hydrophobic cleft is unlikely therefore to include reactive nucleophiles unless the catalytic mechanism proceeds via a covalently modified enzyme intermediate. There is kinetic [18] and stereochemical [19] evidence that this is not the case. The findings of this work are therefore compatible with the sequential mechanisms that have been proposed on kinetic grounds [18].

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Decreased hepatic glutathione *S*-transferase A, AA and L concentration produced by prolonged thyroid hormone administration

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The glutathione *S*-transferases (GST) are a complex group of enzymes that possess several biological functions but the primary function of GST is considered to be one of detoxification [1]. The cytosolic GST forms are comprised of two subunits of approximately similar size and in rat liver the major subunit forms are Ya (M_r 25,500), Yc (M_r 27,500), Yb_1 and Yb_2 (both M_r 26,300). These subunits may combine to produce various GST isoenzymes as summarized in Table 1 but the homodimers of the Ya, Yb_1 and Yc subunits account for approximately 60% of the total hepatic GST [1,2]. Some degree of differentiation of the GST subunits in crude tissue extracts may be obtained by using different substrates [1] but a more reliable approach to quantitating the GST isoenzymes is to measure specifically the concentration of each of the GST using radioimmunoassay.

In the rat, surgical thyroidectomy results in a 30% increase in the hepatic concentration of GST L (YaYa, previously called "ligandin") and normal GST concentrations are restored following daily intraperitoneal

injection of 50 μg/kg of thyroxine (T_4) [3]. Chemically-induced hypothyroidism in mice produces differential effects on the expression of various GST activities but again these effects may be reversed by intraperitoneal administration of 2 mg/kg triiodothyronine for 3 days [4].

In both the above studies [3,4] the doses of the thyroid hormone injected were vastly in excess of what would be required to compensate for thyroid failure. For example, in man the average total amount of T_4 and T_3 produced by the body is only 90 μg/day and 30 μg/day respectively. Also thyroid hormones were given over a period of only a few days, a time-period at which steady state concentrations of thyroid homones would not have been achieved.

We recently studied the effects of prolonged oral thyroid hormone administration on rat hepatic GST L concentration [5]. Thyroid hormones were administered to euthyroid rats at doses which approximated to the equivalent body weight-related doses given to hypothyroid patients and for time-periods sufficient to produce steady-state plasma concentrations. Using these conditions there was a decrease in the hepatic content of GST L; i.e. the opposite of the effect seen in hypothyroidism. We did not study the effects of thyroid hormone administration on the concentration of other GST but such data would be of interest since animals pre-treated with thyroid hormones show an increased susceptibility to drug-induced hepatic damage particularly by hologenated hydrocarbons [6,7].

In the present study we have quantitated, using specific radioimmunoassays, the effect of thyroid hormone administration on the major hepatic forms of GST namely GST L (YaYa), GST A (Yb_1Yb_1) and GST AA (YcYc).

Materials and methods

Radioimmunoassay of GST in hepatic cytosols. The GST were purified as previously described [2] and antisera were raised in rabbits to GST L, GST A and GST AA (subunit composition YaYa, Yb_1Yb_1 and YcYc respectively) using standard immunisation techniques [5]. The preparation of iodinated GST and the radioimmunoassay procedure was as described previously [5]. The specificities (cross-reactivities) of the three antisera were assessed by determining the amount of each GST, compared with the immunogen, which was required to displace 50% of bound label.

Total GST activity. The activity of GST in hepatic cytosols was measured at 37° using a centrifugal analyser with 1 mM 1-chloro-2,4-dinitrobenzene and 2 mM-reduced glutathione as substrates [5].

Plasma measurements. The concentration of total T_3 and total T_4 were measured by radioimmunoassay [8]. The activity of alanine aminotransferase was measured using a Sequential Multiple Analyser with Computer (SMAC) system (Technicon Instrument Corporation, Basingstoke, U.K.).

Effect of T_4 and T_3 administration on hepatic GST levels. Three groups of male Sprague–Dawley rats (each approx. 400 g) were used. Eight animals were given T_4 (7.5 mg/l) in their drinking water *ad libitum* for four week and six animals were given T_3 (750 μg/l) in their drinking water *ad libitum* for two weeks. These doses and times were as used previously [5] and were chosen for the reason stated in the introduction. Eight control animals were also used which were kept on a normal diet for 6 weeks.

Blood was collected after decapitation and plasma prepared. The livers were removed, weighed, placed immediately into 20 mmol/l sodium phosphate buffer, pH 7.4, and kept on ice. Cytosols were prepared within 2 hr of sacrifice as follows:

Livers were individually homogenised in 20 mmol/l sodium phosphate buffer pH 7.4 (25% w/v) and the homogenates centrifuged at 100,000 *g* for 1 hr at 4°. A portion of the supernatants were assayed immediately for GST activity and the remainder were stored at −70° for subsequent analysis. On thawing supernatants were diluted 10,000 times with assay diluent prior to analysis for GST concentration.

Results

Specificity of antisera. The cross-reactivities of the GST antisera with the various rat GST are shown in Table 1. In each case, the antisera showed little or no cross-reactivity with GST other than the immunogen. Thus the 3 RIA developed were specific for GST A (Yb_1Yb_1), GST AA (YcYc) and GST L (YaYa).

Plasma analytes. The changes in the levels of various plasma analytes in these animals have previously been reported in detail [5] and are summarized in Table 2.

The dose of T_4 given to the animals resulted in T_4 being increased approximately 5-fold and T_3 7-fold above the concentrations in control animals. The dose of T_3 given to the animals resulted in suppression of T_4 synthesis but the mean plasma T_3 concentration in these animals was only 60% higher than in the control animals (Table 2).

Hepatic GST concentration and activity. The changes in the hepatic concentration of GST L have previously been reported and are summarized in Table 3 [5].

Administration of T_4 resulted in a significant increase in the concentration of cytosolic protein but no significant difference was found between control and the T_3-treated

Table 1. The cross-reactivities of the antisera for hepatic GST A, AA and L with various GST, as measured by radioimmunoassay

GST	Subunit composition	Family	Cross-reactivities (%) Anti-A (Yb_1Yb_1)	Anti-AA (YcYc)	Anti-L (YaYa)
AA	YcYc	I	<0.1	100	0.1
L	YaYa	I	<0.1	4.1	100
F	YaYa	I	<0.1	1.7	100
B	YaYc	I	<0.1	7.1	<0.1
K	YkYk	I	<0.1	<0.1	<0.1
A	Yb_1Yb_1	II	100	<0.1	<0.1
P	Yb_1Yn	II	3.9	<0.1	<0.1
C	Yb_1Yb_2	II	22.5	<0.1	<0.1
R	Yb_2Yb_3	II	0.9	<0.1	<0.1
D	Yb_2Yb_2	II	<0.1	<0.1	<0.1
N	YnYn	II	<0.1	<0.1	<0.1
S	Yb_2Yn	II	<0.1	<0.1	<0.1
H	YfYf	III	<0.1	<0.1	<0.1

Table 2. Summary of changes in plasma analytes in the various groups of animals

Analyte	Animal group: Control (N = 8)	T_4-treated (N = 8)	T_3-treated (N = 6)
Thyroxine (nmol/l)	68 ± 13	365 ± 57 ($P < 0.001$)	18 ± 7 ($P < 0.001$)
Triiodothyronine (nmol/l)	1.1 ± 0.2	7.6 ± 3.2 ($P < 0.001$)	1.8 ± 0.3 ($P < 0.001$)
Alanine aminotransferase (U/L)	73 ± 15	109 ± 20 ($P < 0.002$)	80 ± 8 (NS)

Full details can be found in Ref. 5.
Means ± SD are shown.

Table 3. The effect of T_3 or T_4 administration on hepatic cytosolic protein concentration, GST concentration and GST activity

Animal treatment	Total protein (g/l)	GST activity (μmol CDNB/min/mg)	GST mass (μg/mg cytosolic protein) A	AA	L
Control	36.5 ± 2.0	1.96 ± 0.2	25.2 ± 1.37	18.9 ± 3.8	20.1 ± 5.8
T_3-treated	39.0 ± 5.8 (NS)	1.03 ± 0.09 ($P < 0.001$)	19.4 ± 3.18 ($P < 0.001$)	14.6 ± 3.7 ($P < 0.05$)	13.3 ± 4.0 ($P < 0.03$)
T_4-treated	46.3 ± 5.0 ($P < 0.001$)	0.61 ± 0.17 ($P < 0.001$)	16.4 ± 2.7 ($P < 0.001$)	12.9 ± 3.2 ($P < 0.01$)	13.3 ± 3.4 ($P < 0.01$)

Mean ± SD are shown and where values differ significantly from control values the significance values are shown in brackets
NS = not significant

animals (Table 3). However, administration of T_4 or T_3 resulted in a significant decrease in the total cytosolic content of GST A (Yb_1Yb_1), AA (YcYc) and L (YaYa) (Table 3). The total enzymic activity of GST in rat liver cytosol was also significantly reduced following thyroid hormone administration (Table 3). In T_3-treated animals GST activity was only 47% of control values and in T_4-treated animals GST activity was reduced to 30% of control values.

Significant differences in GST concentration or activity were found irrespective of whether data were related to cytosolic protein concentration or the wet weight of liver.

We were unable to measure the concentration of GST D (Yb_2Yb_2) or the heterodimeric forms of GST but it seems unlikely that the concentrations of the GST that were not determined were preferentially increased. A plot of total GST against the sum of GST A, AA and L for each of the three groups gave a linear relationship. If thyroid hormone administration had produced preferential induction of GST forms not measured, then such a clear linear relationship would not result. Also since GST A, AA and L were decreased by approximately the same extent in the T_3- and T_4-treated animals it appears highly unlikely that the heterodimers of the Ya, Yb and Yc subunits, not measured by the radioimmunoassays, would be affected disparately.

Discussion

The data presented here show that prolonged administration of T_3 or T_4 at non-pharmacological doses to euthyroid rats results in a marked decrease in hepatic GST A, GST AA and GST L concentration; the isoenzymes that constitute approximately 60% of total liver GST [2].

The decrease in hepatic GST is unlikely to be entirely due to hepatic damage and release of GST from the hepatocyte. The increases in plasma GST L concentration and plasma ALT activity observed in our T_4-treated were small and, the total hepatic cytosolic protein concentration was increased in these animals, probably as a result of T_4-induced protein synthesis. In our T_3-treated rats there was no change in plasma ALT (Table 2) and there was only a modest 2-fold increase in plasma GST L concentration [5] yet hepatic GST L concentration still decreased by approximately 25%. A likely major contributing factor for the decreased hepatic GST concentration is a reduced synthesis or increased turnover of GST as suggested by Arias and his colleagues [4].

It is of relevance to note that the changes in plasma T_3 observed in the T_3-treated animals were similar to the magnitude of the changes in plasma T_3 seen in T_4-treated hypothyroid patients [9]. It seems possible, therefore, that hypothyroid patients receiving thyroid hormone replacement may also have a reduction in hepatic GST that may increase their vulnerability to drug-induced liver damage. In this respect it has been demonstrated that abnormal liver function can occur in T_4-treated hypothyroid patients [9].

In summary, prolonged administration of thyroid hormones at non-pharmacological doses produces a marked decrease in all the major forms of hepatic GST probably as a result of reduced hepatic synthesis or increased turnover. This reduction in GST concentration may explain, at least in part, the increased susceptibility that thyroid hormone-treated animals have to drug-induced liver damage.

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Development and characterization of anti-spiroperidol antibodies*

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Ligand-specific antibodies are valuable tools for several types of applications. For example, antibodies specific for neuroleptic antipsychotic drugs are used to measure serum levels of these drugs in medicated patients [1]. Anti-ligand antibodies can also be used to induce anti-idiotypic antibodies that cross-react with receptors to which the hapten binds [2, 3], and it has been suggested [4] that the use of primary sequence information and X-ray crystallographic studies to construct three-dimensional models of the ligand binding site of an antibody may aid in constructing models of binding sites on receptors that are membrane-bound and difficult to crystallize. To measure serum levels of a drug it is necessary to have antibodies very selective for that drug, but for other applications antibodies that recognize a number of ligands are preferred. Thus, when anti-ligand antibodies are used to induce cross-reactive anti-idiotype antibodies, or as models for receptors, the primary antibody should recognize many ligands, mimicking as closely as possible the pharmacological profile of the receptors.

A panel of anti-aminospiroperidol and anti-*N*-aminophenethyl-spiroperidol (NAPS) monoclonal antibodies with high affinity for the dopamine D-2 receptor ligand spiroperidol and other butyrophenones, and high affinity for the non-butyrophenone D-2 ligand domperidone, has been produced [4]. These antibodies were elicited from mice in an early stage of the immune response by immunizing the mice once, then boosting those mice that had the highest titer 3 days before preparation of hybridomas. We now describe three monoclonal antibodies isolated from a mouse in a later stage of the immune response. These antibodies have the highest affinity for spiroperidol and related ligands of any antibodies produced to date.

Methods

NAPS and NAPS-KLH (keyhole limpet hemacyanin) were synthesized as described previously [4]. Briefly, 0.01 mmol of NAPS was reacted with 0.04 mmol of bromoacetic acid in 1.0 ml of *N,N*-dimethylformamide in the presence of 0.04 mmol of dicyclohexyl carbodiimide. The reaction mixture was incubated for 16 hr at 25°, at which time conversion of NAPS to its *N*-bromoacetyl derivative appeared to be complete. The ether-precipitated bromoacetyl derivative was dissolved in 1.0 ml of dimethylformamide and added to 4.0 ml of a solution containing 5.0 mg/ml of KLH in 0.5 M $NaHCO_3$ buffer at pH 10.0. To solubilize KLH, 0.2 ml of 10% sodium dodecyl sulfate in water was added to the protein solution. The coupling reaction was carried out for 16 hr at 4°. Uncoupled hapten was separated from the NAPS-KLH conjugate using Sephadex G-50 equilibrated in 50 mM sodium phosphate buffer containing 150 mM NaCl and 0.01% NaN_3 at pH 7.4.

Female BALB/c mice (6–8 weeks old) were immunized with equal volumes of Freund's Complete Adjuvant and NAPS-KLH (1.0 mg/ml). Mice received intraperitoneal and multiple subcutaneous injections of a total of 0.4 ml of the emulsion. The mice received subsequent injections of NAPS-KLH and Freund's Incomplete Adjuvant (0.4 ml, i.p.) at approximately 1-month intervals. One month after the third immunization one mouse was treated with NAPS-KLH in saline (0.2 ml, i.p.). Three days after this boost, splenocytes were removed for the preparation of hybridomas using the method of Kennett *et al.* [5] as described previously [4]. A charcoal adsorption assay [4] was used with minor modifications to measure the binding of [^{3}H]-spiroperidol to the monoclonal antibodies. Diluted hybridoma supernatant (50 μl) was incubated for 1 hr at 37° in 1.5-ml microfuge tubes with Tris-buffered saline (50 mM Tris–HCl, pH 7.4, and 0.9% NaCl), [^{3}H]-spiroperidol, and appropriate drugs, in an assay volume of 1 ml. Direct binding experiments were carried out using various concentrations of [^{3}H]-spiroperidol in the absence of an inhibitor. Competition experiments were carried out using six concentrations of inhibitor with approximately 1 nM [^{3}H]-spiroperidol in each assay. Nonspecific binding was determined by replacing hybridoma supernatant with control tissue culture medium. For each competing drug, values for IC_{50} and Hill coefficient were obtained from indirect Hill plots, and K_i was calculated by the method of Cheng and Prusoff [6]. Statistical comparisons of the geometric means of K_i and K_d values were made using Student's *t*-test. Light chain type and isotypes were identified by Dr. Robert Luedtke at the University of Pennsylvania.

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