*Br. J. Pharmac.* (1985), **86**, 475–482

# The binding of [$^3$H]-tiotidine to homogenates of guinea-pig lung parenchyma

J.C. Foreman, D.B. Norris*, T.J. Rising*, & S.E. Webber

Department of Pharmacology, University College London, Gower Street, London WC1E 6BT and Hoechst (U.K.) Ltd*, Walton Manor, Milton Keynes, MK7 7AJ

**1** By use of a rapid filtration assay, the binding of [$^3$H]-tiotidine to homogenates of guinea-pig lung parenchyma was found to be saturable and of a high affinity. Mean values for the $K_D$ and $B_{max}$ were calculated as 8.5 ± 1.5 nM and 28 ± 5 fmol mg$^{-1}$ protein respectively.

**2** The association and dissociation rate constants for [$^3$H]-tiotidine binding at 4°C were calculated to be 0.81 ± 0.06 μM min$^{-1}$ and 0.063 ± 0.005 min$^{-1}$ respectively, yielding a kinetically derived $K_D$ of 7.8 nM.

**3** A wide range of $H_2$-receptor agonist and antagonists displaced [$^3$H]-tiotidine binding from lung parenchyma homogenates in a biphasic manner.

**4** Examination of the first phase of the displacement of [$^3$H]-tiotidine yielded $K_i$ values for the antagonists tested similar to those found in other binding studies using this ligand and similar to $K_B$ values calculated for the antagonists in pharmacological studies.

## Introduction

The measurement of specific binding of high affinity labelled ligands to pharmacological receptors has been employed to obtain information on receptor numbers, distribution and the characteristics of agonist and antagonist binding. With this technique, histamine $H_1$-receptors have been characterized in rat brain (Chang *et al.*, 1978), in guinea-pig intestinal smooth muscle (Hill *et al.*, 1977) and in guinea-pig lung (Carswell & Nahorski, 1982) with [$^3$H]-mepyramine as ligand in each case. Until recently, the majority of investigations into the $H_2$-receptor have been carried out by pharmacological methods (Ash & Schild, 1966; Black *et al.*, 1972) or by studying the effects of $H_2$-compounds on histamine-stimulated adenylate cyclase (Baudry *et al.*, 1975; Hegstrand *et al* 1976). Exceptions to this include work with [$^3$H]-metiamide (Rosenfeld *et al.*, 1976) and [$^3$H]-histamine (Osband & McCaffrey, 1979), both of which proved unsuccessful in labelling the $H_2$-receptor. More recently, results from work with [$^3$H]-cimetidine have suggested a high affinity binding site for this ligand which is unrelated to the pharmacological receptor, but which recognizes the imidazole moiety of this compound (Rising *et al.*, 1980; Smith *et al.*, 1980). [$^3$H]-tiotidine is a more potent $H_2$-antagonist than either ranitidine or cimetidine (Yellin *et al.*, 1979) and has recently been shown to meet the criteria for labelling the $H_2$-receptor in membranes from the guinea-pig cerebral cortex (Gajtkowski *et al.*, 1983).

We describe here the specific binding of [$^3$H]-tiotidine to homogenates of guinea-pig lung parenchyma.

## Methods

### *Preparation of homogenates*

Guinea-pigs (300–500 g) were killed and the heart and lungs removed together and placed on ice. Lung parenchymal strips were prepared as previously described (Drazen & Schneider, 1978) and then homogenized in 10 ml of 150 mM sodium phosphate buffer pH 7.4, in a Polytron blender at setting 8 for 5 × 10 s bursts followed by a Potter homogenizer at the maximum setting for 5 × 10 s bursts. The homogenate obtained was centrifuged at 1000 *g* for 10 min at 4°C in a Sorvall RC-2B centrifuge. The pellet was discarded and the supernatant respun in the same centrifuge at 50000 *g* for 10 min. The pellet resulting from this spin was washed three times by resuspension in 20 ml phosphate buffer followed by recentrifugation at 50000 *g*. The pellet was finally resuspended in 20 ml phosphate buffer pH 7.4 and the protein concentra-

tion of the solution was determined by a modified Lowry method (Lowry et al., 1951). The volume of the solution was adjusted to give a protein concentration of 2.5 mg $ml^{-1}$. The suspension was either used immediately or rapidly frozen in liquid nitrogen and stored at −20°C for up to two weeks during which time there was no evidence of any deterioration in the binding properties of the preparation with respect to the ligand.

*Binding of [$^3$H]-tiotidine to homogenates of guinea-pig lung parenchyma*

One hundred μl of homogenate, containing approximately 250 μg protein was incubated in triplicate with various concentrations (1–24 nM) of [$^3$H]-tiotidine in the presence or absence of 0.1 μM unlabelled tiotidine in a total volume of 250 μl. All the constituents in the assay were made up in sodium phosphate buffer, 150 mM, pH 7.4. Incubations were carried out at 4°C for 40 min and terminated by the addition of 2 ml ice-cold phosphate buffer. The mixture was filtered under reduced pressure onto Whatman GF/B glass-fibre filters followed by three 3 ml washes with room-temperature buffer. The filters were dried in a stream of cold air and allowed to stand for 18 h in soluene/toluene scintillator followed by determination of radioactivity using liquid scintillation counting at an efficiency of 35–40%. Receptor specific binding was obtained by subtracting the binding of [$^3$H]-tiotidine in the presence of 0.1 μM unlabelled tiotidine from the uninhibited binding.

*Kinetics of [$^3$H]-tiotidine binding*

In experiments designed to study the association of [$^3$H]-tiotidine to guinea-pig lung parenchyma, 100 μl of homogenate was incubated in quadruplicate with [$^3$H]-tiotidine, 2 nM, in the presence or absence of unlabelled tiotidine, 0.1 μM, in a total volume of 250 μl. Incubations were carried out at 4°C for various times between 0 and 40 min. The reaction was terminated and the radioactivity in each sample determined by the methods described above. The dissociation of [$^3$H]-tiotidine binding was examined by incubating 100 μl of homogenate at 4°C for 40 min, with [$^3$H]-tiotidine, 2 nM, in a total volume of 250 μl. Unlabelled tiotidine, 0.1 μM, was then added to each tube and the radioactivity was determined at various times between 0 and 60 min thereafter by the methods described above.

*Inhibition of [$^3$H]-tiotidine binding*

One hundred μl of homogenate was incubated in duplicate in preliminary displacement experiments and in quadruplicate in detailed displacement experiments, with [$^3$H]-tiotidine, 2 nM, and various concentrations of displacing compound in a total volume of 250 μl. In each experiment some samples contained no displacing compound and were used to measure the total binding. The incubations were carried out for 40 min at 4°C and were terminated as described above. Radioactivity was determined as described above.

*Materials*

[$^3$H]-tiotidine was obtained from New England Nuclear, Southampton; histamine dihydrochloride from BDH, Poole, Dorset. The following drugs were kindly donated by the companies indicated: mepyramine maleate (May and Baker); burimamide, cimetidine and metiamide (Smith Kline and French); 2-methyl histamine, 4-methyl histamine, tiotidine, YM11170 (3-[[[2-[(diaminomethylene)amino]-4-thiazolyl]methyl]thio]-$N_2$-sulphamoyl propionamidine), ranitidine and dimaprit (Hoechst U.K.).

## Results

*Optimisation of incubation conditions*

In a preliminary displacement experiment, [$^3$H]-tiotidine, 2 nM, bound to homogenate prepared from guinea-pig lung parenchyma was displaced by unlabelled tiotidine, 0.1 nM to 10 μM. The displacement curve obtained was clearly biphasic (Figure 1). The initial displacement represented 200 c.p.m out of a total binding of 800 c.p.m (25%) and occurred with

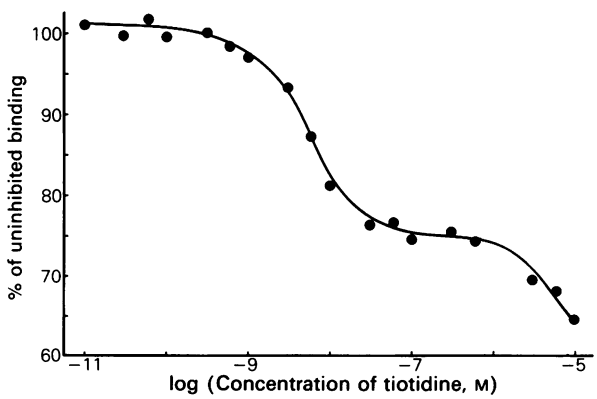


**Figure 1** Inhibition of [$^3$H]-tiotidine binding to homogenates of guinea-pig lung parenchyma by unlabelled tiotidine. [$^3$H]-tiotidine, 2 nM and unlabelled tiotidine were incubated with homogenate as described in the Methods section. The curve represents a single experiment and the points are means of duplicate determinations. Note that the ordinate scale, % of uninhibited binding, does not start at zero. 100% of uninhibited binding is equal to 820 c.p.m.

concentrations of unlabelled tiotidine between 0.1 nM and 50 nM. This initial displacement was considered likely to represent binding to the $H_2$-receptor as it occurred over the concentration range at which tiotidine is pharmacologically active (Yellin *et al.*, 1979). The second displacement occurred with concentrations of unlabelled tiotidine greater than 0.1 μM and was considered to be displacement of non-specific binding. Throughout the remaining experiments specific binding has been defined as the difference in binding obtained in the presence and absence of tiotidine, 0.1 μM, as this concentration of unlabelled ligand displaced all of the high affinity binding thought to be associated with the $H_2$-receptor but did not displace any of the lower affinity binding taken to represent non-receptor binding. Although in some ligand binding studies the displacing agent used has been a drug different from the labelled ligand we could see no reason for not using tiotidine itself as the displacing agent, especially since it gave a clearer distinction between specific and non-specific binding (compare Figures 1 and 5).

The association of specific [$^3$H]-tiotidine binding was examined at three different temperatures, 37°C, 20°C and 4°C. The rate of association was too rapid to measure at 37°C and 20°C, with all specific binding occurring within 1 min, whereas at 4°C specific binding reached a plateau after 25 min. Specific [$^3$H]-tiotidine binding was found to be linearly related to the final protein concentration over the range 0.2 to 2 mg ml$^{-1}$.

On the basis of these results, all subsequent experiments were carried out at 4°C with an incubation time of 40 min and a final protein concentration of 1 mg ml$^{-1}$. Specific binding was destroyed after heat-treating the protein in a boiling water bath for 10 min. Less than 0.5% of the total bound ligand bound to the filters in the absence of tissue.

### *Binding of [$^3$H]-tiotidine to homogenates of guinea-pig lung parenchyma*

The binding of [$^3$H]-tiotidine to homogenates of guinea-pig lung parenchyma, either alone or in the presence of unlabelled tiotidine, 0.1 μM, together with the difference curve taken to represent receptor-specific binding, is shown for a representative experiment in Figure 2. The binding not displaced by tiotidine, 0.1 μM, increased linearly with the concentration of [$^3$H]-tiotidine as is usually observed with non-specific binding, whilst the specific binding (Figure 2b) appeared to saturate over a concentration range of 0–24 nM and is of high affinity. Scatchard analysis (Figure 2c) of the data in Figure 2b yielded values of 39 fmol mg$^{-1}$ protein for the maximum binding of [$^3$H]-tiotidine ($B_{max}$) and 9.2 nM for the dissociation constant ($K_D$). Mean values ± s.e.mean

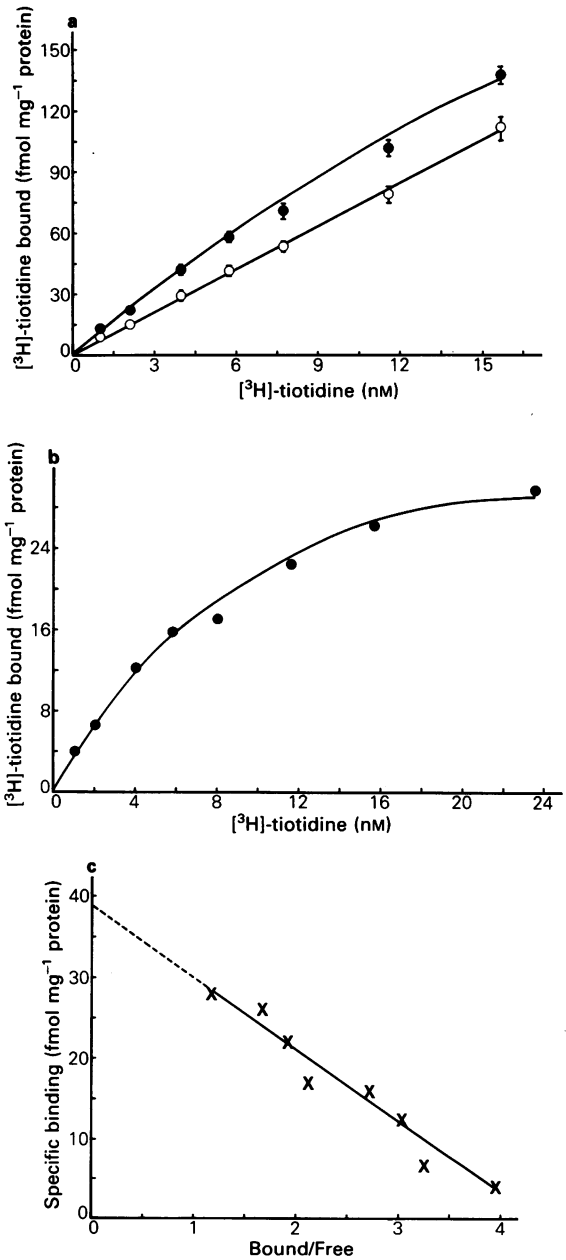


**Figure 2** Binding of [$^3$H]-tiotidine to homogenates of guinea-pig lung parenchyma. (a) Binding of [$^3$H]-tiotidine in the presence (○) or absence (●) of 0.1 μM unlabelled tiotidine. Points are means of triplicate determinations made on a single membrane preparation as described in the Methods section, vertical lines show s.e.mean. (b) Receptor specific binding of [$^3$H]-tiotidine, defined as that binding displaced by unlabelled tiotidine, 0.1 μM. (c) Scatchard transformation of the specific binding.

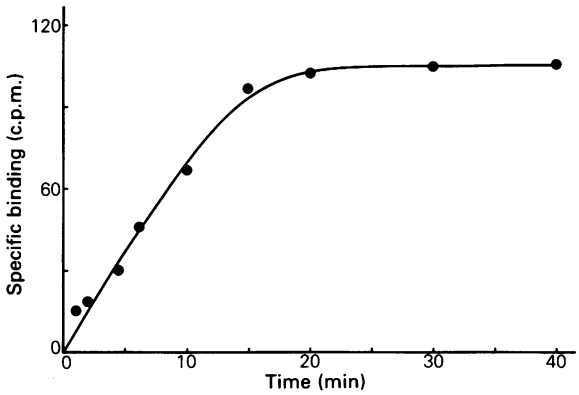


**Figure 3** Rate of association of [$^3$H]-tiotidine with the binding sites. Incubations were carried out on a single homogenate preparation as described in the Methods section. The specific binding at each time point was calculated as described in Figure 2.

for the $K_D$ and $B_{max}$, calculated from the Scatchard analysis of four separate experiments were 8.5 ± 0.5 nM and 28 ± 5 fmol mg$^{-1}$ protein respectively. The mean Hill coefficient was calculated as 0.94 ± 0.07 from Hill plots of the data.

*Kinetics of [$^3$H]-tiotidine binding*

Figure 3 shows the rate of association of specific [$^3$H]-tiotidine binding with guinea-pig lung parenchyma homogenates. Specific binding increased over a time period of 20 min with a doubling time for association of approximately 8 min. The following equation was applied to the data in Figure 3:

$$\ln\left[\frac{x}{x - SB}\right] = K_{obs} \cdot t$$

x is the specific binding at equilibrium, SB the specific binding at time $t$, $K_{obs}$ the observed association rate and $t$ is the time of incubation. The straight line produced has a slope equal to $K_{obs}$. As the dissociation rate constant, $k_2$, is known from independent experiments, the true association rate constant, $k_1$, can be calculated from the equation:

$$K_{obs} = k_1(L) + k_2$$

where (L) is the ligand concentration used in the assay. Using the methods described above, a mean value (± s.e.mean) for the true association rate constant from four separate experiments was calculated to be 0.81 ± 0.06 μM min$^{-1}$.

The dissociation of [$^3$H]-tiotidine binding occurred over a time period of 30 min with a half-time for dissociation of approximately 9 min (Figure 4). The following equation was applied to the data in Figure 4;

$$\ln\left(\frac{SB}{SBeq}\right) = k_2 t$$

SBeq is the specific binding at equilibrium, SB the specific binding at each time point and $t$ is the time of incubation. A straight line was produced with a slope equal to the dissociation rate constant, $k_2$. Using this method, a mean value for the dissociation rate constant from four separate experiments was calculated as 0.063 ± 0.005 min$^{-1}$. The kinetically derived value for

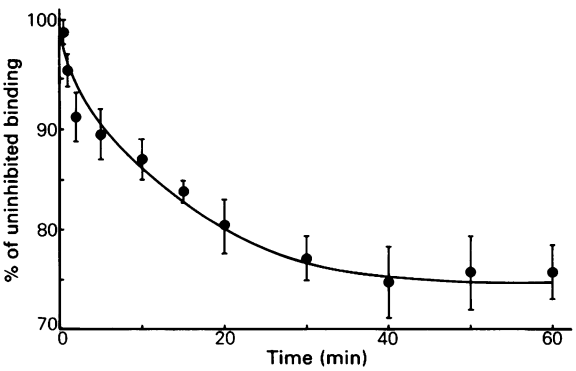


**Figure 4** Dissociation of bound [$^3$H]-tiotidine. Incubations were carried out on a single membrane preparation as described in the Methods section. Points are means of quadruplicate determinations; vertical lines show s.e.mean. Note that the ordinate scale, % of the uninhibited binding, does not start at zero. 100% of uninhibited binding (the absence of the displacing agent: unlabelled tiotidine, 0.1 μM) is equal to 720 c.p.m.

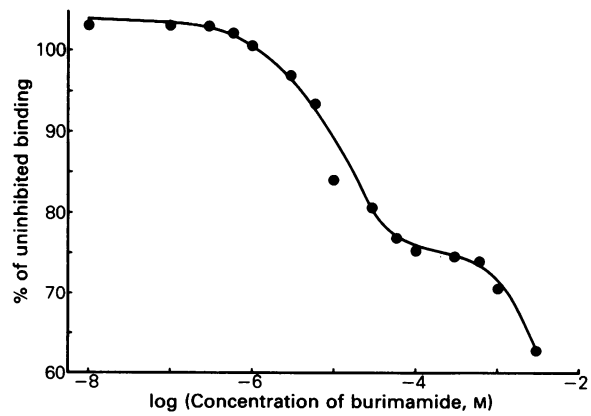


**Figure 5** Inhibition of [$^3$H]-tiotidine binding by burimamide. Incubations were carried out on a single membrane preparation as described in the Methods section. The points are means of duplicate determinations. Note that the ordinate scale, % of uninhibited binding, does not start at zero. The abscissa scale is the log of the molar concentration of burimamide. 100% of uninhibited binding (no displacing ligand) is equal to 800 c.p.m.

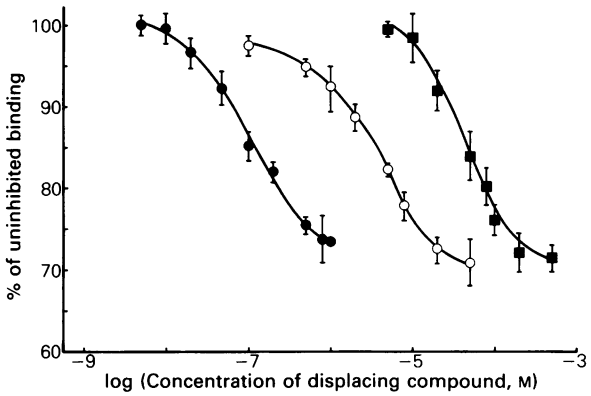


**Figure 6** Inhibition of [$^3$H]-tiotidine binding to homogenates of guinea-pig lung parenchyma by YM11170, burimamide and histamine. [$^3$H]-tiotidine and antagonists were incubated with homogenate as described in the Methods section. Each curve represents a single experiment performed in quadruplicate and the points are means with s.e.mean shown by vertical lines. (●) YM11170; (○) burimamide, (■) histamine. Note that the ordinate scale, % of the uninhibited binding, does not start at zero. 100% of uninhibited binding (no displacing agent) is equal to 700–900 c.p.m.

the equilibrium dissociation constant, $K_D$, calculated as $k_2/k_1$, was found to be 7.8 nM.

*Inhibition of [$^3$H]- tiotidine binding*

To confirm that [$^3$H]-tiotidine does specifically label the $H_2$-receptor, we examined the inhibition of [$^3$H]-tiotidine binding to homogenates of guinea-pig lung parenchyma by several compounds considered to be selective agonists and antagonists at histamine $H_2$-receptors and by mepyramine and 2-methyl histamine, which have their primary effect on histamine $H_1$-receptors. In a series of preliminary competition experiments, all the $H_2$-compounds tested displaced [$^3$H]-tiotidine binding, producing competition curves similar to the curve obtained using unlabelled tiotidine as the displacing agent (Figure 1). All of the competition curves were clearly biphasic: a typical example using the $H_2$-antagonist, burimamide, being shown in Figure 5. For each $H_2$-compound, the first phase displacement was studied in detail as this was taken to represent displacement of [$^3$H]-tiotidine from the $H_2$-receptor, occurring over the pharmacologically active concentration range of each competing $H_2$-compound (Black *et al.*, 1972;Black *et al.*, 1973; Brimblecombe *et al.*, 1975; Yellin *et al.*, 1979; Bradshaw *et al.*, 1979; Gajtkowski *et al.*, 1983). Figure 6 shows the first phase displacement of bound [$^3$H]-tiotidine by histamine, burimamide and YM11170, a selective antagonist at histamine $H_2$-receptors (Takeda *et al.*, 1982). The maximum first phase displacement by each competing $H_2$-compound examined was shown, in a non-paired *t* test, not to be significantly different from that obtained in the presence of unlabelled tiotidine, 0.1 μM, ($P > 0.05$ in each case). The non-specific binding routinely accounted for 70–75% (500–600 c.p.m) of the total binding (700–900 c.p.m). The compounds which have an effect primarily on $H_1$-receptors did not displace [$^3$H]-tiotidine binding even when used at concentrations of 100 μM.

For each $H_2$-compound the concentration of drug

**Table 1** Inhibition constants ($K_i$) for [$^3$H]-tiotidine binding to homogenates of guinea-pig lung parenchyma

| *Compound* | $K_i$(μM) | *Hill slope* |
|---|---|---|
| *$H_2$-antagonists* | | |
| Tiotidine | 0.0064 ± 0.0005 | 1.08 ± 0.06 |
| YM11170 | 0.041 ± 0.011 | 1.05 ± 0.06 |
| Ranitidine | 0.81 ± 0.28 | 1.21 ± 0.10 |
| Cimetidine | 0.82 ± 0.15 | 1.03 ± 0.04 |
| Metiamide | 0.76 ± 0.34 | 1.03 ± 0.10 |
| Burimamide | 4.2 ± 0.8 | 0.94 ± 0.05 |
| *$H_2$-agonists* | | |
| Histamine | 34 ± 15 | 1.06 ± 0.16 |
| Dimaprit | 19 ± 3 | 0.95 ± 0.19 |
| 4-Methyl histamine | 120 ± 50 | 1.05 ± 0.13 |
| *$H_1$-antagonist* | | |
| Mepyramine | >100 | |
| *$H_1$-agonist* | | |
| 2-Methyl histamine | >100 | |

The results are expressed as mean ± s.e.mean where $n = 4$.

that inhibited the specific binding of [$^3$H]-tiotidine by 50% ($IC_{50}$) was obtained from the first phase displacement curves and then converted to a $K_i$ value using the equation;

$$K_i = \frac{IC_{50}}{1 + [L]/K_D}$$

where [L] is the concentration of [$^3$H]-tiotidine used in the assay and $K_D$ is the equilibrium dissociation constant of [$^3$H]-tiotidine calculated from saturation experiments. These calculations assume that the interaction of the competing compound with the receptor can be described by the Law of Mass Action and that equilibrium conditions were achieved. For a simple interaction of one mole of antagonist with one mole of receptor at equilibrium, the Hill slope from a Hill plot of the data will be unity. The $K_i$ values and accompanying Hill slopes for the $H_2$-compounds are summarized in Table 1.

## Discussion

The work presented in this paper shows that the specific binding of [$^3$H]-tiotidine to homogenates of guinea-pig lung parenchyma is reversible, of a high affinity and saturable over a concentration range of 1 to 24 nM. The $K_D$ of 8.5 nM calculated for [$^3$H]-tiotidine from saturation experiments in this study, is similar to the $K_D$ of 17 nM derived for this ligand in a study using guinea-pig cerebral cortex (Gajtkowski *et al.*, 1983). However, the maximum number of binding sites obtained in the lung preparation (28 fmol mg$^{-1}$ protein) is significantly lower than obtained in the cortex (105 fmol mg$^{-1}$ protein). This may reflect a difference in receptor numbers between the two tissues or could suggest that a number of receptor sites were lost in the preparation of the homogenates of lung parenchyma. The value of 7.8 nM for the equilibrium dissociation constant ($K_D$) calculated from kinetic experiments is very similar to the $K_D$ value of 8.5 nM obtained in the saturation experiments.

The criteria of reversibility, saturability and high affinity of ligand binding are far from being decisive in proving that a ligand is labelling a receptor site, since they are also valid for an acceptor site. There are two further criteria that must be fulfilled before a binding site may be considered as a receptor site, namely the displacement of the specifically bound ligand by related compounds for the same receptor type and correlation of the affinity constants obtained in the binding experiments with those obtained from pharmacological studies. In the *in vitro* binding assay the affinity of various agonists and antagonists can be determined by ligand displacement experiments. The affinity should be determined not only for drugs which act at different pharmacological receptors but also for drugs belonging to the same pharmacological class as the labelled ligand but with different chemical structures. If this type of experiment is not attempted then problems in identifying receptors will arise. This was clearly the case when [$^3$H]-cimetidine was reported to label the histamine $H_2$-receptor in rat brain (Burkard, 1978; Kendall *et al.*, 1980), where it was later realized that [$^3$H]-cimetidine was in fact labelling an imidazole recognition site which was completely unrelated to the pharmacological receptor (Rising *et al.*, 1980; Smith *et al.*, 1980). In displacement experiments it is very important to include compounds of the same pharmacological class but with substantial variation of chemical structure to establish that the ligand is labelling a specific pharmacological receptor. In this present study, the binding of [$^3$H]-tiotidine to homogenates of guinea-pig lung parenchyma was displaced by several chemically different compounds but all with pharmacological activity at histamine $H_2$-receptors. The specific binding of tiotidine, which does not contain an imidazole group, was displaced by both agonists and antagonists which contain an imidazole moiety (e.g. cimetidine and histamine) and also compounds which do not contain an imidazole moiety (e.g. ranitidine and dimaprit).

In a series of preliminary experiments, the displacement curves for both $H_2$-agonists and antagonists were biphasic (Figure 1 and 5). The second phase of the displacement occurred at higher concentrations of the $H_2$-compounds than are normally associated with their pharmacological actions at the $H_2$-receptor and this is probably due to displacement of bound [$^3$H]-tiotidine from non-specific binding sites in the homogenate. It should be remembered that this is an homogenate of a very heterogeneous tissue. When the first phase of the displacement for both $H_2$-agonists and antagonists was examined in more detail, the amount of non-specific or second phase binding was, in each case, not significantly different from the amount of binding insensitive to unlabelled tiotidine 0.1 μM. This has been used to justify the use of this concentration of unlabelled tiotidine to define the level of non-specific binding in both the saturation experiments (Figure 2) and the kinetic experiments (Figures 3 and 4). Further, it also shows that all the $H_2$-compounds tested inhibit specific [$^3$H]-tiotidine binding to the same extent. None of the Hill slopes calculated for the first phase displacement are significantly different from one, which is consistent with a simple Mass Action equilibrium between [$^3$H]-tiotidine and competing compound with a single set of binding sites (Table 1). The $K_i$ values calculated from inhibition of [$^3$H]-tiotidine bnding in guinea-pig lung parenchyma and cerebral cortex (Table 2) never differ by as much as an order of magnitude and the correlation between these $K_i$ values suggests a similar binding site for [$^3$H]-tiotidine in these two tissues. This

**Table 2** Comparison of the activity of $H_2$-compounds

| *Compound* | *[$^3$H]-tiotidine binding* *Lung parenchyma* $K_i$(μM) | *Cerebral** *cortex* $K_i$(μM) | *Lung parenchymal** strip* $K_D$(μM) | *Right atrium†* $K_D$(μM) | |
|---|---|---|---|---|---|
| Tiotidine | 0.0064 | 0.034 | 0.0037 | 0.015 | Yellin *et al.* (1979) |
| YM11170 | 0.041 | 0.012 | 0.05 | 0.035 | Gajtkowski *et al.* (1983) |
| Ranitidine | 0.81 | 0.39 | 0.35 | 0.063 | Bradshaw *et al.* (1979) |
| Cimetidine | 0.82 | 0.47 | 0.70 | 0.81 | Brimblecombe *et al.* (1979) |
| Metiamide | 0.76 | 0.51 | 0.91 | 0.92 | Black *et al.* (1973) |
| Burimamide | 4.2 | 3.0 | 5.1 | 7.8 | Black *et al.* (1972) |
| Histamine | 34.0 | 43.0 | — | 0.95 | Gajtkowski *et al.* (1983) |
| Dimaprit | 19.0 | 44.0 | 17.4 | 5.9 | Gajtkowski *et al.* (1983) |
| 4-Methyl histamine | 120.0 | 270 | 4.0 | 2.5 | Gajtkowski *et al.* (1983) |

*Data from Gajtkowski *et al.* (1983); **data from Foreman *et al.* (1985); †From reference indicated.

conclusion is supported by the similar $K_D$ values obtained for the ligand in the two tissues. It should be pointed out, however, that the binding study was also able to discriminate between compounds with their main action at $H_1$ rather than $H_2$-receptors. The $H_2$-agonist, 4-methyl histamine (Black *et al.*, 1972), was able to displace, whereas 2-methyl histamine, which acts primarily on $H_1$-receptors (Black *et al.*, 1972) did not displace. Although tiotidine is not itself an effective $H_1$-antagonist, it was considered to be worthwhile examining the effect of an $H_1$-antagonist on tiotidine binding. The $H_1$-antagonist, mepyramine was, as expected, a very weak inhibitor of [$^3$H]-tiotidine binding in this study, producing less than 10% displacement of specific binding at 100 μM. In contrast, when acting at $H_1$-receptors mepyramine has $K_i$ values of 1.2 nM and 0.56 nM for inhibition of [$^3$H]-mepyramine binding to guinea-pig brain (Hill *et al.*, 1978) and guinea-pig lung parenchyma (Carswell & Nahorski, 1982) respectively.

Together with the characterization of the displacement of bound radioactive ligand, it is necessary to confirm that the observed binding is related to a particular pharmacological receptor and to try to correlate the drug affinity found in the binding assay for a series of compounds with the drug potency obtained in pharmacological tests. The importance of such an approach has been clearly demonstrated by the early work on specific binding of $^3$H-catecholamines which appeared not only to bind to the expected receptor but also to a heterogeneous group of sites including other receptor sites, sites involved in oxidative processes and a variety of other membrane-located acceptor sites (Lefkowitz *et al.*, 1976).

We have compared the inhibition constants obained in this study for a variety of $H_2$-compounds, with $K_B$ values calculated from inhibition of the action of dimaprit in lung parenchyma reported in the preceding paper (Foreman *et al.*, 1985) and from the inhibition of the chronotropic activity of histamine in guinea-pig right atria (Table 2). For the $H_2$-antagonists there is a correlation between the inhibition constants obtained in the present study and the dissociation constants obtained in both the isolated lung strip and the right atria ($r = 0.97$, $P < 0.01$ and $r = 0.95$, $P < 0.01$ respectively). Despite this, the constants shown for tiotidine are not in particularly close agreement, with values ranging from 3.7 nM to 34 nM. Nevertheless, the values obtained in the lung experiments are similar (6.4 nM and 3.7 nM) but these differ from the values obtained in the cerebral cortex and right atria studies. No explanation for this is apparent at present but it should be pointed out that the other $H_2$-antagonists give comparable results. However, ranitidine had a significantly different dissociation constant in the right atria studies compared with the other systems (Table 2). The reason for this discrepancy is unknown but we did note in our own experiments that when ranitidine was used as the displacing agent, there was a less clear plateau between displacement of specific and non-specific binding which may have led to some error in the estimation of $IC_{50}$ values in the various other studies quoted. Ranitidine has previously been shown to be 4 to 10 times more potent than cimetidine at the $H_2$-receptor (Bradshaw *et al.*, 1979) but the lung and cerebral cortex studies shown in Table 2 indicate a smaller potency difference. The $H_2$-agonists do not show such a good correlation between the three systems as the antagonists. The $K_i$ values obtained for histamine, dimaprit and 4-methyl histamine in the binding experiments are between 4 and 50 times higher than the corresponding $EC_{50}$ (50% effective concentration) values obtained for stimulation of the right atria and relaxation of the stimulated lung strip. This, however, is not unusual since inhibition of [$^3$H]-tiotidine binding is an index of the affinity of a compound for the receptor site, whereas the $EC_{50}$ is additionally depen-

dent on the efficacy of the agonists and the extent of the receptor reserve.

In conjunction with the preceding paper (Foreman *et al.*, 1985) we have demonstrated an $H_2$-receptor by binding studies whose interaction with agonists and antagonists quantitatively matches the pharmacological potencies of these drugs on the contractile responses of the lung strip.

S.E.W. holds a C.A.S.E. Award from S.E.R.C. with Hoechst (U.K.) Ltd as industrial sponsors.

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(*Received March 23, 1985.*
*Revised May 11, 1985.*
*Accepted July 1, 1985.*)