applied to AG 1 X2 columns (Bio-Rad, Richmond, CA). After a series of washes with ethanol, acetate buffer, and then 1% and 35% acetic acid as described by Morreale de Escobar et al,[15] the iodothyronines were eluted with six 0.5-mL aliquots of 70% acetic acid. Blanks contained the tracer and reagents but no tissue. The resulting eluates were evaporated to dryness and then reconstituted in radioimmunoassay (RIA) buffer prior to assay for T_3 and T_4 content.

Free Fractions of T_3 and T_4

The free fractions of T_3 and T_4 in serum were estimated essentially as described by Stockigt et al.[17] Trace amounts of ^{125}I-T_3 or ^{125}I-T_4 (500,000 cpm/mL) were added to 0.5 mL serum and dialyzed for 18 hours at 37°C against 10 mL phosphate buffer, and the free fractions of T_3 and T_4 in serum were estimated from the dialysate counts after $MgCl_2$ precipitation. In separate experiments, a range of concentrations of imipramine or desipramine were added to normal untreated rat or human serum and subjected to equilibrium dialysis to determine whether the binding of T_3 or T_4 to serum proteins was directly influenced by the presence of imipramine or its main metabolite. The range of concentrations of imipramine and desipramine were selected to include the total concentration of both compounds as estimated in serum at the time of death of the animals treated with 10 mg/kg/d imipramine, namely 5×10^{-7} μmol/L. In an additional experiment, to replicate the conditions in undiluted serum, the drugs were added to the dialysate compartment in respective concentrations appropriate to achieve, after dialysis, the total and free concentrations of drug in serum equivalent to those that occur in vivo assuming a free fraction of 15%.[18]

Hormone Assays

Rat TSH and GH assays were performed in duplicate by RIAs using materials kindly supplied by the National Institute of Diabetes and Digestive and Kidney Diseases (Bethesda, MD). TSH and GH were labeled with ^{125}I using the iodogen method. Bound hormone was precipitated with a second antibody (anti-rabbit and anti-monkey

immunoglobulin, respectively). Intraassay variation was 7% for GH and 6% for TSH. Assay sensitivity was 40 pg for both GH and TSH using the reference preparation RP-2. Serum T_3 and T_4 were assayed in duplicate by RIAs using ^{125}I-T_3 and ^{125}I-T_4 essentially as described by Obregon et al,[19] with antibody kindly donated by Dr M.J. Obregon (Madrid, Spain). Intraassay variation was 4% for both T_3 and T_4. Assay sensitivity was 1.4 pg/tube and 3.8 pg/tube for T_3 and T_4, respectively. All samples from each experiment were estimated in the same assay.

Drugs and Chemicals

Trypsin and lima bean trypsin inhibitor were obtained from Worthington (Freehold, NJ); DMEM from GIBCO (Grand Island, NY); fetal calf and horse serum from ICN/Flow Laboratories (Costa Mesa, CA); DNase type 1, bisbenzimide (Hoechst 33258), fatty acid–free BSA, L-T_3 (sodium salt), L-T_4 (sodium salt), TRH, imipramine HCl, and desipramine HCl from Sigma (St Louis, MO); GHRH 1-44 amide from Cambridge Research Biochemicals (Harston, Cambridge, UK); penicillin from Glaxo (Boronia, Australia); ^{125}I-sodium iodide, carrier-free (620 GBq/mg) from Australian Radioisotopes (St Lucia, Australia); and ^{125}I-T_3 (44.4 MBq/μg) and ^{125}I-T_4 (46.3 MBq/μg) from NEN-DuPont (Wilmington, DE). All other chemicals were of analytical-grade purity.

Data Analysis

The data are expressed as the mean ± SEM. The experimental groups were compared by one-way ANOVA followed by selected Student's *t* tests where appropriate. A critical value of *P* less than .05 was used.

RESULTS

In Vivo Imipramine Treatment

Treatment with 10 mg/kg, but not 2 mg/kg, imipramine for 14 days caused a significant decrease in serum total T_4 (Fig 1). Treatment with 10 mg/kg imipramine, but not 2 mg/kg, resulted in a reduction in body weight gain over 14 days compared with control levels (8 ± 4 g *v* 66 ± 4 g in imipramine-treated and control rats, respectively, n = 5). The 10-mg/kg dose, but not the 2-mg/kg dose, resulted in plasma levels of imipramine and desipramine (0.3 to 0.5 × 10^{-6} mol/L and 0.3 to 1.0 × 10^{-6} mol/L, respectively) similar to the therapeutic range. This effect of imipramine on total T_4 concentrations appeared to be unrelated to an effect on T_4 secretion, since there was a similar reduction in total T_4 concentrations in thyroidectomized T_4-treated animals (Fig 1). Since imipramine 10 mg/kg increased the free fraction of T_4 from 0.051% ± 0.002% to 0.087% ± 0.008% ($P < .002$, n = 5), the overall effect of imipramine treatment on serum free T_4 concentration was a small but significant increase (Fig 1). Total serum T_3 concentration was reduced by treatment with 10 mg/kg imipramine, but not by the lower dose of 2 mg/kg (Fig 2). In thyroidectomized T_4-treated animals, there was no significant effect on total serum T_3 (Fig 2). The free fraction of T_3 was also increased by imipramine 10 mg/kg from 0.673% ± 0.024% to 0.825% ± 0.040% ($P < .05$, n = 5), so the estimated serum free T_3 concentration was the same in control animals and those treated with imipramine (Fig 2).

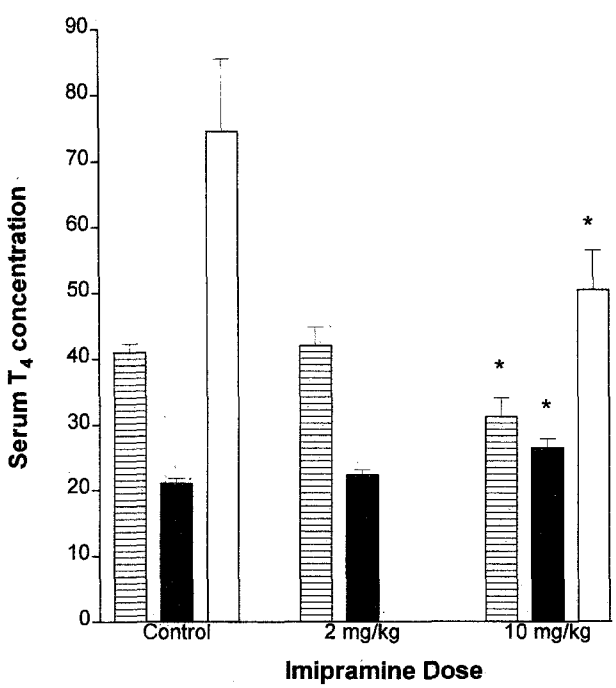

Fig 1. Effect of imipramine treatment on serum T_4 concentrations. Rats were treated with imipramine IP for 14 days. (▤) Total T_4 (×10^{-9} mol/L), (■) free T_4 (×10^{-12} mol/L), and (□) total T_4 in thyroidectomized T_4-treated rats. *$P < .05$, n = 5, *v* control rats, unpaired *t* test.

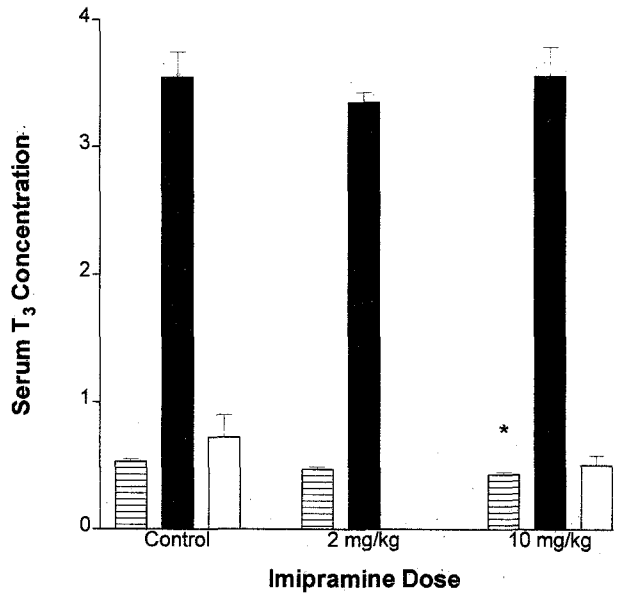

Fig 2. Effect of imipramine treatment on serum T_3 concentrations. Rats were treated with imipramine IP for 14 days. (▤) Total T_3 (×10^{-9} mol/L), (■) free T_3 (×10^{-12} mol/L), and (□) total T_3 in thyroidectomized T_4-treated rats. *$P < .05$, n = 5, *v* control rats, unpaired *t* test.

The effects of imipramine treatment on the free fraction of T_3 and T_4 appear to be indirect, since in vitro addition of imipramine and desipramine 10^{-7} to 10^{-5} mol/L to rat serum or human serum had no significant effect on the free fraction of T_3 or T_4 (Table 1). Whether added directly to the serum before dialysis or to the dialysate to produce a total and free fraction of the drug in serum after dialysis equivalent to that present in undiluted serum before dialysis, neither imipramine nor desipramine had any significant effect on the free fractions of T_3 or T_4.

Serum TSH was unaffected by imipramine treatment at either dose level (Fig 3).

The results shown in Table 2 indicate that there was no

Table 1. Effect of In Vitro Treatment With Imipramine and Desipramine on the Free Fraction of T_4 and T_3 in Normal Serum (% free fractions of thyroid hormones)

Treatment	Human Serum		Rat Serum			
	T_4*	T_3*	T_4*	T_3*	T_4†	T_3†
Control	0.030 ± 0.002	0.38 ± 0.04	0.051 ± 0.002	0.64 ± 0.04	0.061 ± 0.001	0.61 ± 0.04
Imipramine						
10^{-7} mol/L	0.029 ± 0.002	0.48 ± 0.05	0.053 ± 0.002	0.58 ± 0.03	0.062 ± 0.002	0.66 ± 0.03
10^{-6} mol/L	0.030 ± 0.002	0.45 ± 0.04	0.047 ± 0.002	0.58 ± 0.03	0.062 ± 0.002	0.67 ± 0.04
10^{-5} mol/L	0.028 ± 0.002	0.45 ± 0.04	0.048 ± 0.002	0.67 ± 0.04	0.059 ± 0.003	0.70 ± 0.04
Desipramine						
10^{-7} mol/L	0.029 ± 0.001	0.39 ± 0.03	0.044 ± 0.003	0.65 ± 0.03	0.065 ± 0.003	0.65 ± 0.03
10^{-6} mol/L	0.032 ± 0.003	0.40 ± 0.04	0.047 ± 0.002	0.60 ± 0.02	0.065 ± 0.004	0.65 ± 0.03
10^{-5} mol/L	0.033 ± 0.002	0.40 ± 0.03	0.048 ± 0.001	0.69 ± 0.04	0.066 ± 0.004	0.66 ± 0.04

NOTE. Values are the mean ± SEM for determinations in triplicate.
*Drugs added to serum.
†Drugs added to dialysate to produce specified final concentration in serum after equilibrium.

significant effect of the treatment on T_3 or T_4 concentration in the brain, liver, or heart.

Pituitary Cell Cultures

The effect of imipramine and desipramine on TSH secretion by rat anterior pituitary cells is shown in Fig 4. TSH and GH secretion were significantly increased by their respective releasing hormones, TSH from 3.3 ± 0.3 to 15.1 ± 1.8 ng/μg DNA by TRH and GH from 1.1 ± 0.1 to 3.3 ± 0.7 μg/μg DNA by GHRH. When expressed as a percentage of control secretion, both basal TSH and TRH-stimulated TSH secretion were decreased by T_3 and somatostatin (Fig 4A). Imipramine and desipramine 10^{-6} and 10^{-5} mol/L had no significant effect on basal TSH secretion. However, 10^{-5} but not 10^{-6} mol/L imipramine and desipramine significantly inhibited TRH-stimulated TSH secretion.

At 10^{-6} mol/L, neither imipramine nor desipramine had any significant effect on the inhibitory effect of T_3 on either basal or TRH-stimulated TSH secretion. T_3 significantly increased GHRH-stimulated GH secretion but not basal GH secretion (Fig 4B), and somatostatin significantly decreased both basal and GHRH-stimulated GH secretion. Imipramine 10^{-6} mol/L had no significant effect on GH secretion, but 10^{-5} mol/L imipramine and desipramine both inhibited basal and GHRH-stimulated GH secretion. Neither imipramine nor desipramine 10^{-6} mol/L had any significant effect on the stimulatory effect of T_3 on GHRH-stimulated GH secretion.

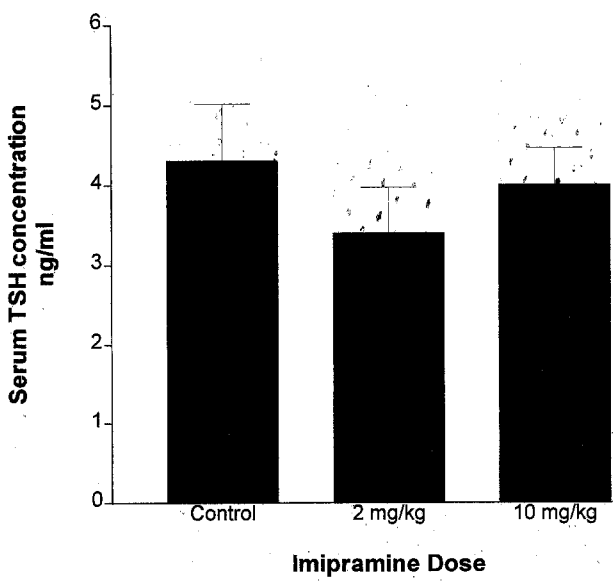

Fig 3. Effect of imipramine treatment on serum TSH concentrations. Rats were treated IP with imipramine for 14 days (n = 5).

Treatment of rats with 10 mg/kg imipramine for 14 days had no significant effect on thyrotrope responsiveness ex vivo (Table 3). There was no significant difference in basal or TRH-stimulated TSH secretion between control and imipramine-treated animals or in the percent reduction of both basal and TRH-stimulated TSH secretion by T_3 and T_4.

DISCUSSION

The present results support an effect of imipramine on the disposition of T_4 and T_3 in the rat. These effects of imipramine were manifest at serum concentrations of the drug similar to that found therapeutically, namely between 0.1 and 1×10^{-6} mol/L. Imipramine pretreatment resulted in a decrease in serum total T_4 and total T_3. This effect on T_4 appeared to be independent of the effects of the drug on the thyroid gland, since it occurred in thyroidectomized animals treated with exogenous T_4. Accordingly, the decrease in total T_4 was probably due to an increased clearance of T_4 under the influence of imipramine. The situation with respect to T_3 is more complex, since T_3 is largely formed by deiodination of T_4 in addition to secretion of T_3 by the thyroid gland.[20] The lack of a significant effect of imipramine treatment on serum total T_3 in thyroidectomized animals may indicate some effect of the drug on T_3 secretion. This might be further clarified by experiments involving T_3 replacement of thyroidectomized animals with exogenous T_3.

The decrease in serum total T_4 and T_3 following imipramine treatment was not reflected in the concentration of free hormones in serum, due to the simultaneous increase in the free fraction of both T_4 and T_3 in serum. The mechanism of this effect of imipramine treatment on the free fraction of T_3 and T_4 has not been elucidated. Potential mechanisms include (1) a direct interaction of imipramine or desipramine on binding to transport proteins by T_3 and T_4 and (2) indirect effects via changes in the concentration of transport proteins in serum. The

Table 2. Effect of Imipramine Treatment on Tissue Content of Thyroid Hormones

Treatment	T_4 (pmol/g)			T_3 (pmol/g)		
	Liver	Heart	Brain	Liver	Heart	Brain
Control (n = 5)	21.6 ± 2.3	4.3 ± 0.8	1.7 ± 0.2	3.6 ± 0.2	2.4 ± 0.2	2.6 ± 0.2
Imipramine 10 mg/kg/d (n = 5)	18.1 ± 0.5	3.9 ± 0.9	1.6 ± 0.1	3.1 ± 0.3	1.9 ± 0.2	2.3 ± 0.3

NOTE. Data were analyzed by unpaired Student's *t* test.

present results indicate that a direct effect of imipramine or its metabolite, desipramine on the binding of T_3 or T_4 to transport proteins in serum is not involved, since addition of these two drugs to normal serum in a concentration range encompassing their in vivo therapeutic serum concentrations had no significant effect on the free fraction of T_3 or T_4. Moreover, our in vitro experiments indicate that an effect on the binding of T_3 and T_4 to their transport proteins at free concentrations of the drug achieved in vivo is also unlikely. These data suggest that imipramine treatment is more likely to act by altering the circulating concentration of transport proteins for thyroid hormones. Considering the fact that transport proteins for thyroid hormones in the rat differ from those in the human in that the adult fed rat does not possess significant amounts of thyroid binding globulin, the effect of imipramine on the free fraction of T_3 and T_4 needs to be investigated more closely in patients. For this reason, experiments are being undertaken to assess serum concentrations of transport proteins for T_3 and T_4 in both rats and humans before and after imipramine treatment, to test the second potential mechanism for the increased free fractions of T_3 and T_4.

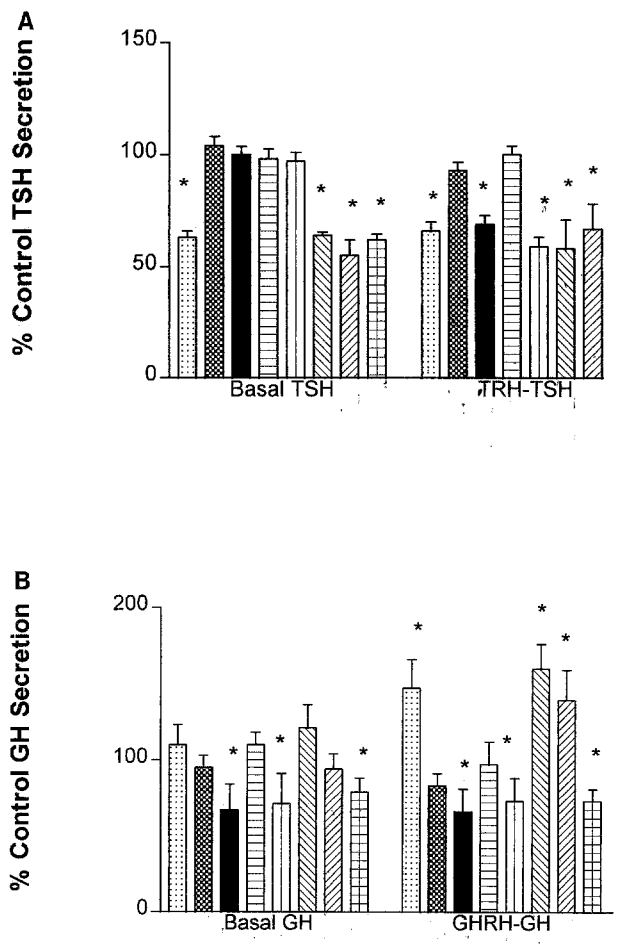

Fig 4. Effect of imipramine and desipramine treatment in vitro on (A) TSH and (B) GH secretion in cultured rat anterior pituitary cells. Cells were exposed to test agents for 18 hours. The effects of 10^{-6} and 10^{-5} mol/L imipramine and desipramine were tested both in control cells and in cells exposed to T_3 10^{-9} mol/L. (▨) T_3, (▩) imipramine 10^{-6} mol/L, (■) imipramine 10^{-5} mol/L, (▤) desipramine 10^{-6} mol/L, (▥) desipramine 10^{-5} mol/L, (▧) imipramine 10^{-6} mol/L and T_3, (▨) desipramine 10^{-6} mol/L and T_3, (⊞) somatostatin 10^{-8} mol/L. *$P < .05$ *v* control secretion, paired *t* test, n = 3 in 4 cultures.

Tissue concentrations of thyroid hormones are determined by the free hormone concentrations in blood, cellular uptake processes, and intracellular bioconversion of T_4 to T_3. Since free hormone concentrations did not decrease, delivery of T_4 and T_3 to the tissues was not reduced by imipramine treatment. Moreover, there appeared to be no effect of imipramine treatment on the uptake of T_4 or T_3 or bioconversion of T_4 to T_3 in the three tissues studied, since T_3 and T_4 concentrations in the brain, liver, and heart were unaffected by drug treatment. This is despite the fact that T_4 clearance from the serum appeared to be enhanced by imipramine treatment. However, the possibility cannot be excluded that treatment may have altered the subcellular distribution of the thyroid hormones.

Imipramine pretreatment had no significant effect on serum TSH concentrations. A lack of effect on TSH is compatible with the fact that free T_3 and free T_4 were not reduced; in fact, free T_4 was increased slightly by imipramine treatment. Moreover, there was no evidence from the in vitro and ex vivo experiments to suggest that the responsiveness of thyrotropes to either stimulation by TRH or inhibition by T_3 and T_4 was altered by imipramine or desipramine. The in vitro experiments also suggested that the direct effects of imipramine and desipramine on TSH secretion, namely a decrease, only occurred at concentrations greater than 10^{-6} mol/L. Since these experiments were conducted in tissue culture media containing only 0.1% albumin, the free concentrations of drugs in these experiments would be much higher than those that occur in plasma in vivo following imipramine treatment, because in vivo imipramine and desipramine are approximately 85% bound to plasma

Table 3. Effect of Imipramine on Ex Vivo TSH Secretion

Parameter	Control	Imipramine 10 mg/kg/d
TSH (ng/μg DNA)*		
Basal	4.87 ± 0.37	5.04 ± 0.39
TRH-stimulated	16.4 ± 2.4	13.5 ± 2.7
TSH (% control)*		
Basal + T_3	67 ± 4	73 ± 3
Basal + T_4	74 ± 6	79 ± 4
TRH + T_3	65 ± 10	67 ± 9
TRH + T_4	67 ± 10	69 ± 12

*n = 4, in 3 cultures.

protein, mainly alpha-1 acid glycoprotein.[18] Potentially, TSH secretion might have been affected in vivo by the effects of imipramine or desipramine on the release of hypothalamic hormones either directly or via an effect on uptake and receptor mechanisms for central neurotransmitters.[21] However, the present experiments provided no evidence of any effect of imipramine treatment on TSH secretion in vivo.

In conclusion, the main interaction demonstrated in the present study between imipramine and thyroid hormones in the rat was an enhanced clearance of T_4. Serum total T_4 and T_3 were decreased by imipramine treatment in the rat, but there were no parallel changes in the free concentrations of thyroid hormones because of simultaneous decreases in the proportion of thyroid hormones bound to transport proteins. Plasma TSH concentrations were unchanged, as were tissue concentrations of T_3 and T_4 in a variety of target tissues, including the brain. These results suggest that imipramine treatment is unlikely to result in significant changes in thyroid-dependent processes in tissues. However, the possibility that the subcellular distribution of thyroid hormones may be altered by imipramine cannot be excluded by the present data. The enhanced clearance of T_4 observed in the present study may well explain the reported decrease in T_4 in treated depressed patients. However, it is difficult to extrapolate results on free hormone concentrations in the rat to the human because of interspecies differences in transport proteins for thyroid hormones. For this reason, the effect of imipramine treatment on the transport of thyroid hormones in plasma needs to be examined in more detail in patients. Such studies are in progress.

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