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Selective effects of thiol reagents on the binding sites for imipramine and neurotransmitter amines in the rat brain

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1 The action of the antithyroid drugs methimazole (MMI) and propylthiouracyl (PTU) on the binding of [^{3}H]-imipramine, [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) (to 5-HT_1-receptors) and [^{3}H]-spiperone (to 5-HT_2-, D_2-receptors) of rat brain membranes has been examined. The synaptosomal uptake of [^{3}H]-5-HT was also studied.

2 Micromolar concentrations of the disulphide bond reducing agents MMI, PTU, dithiothreitol (DTT) and mercaptoethanol increased both the binding of [^{3}H]-imipramine and the uptake of [^{3}H]-5-HT. In contrast, they decreased the number of 5-HT_1-receptors, and did not affect 5-HT_2- and D_2-sites.

3 Reaction with membrane-bound sulphydryl (SH) groups by micromolar concentrations of N-ethylmaleimide (NEM), hydroxymercuribenzoic acid (PCMB), or Ellman's reagent (DTNB) decreased the binding of [^{3}H]-imipramine, the number of 5-HT_1-receptors, and the uptake of [^{3}H]-5-HT. Millimolar concentrations of NEM were necessary in order to decrease partially 5-HT_2- and D_2-receptors.

4 The effects of NEM on imipramine recognition sites and on the uptake of 5-HT could be prevented by DTT; protection was not obtained in other receptor systems.

5 Three groups of receptors have been, thus, postulated, based upon their different sensitivity towards alterations in membrane [disulphide bridges $\rightleftharpoons$ SH] equilibrium: Group I, including imipramine recognition sites and the uptake system for 5-HT; Group II, including 5-HT_1-receptors; Group III, including 5-HT_2- and D_2-receptors.

Introduction

We have previously shown (Vaccari, 1985) that chronic, neonatal administration of the sulphydryl goitrogen methimazole (MMI) decreased the number of [^{3}H]-imipramine binding sites in cerebral cortical membranes from prepubertal hypothyroid rats. A similar antithyroid treatment of adult rats did not affect imipramine 'receptors'. It was, thus, suggested that developmental impairment of cortical binding sites for [^{3}H]-imipramine was due to a deficient sprouting of those (5-hydroxytryptaminergic) nerve endings where imipramine binding sites seem to be located (Palkovits *et al.*, 1981; Fuxe *et al.*, 1983). Furthermore, when the binding reaction of [^{3}H]-imipramine was run in the presence of low concentrations of MMI, there was a consistent increase in the number of brain imipramine binding sites compared to controls. A similar effect could be obtained shortly after an individual injection of MMI to immature rats, when tissue levels of goitrogen were conceivably high. These latter results strongly suggested that goitrogen-induced perturbance of membrane-bound thiol groups might be functionally important for imipramine binding in the brain.

In this paper we have studied in detail the effects of MMI and additional membrane-perturbing reagents on binding characteristics of [^{3}H]-imipramine and on putative receptors for neurotransmitter amines.

Methods

Male Charles River CD rats (250–300 g) were used. For binding experiments, the entire brain (less cerebellum and olfactory bulbs) from individual rats was homogenized in the appropriate buffer, the nuclear pellet was then obtained at 900 *g* for 5 min, and

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discarded. Samples were then centrifuged twice at 48,000 *g* for 10 min; partially purified membranes were suspended 1:26 or 1:50 (w/v) and soon after used for receptor assays.

[^{3}H]-imipramine binding

Membrane aliquots of the 1:26 suspension (200 μl, 1 mg protein ml^{-1}) were incubated with [^{3}H]-imipramine (0.5–32 nM) for 60 min, on ice, in the absence or presence of 100 μM desipramine as the displacer, in a final volume of 500 μl (Raisman *et al.*, 1980). Samples were then rapidly filtered through Whatman GF/B filters, and washed thrice with 5 ml ice cold buffer. Correction for binding of [^{3}H]-imipramine to the filters was performed with direct measurement of the radioactivity specifically bound to the filters in the absence of membranes. After this correction, specific binding of [^{3}H]-imipramine represented 40–50% of total binding.

[^{3}H]-5-hydroxytryptamine binding

The method of Bennet & Snyder (1976) was followed. Aliquots (400 μl) of the 1:26 membrane suspension were preincubated at 37°C for 10 min. Thereafter 50 mM Tris-HCl buffer (pH 7.4 at 37°C) containing 4 mM $CaCl_2$, 0.01% ascorbic acid, and 10 μM pargyline was added up to a volume of 1.0 ml, followed by 2.5 nM [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT), in the absence or presence of 10 μM unlabelled 5-HT as the displacer. After 15 min of incubation at 37°C, the samples were filtered. Specific binding amounted to 60–70% of total binding.

[^{3}H]-spiperone binding

The binding of[^{3}H]-spiperone to 5-HT type-2 (5-HT_2) and dopamine (D_2)-receptors was measured in the whole brain less cerebellum and olfactory bulbs according to List & Seeman (1981), with improvements in the buffer composition (Hartley & Seeman, 1983). Aliquots (200 μl, 0.5 mg protein ml^{-1}) of the 1:50 membrane suspension were incubated for 15 min at 37°C in 50 mM Tris-HCl buffer (pH 7.1 at 37°C) containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 0.5 nM or 1.8 nM [^{3}H]-spiperone in the absence or presence of 10 μM (−)-sulpiride or ketanserin, in order to characterize specific binding at D_2- and 5-HT_2-receptors, respectively. The incubation volume was 1.0 ml. Specific binding was approx 50–60% of the respective total binding.

Membrane-modifying reagents were added to the samples at the start of binding reaction, since we had previously ascertained that this procedure did not substantially differ from that of briefly (for 5–10 min) preincubating membranes at 37°C with thiol reagents. Preincubation of membranes for 30 min at 37°C both in the absence or presence of thiol reagents decreased the specific binding to 5-HT- and D_2-receptors, probably the result of endogenous protease activation.

[^{3}H]-5-hydroxytryptamine uptake

Crude synaptosomes (P_2) were prepared according to Whittaker & Barker (1972). Individual brains (less cerebellum and olfactory lobes) were homogenized in 40 vol of 0.32 M sucrose. After centrifugation at 1000 *g* for 5 min, synaptosomes were isolated by centrifugation of the supernatant at 12,000 *g* for 20 min; they were then suspended in 0.32 M glucose (Raiteri *et al.*, 1977). Triplicate 440 μl aliquots (0.7–0.9 mg protein ml^{-1}) were preincubated for 10 min at 37°C in the presence of 100 μM pargyline, and in the absence or presence of membrane-perturbing reagents. Thereafter, varying volumes of the incubation medium were added up to a final volume of 500 μl, samples were mixed with [^{3}H]-5-HT (10–200 nM), and incubated for 4 min at 37°C. Non specific uptake was assessed with preincubating and incubating samples on ice. Aliquots (100 μl) were filtered on Whatman GF/B filters, and washed thrice with 5 ml of ice cold Krebs-Ringer medium containing 10 mM glucose.

Protein samples were assayed with bovine serum albumin as the standard (Lowry *et al.*, 1951).

Eadie-Hofstee plots for binding or uptake curves were calculated according to Zivin & Waud (1982). Results were analysed by Student's *t* test.

The following drugs were used: methimazole (MMI), DL-dithiothreitol (DTT) and 5-hydroxytryptamine creatinine sulphate were obtained from Sigma Chemical Co. N-Ethylmaleimide (NEM); 6-(*n*-propyl)-2-thiouracyl (PTU); 5,5′-dithiobis (2-nitrobenzoic) acid (Ellman's reagent, DTNB); hydroxy mercuribenzoic acid Na salt (PCMB), and 2-mercaptoethanol were obtained from Serva Feinbiochemica. L-Cystine dimethyl ester 2HCl was from Fluka AG. Desipramine HCl and (−)-sulpiride HCl were kindly donated by Ciba-Geigy and Ravizza, respectively. Ketanserin was a generous gift of Janssen Pharmaceutica. [^{3}H]-imipramine (55 Ci mmol^{-1}); [^{3}H]-spiperone (36 Ci mmol^{-1}), and [^{3}H]-5-hydroxytryptaminine creatinine sulphate (32 Ci mmol^{-1}) were from New England Nuclear.

Results

Effects on [^{3}H]-imipramine binding sites

The thiol goitrogens MMI and PTU at 3.5×10^{-6} M consistently (by 61 and 29%, respectively) increased the specific binding of [^{3}H]-imipramine (4nM) (Figure 1). Additional disulphide bond-reducing agents such

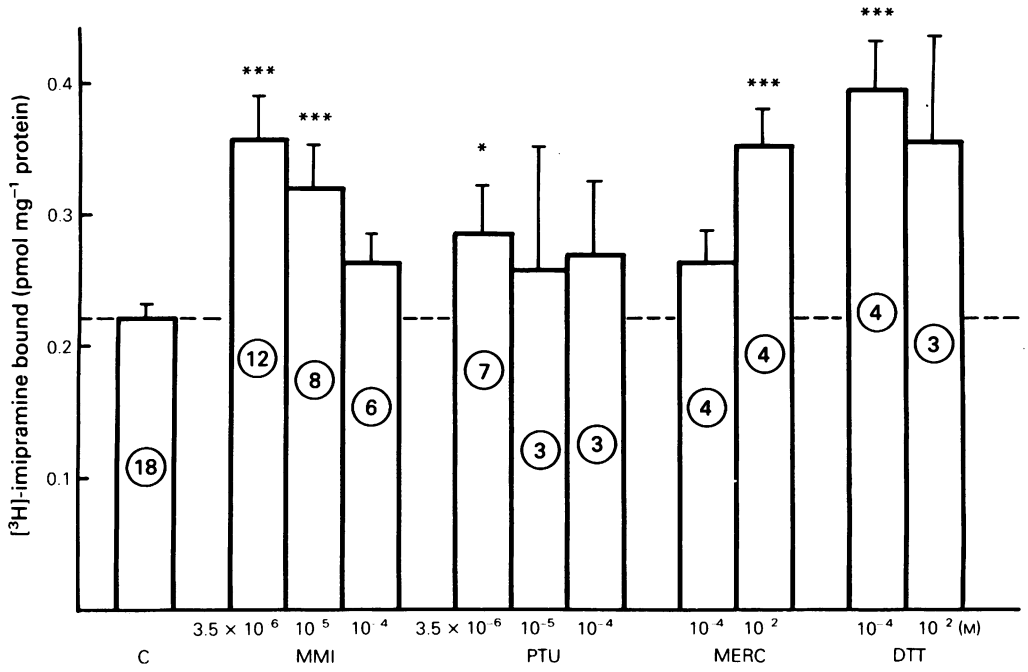
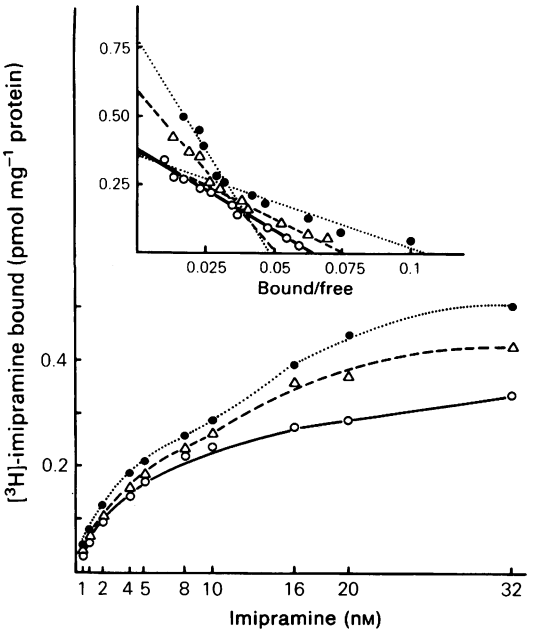


Figure 1 Effects of disulphide bond-reducing reagents on the specific binding of 4 nM [^{3}H]-imipramine to whole brain (less cerebellum and olfactory bulbs) membranes. The binding reaction was performed in the absence (C) or presence of methimazole (MMI), propylthiouracyl (PTU), mercaptoethanol (MERC) or dithiothreitol (DTT). Results are means with vertical lines showing s.e.mean from n experiments (figures in the columns) run in triplicate. * $P<0.05$; *** $P<0.001$, as compared to control.



as mercaptoethanol or DTT in the range 10^{-4}–10^{-2} M also markedly (by 20–80%) increased the binding of [^{3}H]-imipramine compared to controls (Figure 1). Analysis of saturation curves for [^{3}H]-imipramine (0.5–32 nM) revealed a clearly upward-concave Eadie-Hofstee plot in the presence of 3.5×10^{-6} M MMI, thus indicating that the goitrogens lead imipramine to bind preferentially at the low-affinity site which is normally evident with the ligand present at a concen-

Figure 2 Binding profile of [^{3}H]-imipramine to whole brain (less cerebellum and olfactory bulbs) membranes in the absence (control, ○) or presence of 3.5×10^{-6}M methimazole (MMI, ●) or propylthiouracil (PTU, △). Data points are means of 2 experiments performed in triplicate. The inset shows Eadie-Hofstee plots for the high-affinity (in the range 0.5–10 nM ligand) and low-affinity (10–32 nM) components. High-affinity binding parameters were: B_{max} = 366, 354 and 379 fmol mg^{-1} protein; K_D = 5.67, 3.36 and 5.07 nM in controls, MMI and PTU samples, respectively. Binding parameters for the low-affinity component were: B_{max} = 401, 774 and 593 fmol mg^{-1} protein; K_D = 7.27, 16.1 and 11.0 nM in controls, MMI and PTU samples, respectively.

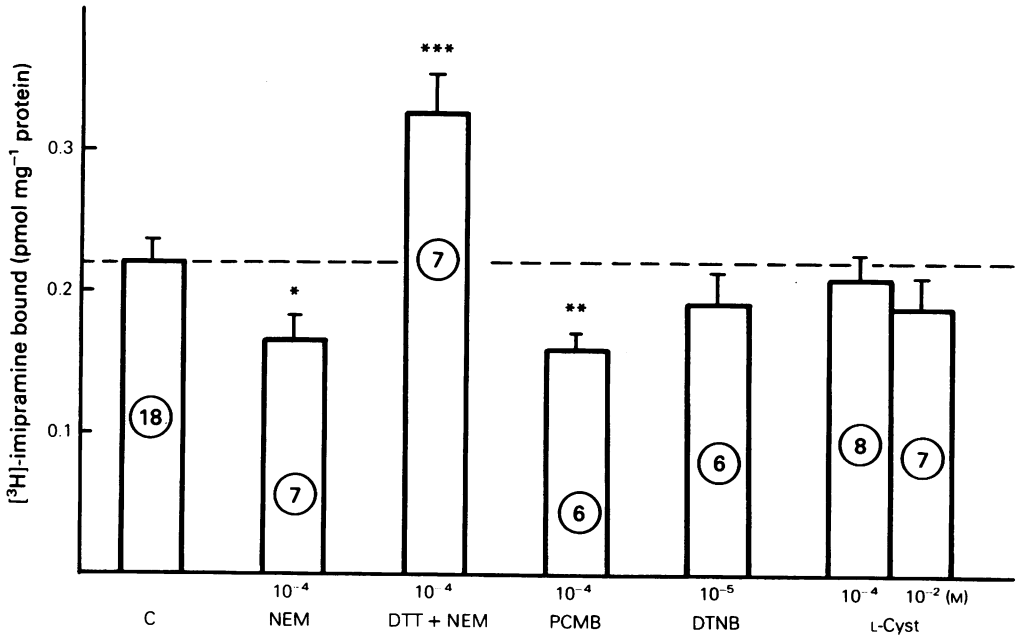


Figure 3 Effects of sulphydryl-reactive agents on the specific biding of 4 nM [^{3}H]-imipramine to whole brain (less cerebellum and olfactory bulbs) membranes. The binding reaction was performed in the absence (C) or presence of N-ethylmaleimide (NEM), hydroxymercuribenzoic acid (PCMB), Ellman's reagent (DTNB) or L-cystine (L-Cyst). Results are means with vertical lines showing s.e.mean from n experiments (figures in the columns) performed in triplicate. * $P<0.05$; ** $P<0.02$, as compared to C. *** $P<0.001$, compared to NEM.

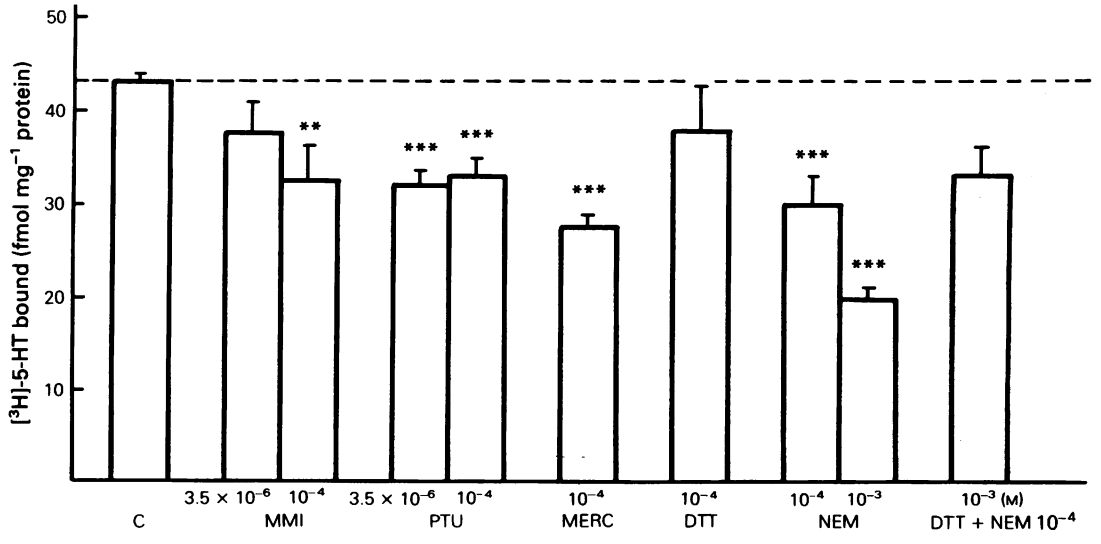


Figure 4 Effects of sulphydryl reagents on the specific binding of 2.5 nM [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) to 5-HT_1-receptors in whole brain (less cerebellum and olfactory bulbs) membranes. The binding reaction was performed in the absence (C) or presence of SH reagents; 10 μM 5-HT was the displacer. Results are means with vertical lines showing s.e.mean from $n = 6$ experiments performed in triplicate. ** $P<0.02$; *** $P<0.005$ or <0.001, as compared to control.

tration above 10 nM. The low-affinity component in the presence of MMI was characterized by an approx 2 fold higher B_{max}, and a 2 fold lower affinity, as compared to controls (Figure 2). Covalent alkylation of accessible sulphydryl (SH) groups with 10^{-4} M NEM or PCMB decreased the binding of [^{3}H]-imipramine by 25 and 28%, respectively, compared to controls (Figure 3). When the binding reaction was run in the presence of 10^{-4} M DTT, the effect of NEM was totally prevented, and [^{3}H]-imipramine was bound by 48% more than in controls (Figure 3). Reoxidation of SH groups by 10^{-5} M DTNB, or L-cystine in the range $10^{-4}-10^{-2}$ M also tended to decrease the binding of [^{3}H]-imipramine, though differences were not statistically relevant (Figure 3).

Effects on [^{3}H]-5-hydroxytryptamine (5-HT$_1$) receptors

Both thiol-reducing goitrogens decreased (by over 20%) the binding of [^{3}H]-5-HT (2.5 nM) to 5-HT$_1$-receptors; a decrease occurred also with mercaptoethanol (−36%) and DTT (−12%) (Figure 4). On the other hand, 10^{-4} M NEM reduced by 30% the density of 5-HT$_1$-receptors, an effect that could not be prevented even at millimolar concentrations of DTT (Figure 4). Approximate binding parameters for [^{3}H]-5-HT (0.05–10 nM; mean values from 2 experiments) were: B_{max} = 197, 152 and 148 fmol mg^{-1} protein; K_D = 2.4, 3.1 and 3.4 nM in controls, and in the presence of 10^{-4} M MMI or NEM, respectively.

Effects on [^{3}H]-spiperone binding (5-HT$_2$-and D$_2$-receptors)

The binding of [^{3}H]-spiperone (1.8 nM) to 5-HT$_2$-receptors was not affected by MMI, PTU, mercaptoethanol or DTT (Figure 5). High concentrations of NEM (10^{-3} M) decreased by 43% the density of 5-HT$_2$-receptors, an effect that was not reversed by DTT (Figure 5). Like 5-HT$_2$ sites, the binding of [^{3}H]-spiperone (0.54 nM) to D$_2$-receptors was not affected by reducing reagents, and was decreased (−83%) by 10^{-3} M NEM, compared to controls (Figure 6).

Approximate binding parameters for 5-HT$_2$-receptors ([^{3}H]-spiperone 0.06–6.5 nM; mean values from 2 experiments) were: B_{max} = 97 and 49 fmol mg^{-1} protein; K_D = 0.72 and 0.34 nM, in the absence or presence of 10^{-3} M NEM, respectively. For D$_2$-receptors ([^{3}H]-spiperone 0.06–6.5 nM; mean values from 2 experiments) approximate binding parameters were: B_{max} = 53 and 21 fmol mg^{-1} protein; K_D = 0.40 and 0.23 nM, in the absence or presence of 10^{-3} M NEM, respectively.

Effects on the uptake of [^{3}H]-5-hydroxytryptamine

The uptake of [^{3}H]-5-HT (10–200 nM) in the P$_2$ fraction is shown in Figure 7. MMI, PTU and DTT tended to increase the uptake of 5-HT, while NEM markedly decreased it, an effect that could be prevented by DTT.

Eadie-Hofstee analysis of uptake curves revealed

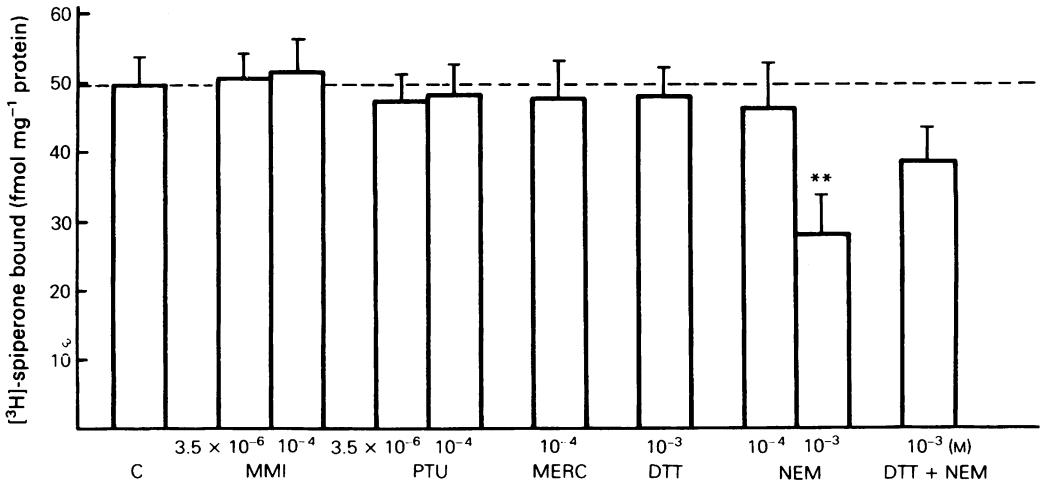


Figure 5 Effects of sulphydryl reagents on the specific binding of 1.8 nM [^{3}H]-spiperone to 5-HT$_2$-receptors in whole brain (less cerebellum and olfactory bulbs) membranes. The binding reaction was performed in the absence (C) or presence of SH reagents (for abbreviations, see Figures 1 and 3); 10 μM ketanserin was the displacer. Results are means with vertical lines showing s.e.mean from n = 6 experiments performed in triplicate. ** $P < 0.02$, as compared to control.

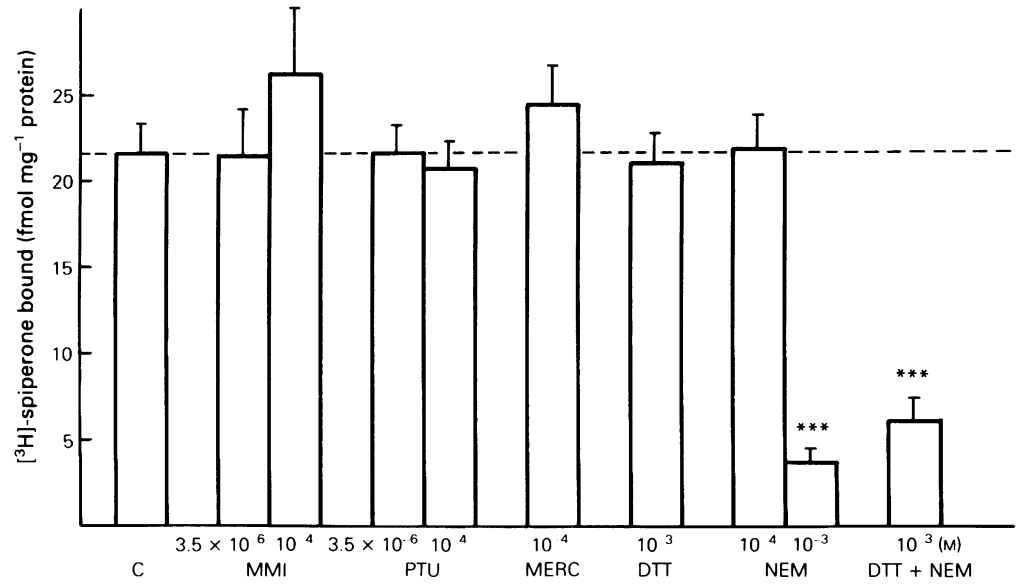


Figure 6 Effects of sulphydryl reagents on the specific binding of 0.54 nM [^{3}H]-spiperone to D_2-receptors in whole brain (less cerebellum and olfactory bulbs) membranes. The binding reaction was performed in the absence (C) or presence of SH reagents (for abbreviations see Figures 1 and 3); 10 μM sulpiride was the displacer. Results are means with vertical lines showing s.e.mean from $n = 6$ experiments performed in triplicate. *** $P < 0.001$, as compared to control.

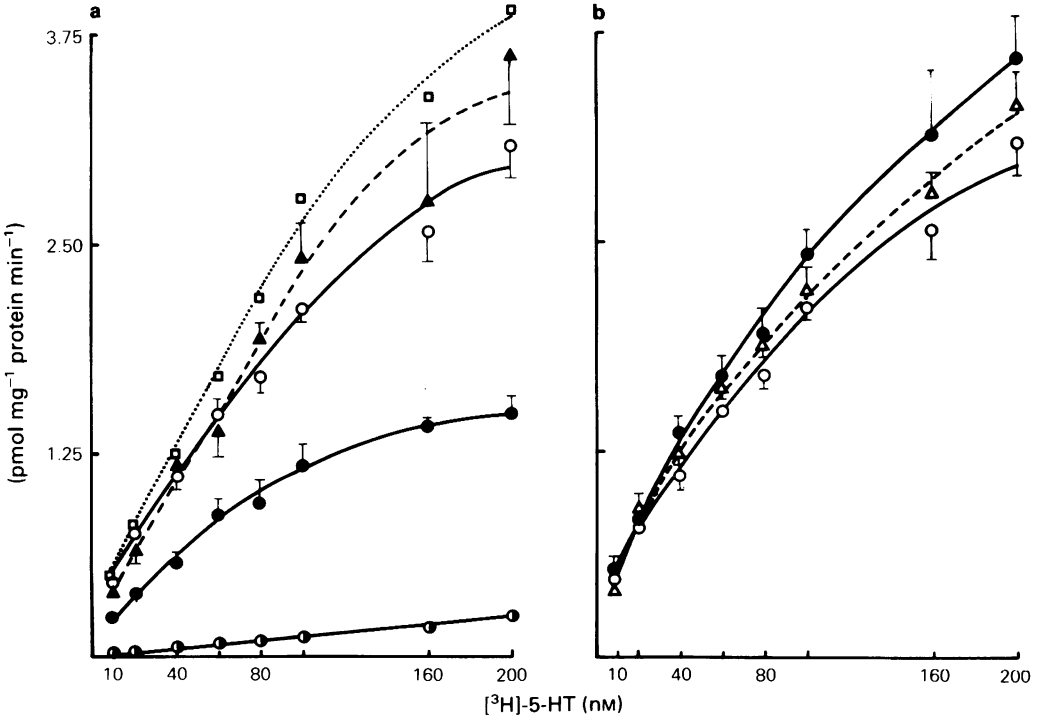


Figure 7 Uptake of [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) in whole brain (less cerebellum and olfactory bulbs) crude synaptosomes. Data points are means with vertical lines showing s.e.mean from 2–6 experiments performed in triplicate. Samples were preincubated at 37°C for 10 min in the absence (○) or presence of 1×10^{-3}M DTT (□), 1×10^{-4}M (●) and 1×10^{-3}M (◑) NEM, 1×10^{-3}M DTT + 1×10^{-4}M NEM (▲) (a), or 1×10^{-4}M MMI (●) and 1×10^{-4}M PTU (△) (b). For abbreviations see Figures 1 and 3. Uptake parameters are shown in Tables 1 and 2.

Table 1 Effects of sulphydryl reagents on synaptosomal high-affinity uptake of [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT)

Reagents	n	K_m	V_{max}
Controls	6	35.5 ± 4.4	2.2 ± 0.1
MMI 1×10^{-4} M	5	60.3 ± 19.9	3.4 ± 0.7**
PTU 1×10^{-4} M	5	55.9 × 4.0**	2.9 ± 0.1***
DTT 1×10^{-3} M	2	43.6 ± 6.3	2.5 ± 0.4
NEM 1×10^{-4} M	4	31.2 ± 9.5	1.2 ± 0.2***
NEM 1×10^{-3} M	2	27.6 ± 2.3	0.1 ± 0.03
NEM 1×10^{-4} M + DTT 1×10^{-3} M	3	52.3 ± 6.8	2.5 ± 0.4

K_m = nM; V_{max} = pmol mg^{-1} protein min^{-1}. values are means ± s.e.mean from *n* experiments. In each experiment, the P_2 fractions obtained from the entire brain (less cerebellum and olfactory bulbs) of two rats were used. Triplicate 440 µl aliquots were preincubated at 37°C for 10 min in the absence or presence of sulphydryl reagents, and then incubated for 4 min after the addition of 10–200 nM [^{3}H]-5-HT. High-affinity uptake parameters (Eadie-Hofstee analysis) were calculated in the range 10–80 nM.
MMI = methimazole; PTU = propylthiouracil; DTT = dithiothreitol; NEM = N-ethylmaleimide.
** $P < 0.01$; *** $P < 0.005$ or < 0.001, compared to controls.

biphasic kinetics, thus indicating a high (HA) and a low-affinity (LA) component for 5-HT. Membrane perturbations mostly altered the maximum velocity (V_{max}) of the uptake reaction, with only sporadic changes occurring in the affinity (K_m) for 5-HT of both HA (in the range 10–80 nM 5-HT), and LA (80–200 nM) uptake compartments (Tables 1 and 2).

Table 2 Effects of sulphydryl reagents on synaptosomal low-affinity uptake of [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT)

Reagents	n	K_m	V_{max}
Controls	6	113 ± 18	4.4 ± 0.5
MMI 1×10^{-4} M	5	155 ± 50	5.9 ± 0.8
PTU 1×10^{-4} M	5	207 ± 75	6.7 ± 1.5
DTT 1×10^{-3} M	2	176 ± 26	7.0 ± 0.5
NEM 1×10^{-4} M	4	98 ± 42	2.1 ± 0.4**
NEM 1×10^{-4} M + DTT 1×10^{-3} M	3	201 ± 112	7.1 ± 3.1

K_m = nM; V_{max} = pmol mg^{-1} protein min^{-1}. Values are means ± s.e.mean from *n* experiments. Low affinity uptake parameters (Eadie-Hofstee analysis) were calculated in the range 80–200 nM [^{3}H]-5-HT. For further details see Table 1.
** $P < 0.02$, compared to controls.

Discussion

In a companion paper (Vaccari, 1985) we have described the effects of a chronic antithyroid treatment with MMI on brain imipramine binding sites. Since MMI is a thiol compound (1-methyl-2-mercaptoimidazole), and alterations of either disulphide bridges and/or SH groups in the receptor subunit or on associated proteins, may be functionally significant for ligand-receptor interactions (Bennett & Snyder, 1976; Fillion & Fillion, 1980; Suen *et al.*, 1980; Wennogle *et al.*, 1981; Hamon *et al.*, 1983; Peterson & Bartfai, 1983; Woodruff *et al.*, 1984), the *in vitro* effects of membrane-active reagents on central [^{3}H]-imipramine binding sites and on additional putative receptors have been studied here in detail.

The present results have enabled us to discriminate between three groups of receptors, based upon their differential sensitivity to alterations in membrane homeostasis.

Group 1

In this group, reduction of disulphide bridges provoked by the two goitrogens MMI or PTU, or by DTT or mercaptoethanol consistently stimulated both [^{3}H]-imipramine binding and [^{3}H]-5-HT uptake processes. By contrast, covalent alkylation of SH groups with NEM or PCMB, or their oxidation with DTNB or cystine, descreased both processes. The inhibitory activity of NEM was prevented by DTT.

These results in brain membranes strictly reflected those occurring in human platelets (Davis, 1984b), thus implying a structural similarity for imipramine binding sites in the rat brain and human platelets.

A consistent part of the increased binding of [^{3}H]-imipramine induced by MMI and similar reagents involved the low-, rather than the high-affinity compartment for this ligand. This argues for a conformational modification of imipramine recognition sites (Dumbrille-Ross *et al.*, 1983; Davis, 1984a) taking place with a greater exposure and/or availability of accessible SH groups (Strauss, 1984). Although a *de novo* synthesis of the imipramine recognition subunit and of the carrier for 5-HT cannot be excluded, it seems more reasonable to assume that reduction of disulphide bridges facilitates the access of imipramine or 5-HT at otherwise 'cryptic' sites, whereas inactivation of SH groups would hinder ligands from reaching their normally accessible sites (Figure 8). Steric modifications, if any, in the receptor microenvironment may be related to changes in the membrane microviscosity. It is well known, that membrane viscosity regulates synaptic functions (Loh & Hitzemann, 1981). Furthermore, several receptors such as the dopamine (Le Fur *et al.*, 1983), and 5-HT-receptors (Heron *et al.*, 1980) in the central nervous system are unmasked and rendered available for binding

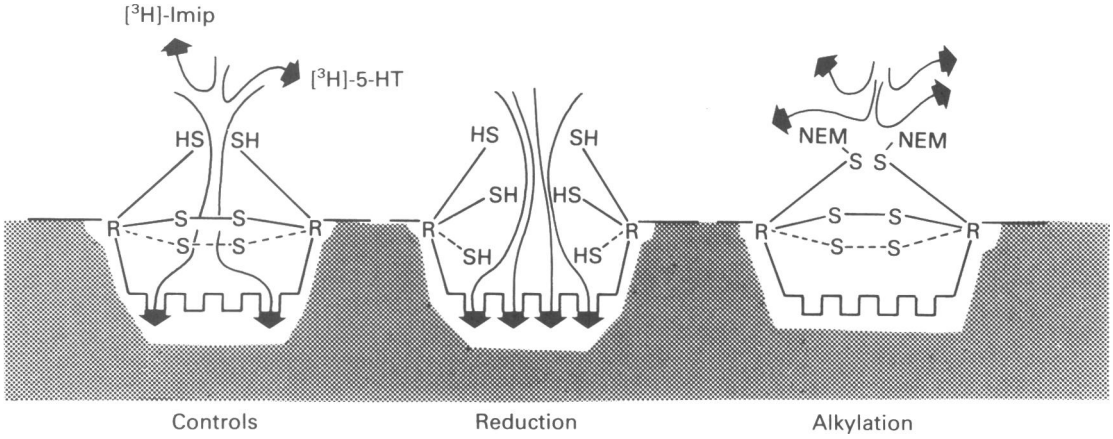


Figure 8 Speculative interpretation for thiol-dependent changes in [^{3}H]-imipramine ([^{3}H]-Imip) binding to brain membranes and in synaptosomal uptake of [^{3}H]-5-HT. Both Ligands (↓), which normally reach accessible sites only, can interact with additional, otherwise 'cryptic' sites, having been unmasked by the reduction of membrane-bound disulphide bridges. In contrast, alkylation by N-ethylmaleimide (NEM) of free SH groups and consequent steric alterations would hinder both ligands from occupying their normally accessible sites.

when the membrane fluidity is mildly increased. Incubations at 4°C for the binding of [^{3}H]-imipramine, and at 37°C for binding and uptake of [^{3}H]-5-HT might have influenced membrane viscosity differently, thus rendering disulphide bonds less or more accessible to thiol reagents. It was not feasible to repeat at 37°C or 4°C those assays whose optimal conditions were at 4°C or 37°C, respectively. [^{3}H]-imipramine binding in cerebral tissues is heat-labile, and consistent results cannot be obtained at 37°C, when a loss of receptors and a marked decrease in affinity occur (Kinnier *et al.*, 1981; Dumbrille-Ross *et al.*, 1983; Davis, 1984a). On the other hand, synaptosomal uptake of [^{3}H]-5-HT is almost totally abolished at 4°C. A similar problem arises with the binding reaction of [^{3}H]-5-HT, which develops very slowly at 4°C (Bennett & Snyder, 1976). Nevertheless there are indications that incubation of membranes at low or near-physiological temperatures does provoke similar, though quantitatively different effects. Thus, when platelet membranes were preincubated and then assayed at either 4°C or 23°C with NEM (which decreased [^{3}H]-imipramine binding), and dithioerythreitol (the stereoisomer of DTT, which enhanced the binding), the only temperature-related effect seen was a higher sensitivity of disulphide bonds to thiol reagents at 23°C, compared to that at 4°C (Davis, 1984b).

Taken together, the present results suggest that imipramine binding and 5-HT transport systems share a similarly reacting protein. This is of further relevance for the purported functional and anatomical association of imipramine binding sites with the 5-HT presynaptic uptake system (Langer *et al.*, 1980; Paul *et al.*, 1981; Barbaccia *et al.*, 1983). Preliminary experiments with the noradrenaline uptake system seem to indicate that changes in [^{3}H]-5-HT uptake were specific (unpublished results).

Group II

This group included binding of [^{3}H]-5-HT at 5-HT$_1$-receptors. Reduction of protein disulphide bonds, or reaction with SH groups both decreased the number of 5-HT$_1$-receptors, and this effect was not prevented by DTT.

NEM, a well-known inhibitor of [^{3}H]-5-HT binding in membranes from rat, horse, and human brains (Bennett & Snyder, 1976; Fillion & Fillion, 1980; Kienzl *et al.*, 1981; Hamon *et al.*, 1983) and DTT or mercaptoethanol, have been shown to inhibit similarly the binding of [^{3}H]-5-HT to the brain 'Serotonin Binding Protein' (SBP) (Tamir & Liu, 1982). Thus, SBP has some characteristics of 5-HT$_1$-receptors.

Group III

In this group, binding of [^{3}H]-spiperone to 5-HT$_2$- and D$_2$-receptors was affected little or not at all by protein-reactive agents, apart from millimolar concentrations of NEM. A low sensitivity of striatal D$_2$-receptors toward DTT or NEM also resulted from use of [^{3}H]-sulpiride as a ligand (Freedman *et al.*, 1982). As a matter of fact, the present results indicate that protein(s) functionally important for 5-HT$_1$- and 5-HT$_2$-receptors do respond differently to cleavage of

disulphide bridges, perhaps the result of structural differences.

Four main conclusions can be drawn from the present results:
(1) Protein(s) functionally significant for pre- and postsynaptic receptors can be selectively influenced by reagents which affect the [disulphide bond ⇌ SH] equilibrium. (2) Recognition sites for [^{3}H]-imipramine and the transport system for [^{3}H]-5-HT probably share the same protein. (3) Differences in sensitivity of 5-HT_1- and 5-HT_2-receptors argue for the existence of two separate recognition entities for 5-HT. (4) Membrane effects of thiol goitrogens may be relevant for side effects occurring in the therapy of hyperthyroidism (Jubitz, 1979).

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