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[^{3}H]-(+)-N-methyl-4-methyldiphenhydramine, a quaternary radioligand for the histamine H_1-receptor

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1 A series of derivatives of 4-methyldiphenhydramine have been examined as potential quaternary radioligands for the histamine H_1-receptor.

2 [^{3}H]-(+)-N-methyl-4-methyldiphenhydramine ([^{3}H]-QMDP), 83 Ci mmol^{-1}, was synthesized by methylation of the tertiary analogue and purified by high-voltage electrophoresis.

3 [^{3}H]-QMDP bound to H_1-receptors in a washed homogenate from guinea-pig cerebellum with an affinity constant, K_a, of $1.14 \pm 0.03 \times 10^9$ M^{-1}. The proportion of non-specific binding of 0.3–0.6 nM [^{3}H]-QMDP, defined by 0.4 μM mepyramine, was usually in the range 15–45%, depending on the method of measurement of binding. The affinity of [^{3}H]-QMDP was similar in guinea-pig cerebellum, cerebral cortex and hippocampus, but was lower, 1.4×10^8 M^{-1}, in rat cerebral cortex.

4 Evidence was obtained for the presence of a secondary, non-muscarinic, binding site for [^{3}H]-QMDP in guinea-pig cerebellum, approximate K_a 1.5×10^7 M^{-1}, accounting for *circa* 4% of the total binding of 1 nM [^{3}H]-QMDP.

5 There was a very good correlation between the affinities of 15 compounds for the H_1-receptor determined from inhibition of [^{3}H]-QMDP binding and from inhibition of [^{3}H]-mepyramine binding.

6 The potential utility of [^{3}H]-QMDP for studies of H_1-receptors in the plasma membrane of cells in culture is discussed.

Introduction

Several compounds have been used successfully as radioligands for the histamine H_1-receptor (reviewed in Schwartz *et al.*, 1986). Of these [^{3}H]-mepyramine (Hill *et al.*, 1977; Tran *et al.*, 1978), which seems to be less prone to bind to secondary sites than [^{3}H]-doxepin (Tran *et al.*, 1981; Taylor & Richelson, 1982), appears to be the ligand of choice for most purposes, although in certain circumstances the iodinated ligand [^{125}I]-iodobolpyramine (Korner *et al.*, 1986) may have distinct advantages. However, all of these compounds are tertiary amines and are presumably able to cross cell membranes. Consequently they are much less well suited for studies of the binding characteristics of histamine H_1-receptors on the plasma membrane of intact cells. The problems that can arise with [^{3}H]-mepyramine in experiments of this kind have been described by Maloteaux *et al.* (1983). As a first step towards the development of an H_1-ligand suitable for use with intact cells we have investigated the properties of a series of quaternary amines.

In general, quaternisation of H_1-antihistamines leads to a fall in the affinity for the H_1-receptor (Nauta & Rekker, 1978), although not necessarily great, and a rise in the affinity for the muscarinic receptor. This is well demonstrated by the properties of the quaternary N-methyl derivative of promethazine, thiazinamium chloride, a bronchodilator drug which is practically equipotent in inhibiting the binding of [^{3}H]-mepyramine to H_1-receptors and [^{3}H]-quinuclidinyl benzilate to muscarinic receptors (Muth *et al.*, 1985). Aprobit, N-2-hydroxyethyl-promethazine, has similar properties (Albanus *et al.*, 1961). A more promising starting point for the development of a quaternary radioligand appears to be in the series of compounds related to 4-methyldiphenhydramine. The correlation between structure and H_1-antihistamine and antimuscarinic activity in this group has been studied in detail (Harms *et al.*, 1975; Nauta & Rekker, 1978) and at least one quaternary derivative in this series, pirdonium, has an equal or higher affinity for the

H_1-receptor than its tertiary precursor, while retaining a good H_1 : muscarinic selectivity. A review of the properties of some of these compounds has led us to prepare and examine the properties of [^{3}H]-(+)-N-methyl-4-methyldiphenhydramine ([^{3}H]-QMDP) as a quaternary radioligand for the histamine H_1-receptor. Some of these results have been presented in preliminary form to the British Pharmacological Society (Treherne & Young, 1986).

Methods

Preparation of homogenates of guinea-pig and rat brain tissue

Guinea-pigs (Dunkin–Hartley strain, males, 300–600 g) were killed by cervical dislocation and brain regions (cerebellum, cerebral cortex and hippocampus) dissected out on ice. Tissues were either homogenized immediately or stored in Krebs-Henseleit solution at −20°C for up to 6 months. There is no obvious change in the properties of [^{3}H]-mepyramine binding to tissue stored in this way (Aceves *et al.*, 1985). Tissues were homogenized in 7–10 volumes of ice-cold 50 mM Na–K phosphate buffer (37.8 mM Na_2HPO_4, 12.2 mM KH_2PO_4), pH 7.5, by use of a teflon-glass homogenizer with a motor-driven pestle (300 r.p.m., 5 up and down strokes) and then centrifuged at 17,000 *g* for 30 min. The pellet was resuspended in phosphate buffer and then centrifuged again at 17,000 *g* for 30 min. The resulting pellet was resuspended in buffer to give a protein concentration of 6–10 mg ml^{-1} and stored at −20°C until required for use. Protein was determined essentially as described by Lowry *et al.* (1951) with bovine serum albumin as standard.

Homogenates of rat (Sprague–Dawley strain, males, 300–400 g) cerebral cortex were prepared in the same way as the homogenates of guinea-pig tissues (final protein concentration 5–8 mg ml^{-1}). In some experiments with [^{3}H]-QMDP the rat homogenate was subjected to further washing following the procedure of Steinberg *et al.* (1985). The homogenate was thawed, incubated at 37°C for 30 min and then diluted with 1.7 volumes of 50 mM phosphate buffer containing 1 mM EGTA (ethylene glycol-*bis*-(β-aminoethyl)-N,N,N′,N′-tetra-acetic acid). The diluted homogenate was centrifuged at 125,000 *g* for 20 min and the pellet then resuspended in the same volume of 50 mM Na–K phosphate/1 mM EGTA buffer. These steps were repeated 4 times, except that the final pellet was resuspended in a volume of buffer such that the final protein concentration was 5–6 mg ml^{-1}. The washed homogenate was used immediately.

Measurement of ^{3}H-ligand binding

Incubations in 50 mM Na–K phosphate buffer, pH 7.5, contained the ^{3}H-ligand, inhibitor (where appropriate) and homogenate (normally 0.30–0.35 mg protein) in a total volume of 1.00–1.11 ml (5–6 replicates at each inhibitor concentration, 20–25 replicates in the absence of inhibitor, spread through the experiment). Equilibration was for 60 min at 30°C and was terminated by addition of 4 ml ice-cold buffer. The mixture was filtered immediately through Whatman GF/B glass fibre filters using either a Shearline R&D (Cambridge, U.K.) 10-place filtration block (25 mm diameter filters) or a Brandel (Gaithersburg, Md, U.S.A.) 24-place cell harvester (effective filter diameter 20 mm). The filters were transferred to scintillation vials and 10 ml scintillator added (25 mm filters) or to insert vials and treated with 4.8 ml scintillator (20 mm filters) and allowed to stand overnight before determination of the tritium by liquid scintillation counting. The scintillator was either Unisolve E (Koch-Light)/water, 95 : 5 (v/v) or Quickszint 212 (Zinnser)/water, 95 : 5 (v/v). In the great majority of the experiments with [^{3}H]-mepyramine and [^{3}H]-QMDP the glass-fibre filters were pre-soaked in 0.3% (v/v) aqueous polyethylenimine (PEI) for 5–16 h. Filter binding of [^{3}H]-QMDP, measured in the absence of homogenate, was usually 3–7% of the total binding in the presence of homogenate.

In a number of the experiments in which inhibition of ^{3}H-ligand binding was measured, a check on the level of non-specific binding was made by including 5–6 incubations with 2 μM promethazine (in experiments with [^{3}H]-mepyramine), 1 μM methylatropine ([^{3}H]-NMS) or 0.4 μM mepyramine or 1 μM temelastine ([^{3}H]-QMDP). The final concentration of radioligand in each experiment was determined by counting an aliquot of the equilibration medium (buffer + ^{3}H-ligand) kept in the incubation tubes (scintillation vial inserts, Hughes & Hughes, or 75 × 12 mm tubes, Sarstedt) for approximately 60 min.

Analysis of binding data

Curves of the amount of [^{3}H]-QMDP bound *versus* the concentration of [^{3}H]-QMDP, after subtraction of the binding of [^{3}H]-QMDP not inhibited by 0.4 μM mepyramine, were fitted to the equation:

$$[^3\text{H}]\text{-QMDP bound} = \frac{B_{max} \times A^n}{A^n + EC_{50}^n}$$

where B_{max} is the maximum binding of [^{3}H]-QMDP, A is the concentration of [^{3}H]-QMDP, EC_{50} is the concentration of [^{3}H]-QMDP giving

half-maximal binding, and n is the Hill coefficient. The best-fit values of B_{max}, EC_{50} and n were obtained by weighted non-linear regression analysis using the Harwell library routine VB01A, as implemented on the Cambridge IBM 3081/3084. All points were weighted by the reciprocal of the variance associated with them.

Curves of inhibition of ^{3}H-ligand binding were fitted in the same way to the equation:

% of the uninhibited binding of the ^{3}H-ligand

$$= \frac{100 - NS}{((A/IC_{50})^n + 1)} + NS$$

with IC_{50}, n and NS as variables. A is the concentration of inhibitor, IC_{50} the inhibitor concentration giving 50% inhibition of the inhibitor-sensitive binding, n the Hill coefficient and NS the percentage of the binding of the ^{3}H-ligand insensitive to the inhibitor ('non-specific' binding). The affinity constant of the inhibitor was calculated from the relationship: $K_a = ([^3\text{H-ligand}] \times K_L + 1)/IC_{50}$, where K_L is the affinity constant of the ^{3}H-ligand. K_L was taken to be 1.6×10^9 M^{-1} (guinea-pig) or 3.5×10^8 M^{-1} (rat) for [^{3}H]-mepyramine (Aceves *et al.*, 1985), 6×10^9 M^{-1} for [^{3}H]-NMS (from measurement of NMS inhibition of [^{3}H]-NMS binding to guinea-pig cerebral cortical homogenate) and 1.0×10^9 M^{-1} for [^{3}H]-QMDP (the mean of 5 different methods of measurement). In the special case where the inhibitor is the unlabelled ^{3}H-ligand, the expression for K_a simplifies to $K_a = 1/(IC_{50} - [^3\text{H-ligand}])$.

A Hill equation (logistic equation) was assumed for binding in fitting inhibition curves in order to check that the Hill coefficient, n, did not differ significantly from unity. To check the validity of K_a values calculated from best-fit values of IC_{50} obtained in this way, a number of the inhibition curves were also fitted with n constrained to be unity. In no case did the value of K_a obtained differ significantly from that obtained with n as an unconstrained variable. The mean difference ± s.e. mean in the estimates of K_a from 28 curves which were fitted both ways was 2.5 ± 0.5%. This is less than the error on the value determined for the specific activity of [^{3}H]-QMDP, 83 ± 5 Ci mmol^{-1} (see under Results).

Organ bath experiments

Longitudinal muscle strips from guinea-pig small intestine were suspended in 10 ml Krebs–Henseleit solution (mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5 and D-glucose 5.5) gassed with 95% O_2/5% CO_2 at 37°C in a conventional organ bath. Agonist-induced contractions were recorded isotonically. Antagonists were added to the Krebs–Henseleit solution in the reservoir and were allowed to equilibrate with the muscle strips for 45–60 min. Antagonist affinities were calculated from the relationship: concentration-ratio = $A \times K_a + 1$, where A is the concentration of the antagonist, K_a its affinity constant and the concentration-ratio is the ratio of the concentration of the agonist required for a given response in the presence of antagonist to the concentration required for the same response in the absence of antagonist.

High-voltage electrophoresis

High-voltage electrophoresis was carried out on a Shandon–Southern Model L24 apparatus using Whatman No 1 (for analytical purposes) or Whatman 3MM (for preparative work) paper and 0.2 M borate buffer, pH 8.6 (0.15 M boric acid, 0.05 M Na borate). The applied voltage was 3 kV (40 V cm^{-1}). The paper was allowed to air-dry and radioactivity detected either with a Packard Model 7200 radiochromatogram scanner or by liquid scintillation counting of 5 mm sections of a 1 cm strip running the length of the paper. Non-labelled compounds run as standards (compounds II and III and choline) were visualised by spraying with Dragendorff's reagent (Thies & Reuther, 1954) or iodoplatinate (Marchbanks, 1968). In analytical runs with [^{3}H]-QMDP, unlabelled (±)-QMDP was usually added as a carrier.

Drugs

[^{3}H]-mepyramine (26 and 28 Ci mmol^{-1}) and [^{3}H]-N-methylscopolamine ([^{3}H]-NMS) (80 Ci mmol^{-1}) were obtained from Amersham International. Carbachol (carbamylcholine chloride), choline chloride and histamine dihydrochloride were purchased from B.D.H. Chemicals Ltd; mepyramine maleate from May & Baker Ltd; chlorpromazine hydrochloride, methylatropine bromide, promethazine hydrochloride and verapamil hydrochloride from Sigma; (+)-tubocurarine chloride from Burroughs Wellcome plc; phentolamine mesylate from CIBA-Geigy; and clonidine hydrochloride from Boehringer Ingelheim GmbH. Nimodipine, PY 108068 and Bay K 8644 were kindly made available by Dr E.K. Matthews. Racemic QMDP iodide was synthesized by Dr D.H. Marrian and benzilylcholine chloride by Mr B. Peck. Gifts of: compounds II–IX (Figure 1) ((+)-II, (−)-II, IV and VI were hydrochlorides, III, V and VII iodides, VIII maleate and IX bromide salts) from Gist-Brocades NV; cimetidine, icotidine (SK&F 93319), temelastine (SK&F 93944) and 2-thiazolylethylamine (2-(2-aminoethyl)-thiazole) dihydrochloride from Smith, Kline & French Research Ltd; (+)- and (−)-chlorpheniramine maleate from

Table 1 Affinity constants of diphenhydramine derivatives for the histamine H_1-receptor

Compound			*Affinity constant* (M^{-1}) v. [^3H]-*mepyramine*	*guinea-pig ileum* (ref.)
I	—	(Diphenhydramine)	$8.6 \pm 0.1 \times 10^7$	4.0×10^7 (a), 1.0×10^8 (b)
II	(−)-isomer		$1.1 \pm 0.2 \times 10^7$	6.3×10^6 (a), 7.9×10^6 (b)
II	(+)-isomer		$1.1 \pm 0.1 \times 10^9$	1.0×10^9 (a), 5.0×10^8 (b)
III	(+)-isomer	(QMDP)	$7.2 \pm 0.1 \times 10^8$	$1.8 \pm 0.2 \times 10^9$ (10) 1.0×10^9 (a)
IV	racemate		$2.0 \pm 0.1 \times 10^8$	4.0×10^8 (a)
V	racemate		$3.6 \pm 0.1 \times 10^7$	—
VI	racemate		$6.5 \pm 0.2 \times 10^8$	4.0×10^9 (a)
VII	racemate		$2.9 \pm 0.1 \times 10^8$	—
VIII	racemate		$5.5 \pm 0.1 \times 10^8$	7.9×10^8 (b)
IX	racemate	(Pirdonium)	$1.0 \pm 0.1 \times 10^9$	$7.8 \pm 1.2 \times 10^8$ (3) 6.3×10^9 (a), 7.9×10^8 (b), 6.3×10^9 (c)

The structures of the compounds examined (I–IX) are shown in Figure 1. Affinity constants from inhibition of the binding of 0.2–0.5 nM [^{3}H]-mepyramine to a membrane fraction from guinea-pig cerebellum were determined as described under Methods (17–21 points on each inhibition curve). Each value is the weighted mean ± s.e. mean from 3 measurements, except for QMDP (4) and the isomers of compound II (1 each). For all compounds the percentage of the binding of [^{3}H]-mepyramine insensitive to inhibition by the compound, obtained from weighted non-linear regression (see Methods), did not differ significantly from the percentage of [^{3}H]-mepyramine binding insensitive to inhibition by 2 μM promethazine, measured in the same experiment. Best-fit values of the Hill coefficient were not significantly different from unity. Affinity constants from the inhibition of histamine-induced contraction of guinea-pig small intestine at 30°C were either determined in this study using longitudinal muscle strips (mean ± s.e. mean given, with the number of determinations in parentheses) or taken from the literature (measured on segments of ileum). Literature values are taken from: (a) Harms *et al.*, 1975; (b) Nauta & Rekker, 1978 and (c) Rekker (1982).

Schering; diphenhydramine hydrochloride from Parke, Davis & Co; methapyrilene hydrochloride from Eli Lilley & Co; (−)-propranolol hydrochloride from ICI plc; and spiperone (spiroperidol) from Janssen Pharmaceuticals are gratefully acknowledged.

Results

Choice of diphenhydramine derivative for labelling

The quaternary 4-methyldiphenhydramine derivatives which appeared to have the high affinity and selectivity required for a ^{3}H-ligand for the histamine H_1-receptor (Harms *et al.*, 1975) were compounds III, VII (on the basis of the affinity of VI) and IX (structures in Figure 1). The affinities of these compounds for the H_1-receptor, together with those of their tertiary amine precursors and certain derivatives lacking the 4-methyl substituent, determined from inhibition of [^{3}H]-mepyramine binding to cerebellar membranes, are given in Table 1. In general, the values obtained agree reasonably well with the values given by Harms *et al.* (1975) and Nauta & Rekker (1978), except for compounds VI and IX (pirdonium). The 4-methyl-pyrrolidino derivative VI was some 6 fold lower in affinity than the value cited, although the affinity of IV, which lacks the 4-methyl substituent, was in reasonable accord with the literature value. The fall in affinity on quaternisation of VI is less than that with IV (K_a quaternary/K_a tertiary = 0.4 for VII/VI and 0.2 for V/IV), but the relatively low affinity of VII, $3 \times 10^8\,M^{-1}$, makes it unattractive as a potential ^{3}H-ligand.

Pirdonium (IX) does have a higher affinity than its tertiary precursor VIII (K_a IX/K_a VIII = 1.8), but the very high value for the affinity constant of pirdonium quoted in some reports, $6 \times 10^9\,M^{-1}$ (Harms *et al.*, 1975; Rekker, 1982), is probably a typographical error, since the value in Nauta & Rekker (1978), pA_2 8.9 rather than 9.8, is similar to the value measured against [^{3}H]-mepyramine and confirmed on the guinea-pig ileum (Table 1). The affinity of pirdonium (IX) is still high, but the presence of two chiral centres is a potential disadvantage. No resolved samples of pirdonium (IX) were available, but measurements on two partially resolved samples of the precursor (VIII) of unknown enantiomeric composition yielded K_a values of $3.0 \pm 0.1 \times 10^8$ and $6.3 \pm 0.1 \times 10^8\,M^{-1}$ (weighted means ± s.e. mean of 3 determinations). The difference is not great, but it

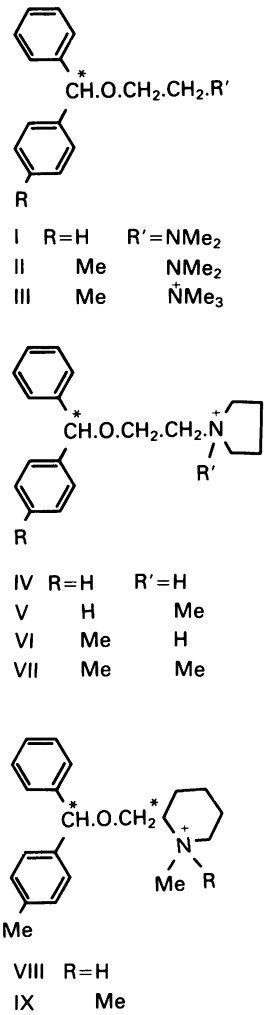

Figure 1 Structures of diphenhydramine derivatives examined. Chiral centres are marked with an asterisk.

does suggest that there are likely to be differences in affinity between the enantiomers of pirdonium (IX), as would be anticipated (Nauta & Rekker, 1978).

The stereo-selectivity of the isomers of compound II (Harms *et al.*, 1975) is fully confirmed. In addition quaternisation does not lead to any marked change in affinity. The affinity of the (+)-isomer of III (QMDP) determined from inhibition of the contractile response to histamine, 1.8×10^9 M^{-1}, is in good agreement with the value of 2.0×10^9 M^{-1} from inhibition of [^{3}H]-mepyramine binding to a membrane fraction from guinea-pig intestinal smooth muscle (Hill & Young, 1981), although a little higher than the value of 7.2×10^8 M^{-1} obtained from measurements on the cerebellar preparation (Table 1). The (+)-isomer of III (QMDP) thus has an acceptable affinity and the advantage that the tertiary precursor has been resolved (Nauta & Rekker, 1978).

The affinity constants for the binding of the (+)-isomer of III (QMDP) and its close relatives to muscarinic receptors in a membrane fraction from guinea-pig cerebral cortex are given in Table 2. The values obtained from inhibition of the binding of [^{3}H]-N-methylscopolamine ([^{3}H]-NMS) are all in reasonable accord with published values from measurements on the guinea-pig ileum (Table 2). The increase in muscarinic receptor affinity on quaternisation (2.7 fold, III/II, (+)-isomers, Table 2), coupled with the decrease in affinity for the H_1-receptor, has the result that the selectivity of QMDP for the H_1-receptor, 65 (K_a H_1/K_a muscarinic) is distinctly less than that of its tertiary precursor (II, (+)-isomer), 297. However, the selectivity of QMDP, 65, is still greater than that of pirdonium, 40 (calculated from the data of Harms *et al.*, 1975) and of the compounds examined QMDP appears to be the best candidate as a quaternary ^{3}H-ligand for the H_1-receptor.

Preparation and purification of [^{3}*H*]*-QMDP*

[^{3}H]-QMDP was prepared (Amersham International) by methylation of (+)-4-methyldiphenhydramine (II, (+)-isomer) with [^{3}H]-MeI (specific activity 85 Ci mmol^{-1}) in methanolic $NaHCO_3$. The reaction product, supplied as an ethanolic solution, was purified by high-voltage electrophoresis in borate buffer, pH 8.6. At this pH the precursor tertiary amine (II), pK_a 8.5 (Knobloch *et al.*, 1954), is only partially protonated and consequently migrates at a slower rate than the quaternary derivative, [^{3}H]-QMDP (Figure 2a). The major impurity in the crude mixture appears to be [^{3}H]-choline, since it migrates with the same mobility as authentic unlabelled compound (Figure 2a). The fraction corresponding to QMDP, eluted with distilled water, gave a single peak of radioactivity on electrophoresis (Figure 2a).

Evidence that the tritium is associated solely with the N-methyl residue was obtained by hydrolysis of [^{3}H]-QMDP in 5 M HCl at 60°C for 2 h, conditions known to cause cleavage of the ether bond in 4-methyldiphenhydramine (II) (Knobloch *et al.*, 1954; de Roos *et al.*, 1963). Comparison of scans of radioactivity demonstrates that after hydrolysis the tritium is associated solely with a peak with the same mobility as choline (Figure 2b). The hydrolysed samples of [^{3}H]-QMDP were also examined by thin layer chromatography (silica-gel, butanol/acetic acid/water/ethanol, 8/1/3/2, v/v/v/v) and descending paper chromatography (Whatman No 1 paper,

Table 2 Affinities of diphenhydramine derivatives for muscarinic receptors

Compound		K_a (M^{-1}) v. [^3H]-*NMS*	K_a (M^{-1}) *Guinea-pig ileum*	$\frac{K_a \text{ Histamine } H_1\text{-receptor}}{K_a \text{ muscarinic receptor}}$
I	(Diphenhydramine)	$4.3 \pm 0.3 \times 10^6$	(5.0×10^6)	16
II	(−)-isomer	$1.8 \pm 0.2 \times 10^6$	(6.3×10^5)	6
II	(+)-isomer	$4.1 \pm 0.2 \times 10^6$	1.0×10^6 (1.3×10^6)	297
III	(+)-isomer (QMDP)	$1.1 \pm 0.5 \times 10^7$	9.0×10^6 (1.0×10^7)	65

The structures of compounds I–III are shown in Figure 1. Values ± s.e. mean of affinity constants from inhibition of the binding of 0.2–0.7 nM [^{3}H]-NMS to a membrane fraction from guinea-pig cerebral cortex were determined from best-fit values of IC_{50} as described under Methods (13–15 points on each curve). Hill coefficients did not differ significantly from unity and best-fit values of the inhibitor-insensitive binding were in close agreement with the percentage of [^{3}H]-NMS binding insensitive to inhibition by 1 μM methylatropine measured in the same experiment. Values of affinity constants obtained from inhibition of agonist-induced contraction are either taken from Harms *et al.* (1975) (Values in parentheses, measured on segments of guinea-pig ileum) or are values from single measurements on intestinal muscle strips (see Methods). The ratio of the affinity constants (K_a) of compounds for the histamine H_1-receptor and the muscarinic receptor were calculated from the values determined from inhibition of the binding of [^{3}H]-mepyramine (Table 1) and [^{3}H]-NMS.

pyridine/butanol/water, 1/1/1, v/v/v). In each system the peak of radioactivity had the same R_F value, 0.38 and 0.34, respectively, as a choline standard. The corresponding R_F values for (±)-QMDP were 0.10 and 0.80.

[^{3}H]-QMDP eluted from electrophoretograms was stored at 4°C. Under these conditions hydrolysis to give 4-methyldiphenylmethanol and [^{3}H]-choline is slow, but significant. After 3 months of storage the [^{3}H]-choline present, determined after separation from [^{3}H]-QMDP by electrophoresis, was 4%. After storage for a year this had increased to 12%.

Specific activity of [^{3}*H*]*-QMDP*

In view of the relatively weak u.v. absorbance of diphenylmethane derivatives (QMDP: $E_{259,\text{ethanol}}$ 485), the concentration of [^{3}H]-QMDP in the sample purified by high-voltage electrophoresis was determined by bioassay. Measurements were made of the inhibition of histamine-induced contractions of longitudinal strips from guinea-pig small intestine at 37°C. At this temperature, equilibration between (+)-QMDP and the agonist is more rapid than at lower temperatures (see Treherne & Young, 1988, accompanying paper). The affinity constant of (+)-QMDP, determined from a total of 11 parallel shifts of the histamine concentration-response curve in 8 independent experiments was $1.2 \pm 0.1 \times 10^9\ M^{-1}$. Using this constant and the same experimental conditions, the concentration of [^{3}H]-QMDP was calculated from a single shift of the concentration-response curve to histamine in each of 6 independent experiments in which the tritium content of the [^{3}H]-QMDP solution was also determined. The mean specific activity ± s.e. mean was 83 ± 5 Ci mmol^{-1}, in close agreement with the value given, 85 Ci mmol^{-1}, for the specific activity of the [^{3}H]-MeI used in the synthesis of [^{3}H]-QMDP.

Characteristics of the binding of [^{3}*H*]*-QMDP to guinea-pig cerebellar membranes*

To find an appropriate method for determining the level of non-H_1-receptor binding of [^{3}H]-QMDP, measurements were made of the inhibition of [^{3}H]-QMDP binding by a series of H_1-antagonists. The level of antagonist-insensitive binding in these experiments was high. The percentage of the binding of 0.4–0.6 nM [^{3}H]-QMDP insensitive to inhibition by 0.4 μM mepyramine was mostly in the range 50–65%. This was reduced to 30–45% by pre-soaking the glass-fibre filters in 0.3% polyethylenimine (PEI) (Hampton *et al.*, 1982; Bruns *et al.*, 1983). Bovine serum albumin (BSA), 0.5%, added alone produced a smaller decrease in the 0.4 μM mepyramine-insensitive binding (56 ± 2% control, 47 ± 1% with BSA) than 0.3% PEI (40 ± 1%, measured in the same experiment), but the combination of 0.3% PEI + 0.5% BSA was no more effective than 0.3% PEI alone. The addition of 100 mM NaCl to the buffer decreased the mepyramine-insensitive binding, but also inhibited the binding of [^{3}H]-QMDP sensitive to 0.4 μM mepyramine. Pretreatment of filters with 0.3% PEI was therefore used routinely to minimise non-specific binding. Under these conditions 'specific' binding of 0.44 nM [^{3}H]-QMDP was a linear function of the protein concentration in the range 0–0.6 mg ml^{-1} (data not shown). In more recent experiments in which filtration has

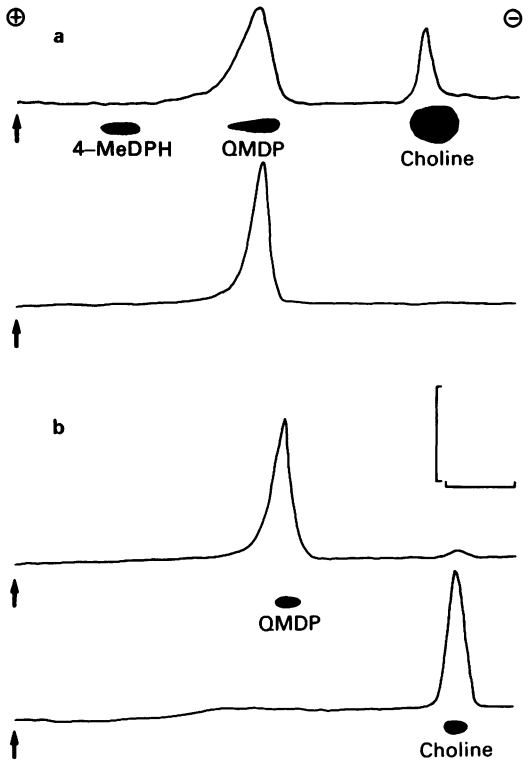

Figure 2 (a) High-voltage electrophoresis of [^{3}H]-(+)-N-methyl-4-methyldiphenhydramine ([^{3}H]-QMDP). Conditions: Whatman No 1 paper, 0.2 M borate buffer, pH 8.6, 40 V cm^{-1}, 45 min. The traces are scans for radioactivity. Calibration bars are 10^5 c.p.m. (vertical) and 5 cm (horizontal). The origin is indicated by the arrows. Upper trace, unpurified reaction mixture; lower trace, fraction corresponding to QMDP. The position of the unlabelled compounds run as markers was revealed by staining with iodoplatinate. Samples were run simultaneously on parallel tracks. 4-MeDPH is (±)-4-methyldiphenhydramine (compound II, Figure 1). (b) High-voltage electrophoresis of the hydrolysis product from [^{3}H]-QMDP. Electrophoresis conditions as in (a). The traces are scans for radioactivity before (upper trace) and after (lower trace) incubation of [^{3}H]-QMDP in 5 M HCl for 2 h at 60°C. Calibration bars as in (a).

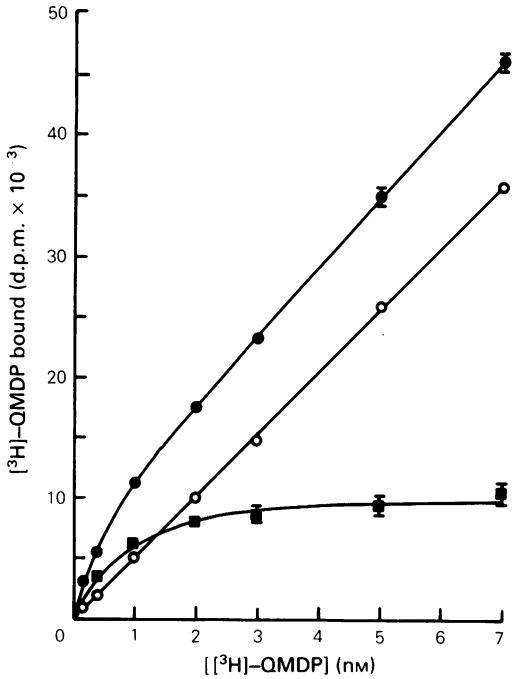

Figure 3 [^{3}H]-(+)-N-methyl-4-methyldiphenhydramine ([^{3}H]-QMDP) binding to guinea-pig cerebellar membranes. The binding of [^{3}H]-QMDP to cerebellar membranes (0.35 mg protein ml^{-1}) was measured in 50 mM Na–K phosphate buffer as described under Methods. Filters were pre-soaked in 0.3% PEI. Each point is the mean of 5 replicate determinations with s.e. mean shown by vertical bars. Where no error bars are shown the error was within the size of the symbol. Specific binding (■) was obtained by subtracting the binding in the presence of 0.4 μM mepyramine (○) from binding with no inhibitor present (●).

been carried out using a cell harvester rather than a conventional filter-block the binding of 0.4–0.6 nM [^{3}H]-QMDP insensitive to 0.4 μM mepyramine has been reduced from 30–45% to approximately 15–30%.

N-methylatropine, in the concentration range 0.1 nM–1 μM, gave no significant inhibition of the binding of [^{3}H]-QMDP. This is consistent with the relatively low density of muscarinic receptors in guinea-pig cerebellum (128 ± 15 pmol g^{-1} protein, 41% of the H_1-receptor density, in 3 experiments on different membrane preparations in which both [^{3}H]-NMS and [^{3}H]-mepyramine binding was measured) and the 65 fold selectivity of (+)-QMDP (Table 2). However, a characteristic of several of the antagonist inhibition curves was that after reaching an initial plateau, higher concentrations of the antagonists caused some secondary inhibition of [^{3}H]-QMDP binding. With mepyramine this effect was only apparent at concentrations $\geqslant 1$ μM in experiments in which the concentration of [^{3}H]-QMDP was *circa* 0.5 nM and on this basis 0.4 μM mepyramine was selected initially as a marker for the non-specific binding.

The binding of [^{3}H]-QMDP in the presence and absence of 0.4 μM mepyramine is shown in Figure 3. The weighted mean values of the affinity constant and the Hill coefficient of the 0.4 μM mepyramine-

sensitive component from 8 such experiments were $1.14 \pm 0.03 \times 10^9\,M^{-1}$ and 0.87 ± 0.03, respectively. The H_1-receptor density inferred from these experiments varied between preparations (162–280 pmol g^{-1} protein), with a mean value of 188 ± 4 pmol g^{-1} protein. The value of the Hill coefficient less than unity may imply some binding of [^{3}H]-QMDP to secondary sites, but the value of K_a is in good agreement with that, $1.8 \pm 0.2 \times 10^9\,M^{-1}$, determined from (+)-QMDP inhibition of histamine-induced contraction of the guinea-pig ileum at 30°C.

Affinity constants for a number of compounds determined from curves of inhibition of [^{3}H]-QMDP binding are set out in Table 3. The value for histamine is an apparent K_a, since the Hill coefficient is clearly less than unity. In part, this is a consequence of some inhibition of the non-specific binding of [^{3}H]-QMDP by the high concentrations needed to define the foot of the curve. However, the apparent K_a, $2.4 \times 10^4\,M^{-1}$, is similar to that, $3.7 \times 10^4\,M^{-1}$, obtained from inhibition of [^{3}H]-mepyramine binding (Carswell & Young, 1986).

Comparison of antagonist affinities determined from inhibition of [^{3}H]-QMDP binding and [^{3}H]-mepyramine binding

Affinity constants for the H_1-receptor for most of the antagonists used in the study with [^{3}H]-QMDP

Table 3 Characteristics of curves of inhibition of [^{3}H]-(+)-N-methyl-4-methyldiphenhydramine ([^{3}H]-QMDP) binding

Ref†	*Inhibitor*	K_a (M^{-1})	*Hill coefficient*	*Binding (%) insensitive to* *Inhibitor*‡	*0.4 μM mepyramine*
1	Mepyramine	$1.2 \pm 0.1 \times 10^9$ (13)	1.28 ± 0.19	54 ± 2*	—
		$1.1 \pm 0.1 \times 10^9$ (15)	1.00 ± 0.04	41 ± 1	—
		$0.9 \pm 0.1 \times 10^9$ (15)	1.11 ± 0.04	20 ± 1**	—
2	(+)-Chlorpheniramine	$1.1 \pm 0.1 \times 10^9$ (10)	1.27 ± 0.11	32 ± 1**	32 ± 1
3	(−)-Chlorpheniramine	$4.5 \pm 0.5 \times 10^6$ (9)	0.95 ± 0.09	19 ± 2**	21 ± 1
4	Chlorpromazine	$1.2 \pm 0.1 \times 10^9$ (10)	1.10 ± 0.05	30 ± 1**	29 ± 1
5	Methapyrilene	$2.1 \pm 0.2 \times 10^8$ (17)	0.86 ± 0.06	45 ± 1	45 ± 1
6	Promethazine	$2.1 \pm 0.2 \times 10^9$ (9)	1.16 ± 0.09	29 ± 1**	26 ± 1
7	Diphenhydramine	$7.3 \pm 0.4 \times 10^7$ (12)	1.11 ± 0.05	43 ± 1	44 ± 1
8	(+)-4-Methyldiphenhydramine	$9.4 \pm 0.8 \times 10^8$ (14)	0.85 ± 0.06	37 ± 1	39 ± 1
9	(−)-4-Methyldiphenhydramine	$9.7 \pm 0.1 \times 10^6$ (15)	0.95 ± 0.04	31 ± 1	43 ± 1
10	(+)-QMDP	$8.0 \pm 0.2 \times 10^8$ (9)	0.97 ± 0.02	24 ± 1**	26 ± 1
11	Icotidine (SK&F 93319)	$6.7 \pm 0.3 \times 10^6$ (16)	1.08 ± 0.06	44 ± 1	38 ± 1
12	Temelastine (SK&F 93944)	$1.2 \pm 0.1 \times 10^9$ (21)	0.93 ± 0.05	54 ± 1	54 ± 2
13	Histamine	$2.4 \pm 0.4 \times 10^4$ (13)††	0.61 ± 0.06	23 ± 3	35 ± 1
14	Phentolamine	$1.3 \pm 0.1 \times 10^6$ (12)	1.19 ± 0.09	44 ± 2	45 ± 1
15	Spiperone	$5.0 \pm 1.7 \times 10^5$ (10)	0.85 ± 0.13	42 ± 7	45 ± 1
16	N-methylatropine	$4.7 \pm 0.8 \times 10^4$ (21)	0.91 ± 0.08	29 ± 5	—

Compounds giving < 10% inhibition of [^{3}H]-QMDP binding

Cimetidine (100 μM), clonidine (10 μM), propranolol (1 μM), (+)-tubocurarine (10 μM), benzilycholine (1 μM), choline (1 μM), verapamil (1 μM), nimodipine (1 μM), PY 108068 (1 μM) and Bay K 8644 (1 μM).

Best-fit values ± estimated s.e. of the IC_{50}, the Hill coefficient and the level of inhibitor-insensitive binding of 0.3–0.6 nM [^{3}H]-QMDP were obtained from non-linear regression analysis as described under Methods with the number of points on each curve in parentheses. K_a was calculated from the IC_{50} as described under Methods, taking the K_a for [^{3}H]-QMDP to be $1.0 \times 10^9\,M^{-1}$, the mean value from different methods of determination. Filters were treated with 0.3% PEI, except in one experiment with mepyramine. The percentage of [^{3}H]-QMDP binding insensitive to 0.4 μM mepyramine (mean ± approximate s.e. mean) was determined in parallel incubations in each experiment. The data are from single representative experiments. Weighted means from multiple determinations are shown in Figure 4.

† Reference number of inhibitor in Figure 4.

‡ Best-fit value from non-linear regression.

* Filters not treated with PEI.

** Samples filtered on a cell harvester.

†† The value of K_a for histamine is an apparent K_a (Hill coefficient < 1). If the curve is assumed to represent binding to 2 sites (double hyperbola), then the best-fit values of K_a and the percentage of the total binding of 0.49 nM [^{3}H]-QMDP associated with each site are: $6.2 \pm 0.1 \times 10^4\,M^{-1}$ ($58 \pm 1\%$) and $5.4 \pm 0.2 \times 10^2\,M^{-1}$ ($23 \pm 1\%$) and, hence, $19 \pm 1\%$ histamine-insensitive binding.

(Table 2) were also determined from inhibition of the binding of [^{3}H]-mepyramine. Non-specific binding was lower with [^{3}H]-mepyramine than with [^{3}H]-QMDP, but was reduced to approximately 7–10% by pretreating the filters with 0.3% PEI. For all the antagonists studied, Hill coefficients for the curves of inhibition of [^{3}H]-mepyramine binding were not significantly different from unity. Values of K_a obtained, or published values of K_a for compounds measured in earlier studies, are compared in Figure 4 with those obtained from inhibition of the binding of [^{3}H]-QMDP. There is a good correlation ($r = 0.997$, slope of regression line 0.99 ± 0.02) over 4 orders of magnitude of K_a and there can be little doubt that the binding of [^{3}H]-QMDP sensitive to inhibition by 0.4 μM mepyramine is to the histamine H_1-receptor.

Affinity of QMDP in other guinea-pig and rat brain tissues

Affinity constants determined for QMDP binding to membrane preparations from guinea-pig cerebral cortex and hippocampus and rat cerebral cortex are set out in Table 4, with the values determined in cerebellum given for comparison. With the exception of the value from [^{3}H]-QMDP binding in cerebellum (cf. above), Hill coefficients did not differ significantly from unity. The level of the binding of 0.5 nM [^{3}H]-QMDP insensitive to 0.4 μM mepyramine was approximately 58% in both guinea-pig cerebral cortex and hippocampus (3 determinations in cerebral cortex, 2 in hippocampus). The values of K_a agree well between the two methods of measurement and between the three regions of guinea-pig brain examined (Table 4). Parallel measurements of mepyramine inhibition of the binding of [^{3}H]-QMDP gave values of K_a for mepyramine of $1.0 \pm 0.1 \times 10^9$, $1.9 \pm 0.2 \times 10^9$ and $1.2 \pm 0.1 \times 10^9$ M^{-1} in guinea-pig cerebellum, cerebral cortex and hippocampus, respectively.

The affinity of (+)-QMDP in rat cerebral cortex appears to be appreciably lower than in guinea-pig brain (Table 4). However, the level of binding of 0.3–

Table 4 Affinity constants for (+)-N-methyl-4-methyldiphenhydramine ((+)-QMDP) binding to histamine H_1-receptors in regions of guinea-pig and rat brain

	K_a *for (+)-QMDP* (M^{-1}) *from*	
Brain region	[^{3}H]-*QMDP binding*	*Inhibition* [^{3}H]-*mepyramine*
Guinea-pig		
Cerebellum	$1.1 \pm 0.1 \times 10^9$	$0.7 \pm 0.1 \times 10^9$
Cerebral cortex	$0.8 \pm 0.1 \times 10^9$	$0.7 \pm 0.1 \times 10^9$
Hippocampus	$1.0 \pm 0.1 \times 10^9$	$1.0 \pm 0.1 \times 10^9$
Rat		
Cerebral cortex	$1.0 \pm 0.2 \times 10^8$ $1.7 \pm 0.2 \times 10^{8*}$	$1.6 \pm 0.2 \times 10^8$

Affinity constants for (+)-QMDP binding in guinea-pig brain regions were determined from binding curves for [^{3}H]-QMDP, such as that shown in Figure 3, with non-specific binding defined by 0.4 μM mepyramine. The value for cerebellum is the weighted mean ± s.e. mean from 8 measurements, that for cerebral cortex from 2. In rat cerebral cortex K_a was determined from (+)-QMDP inhibition of the binding of 0.29 or 0.35 nM [^{3}H]-QMDP. Values under [^{3}H]-mepyramine were obtained from (+)-QMDP inhibition of the binding of 0.21–0.52 nM [^{3}H]-mepyramine and are the weighted means of 2 independent experiments (cerebellum 4 experiments). Methylatropine (1 μM) was present in all incubations with [^{3}H]-QMDP in guinea-pig or rat cerebral cortex.

* Membrane preparation washed extensively as described under Methods.

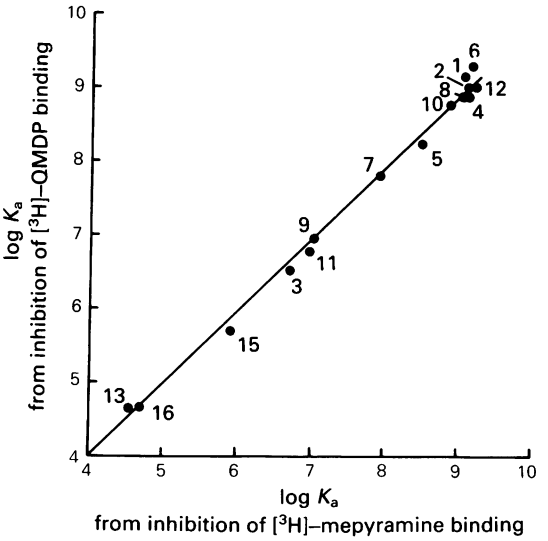

Figure 4 Comparison of affinity constants determined from inhibition of the binding of [^{3}H]-(+)-N-methyl-4-methyldiphenhydramine ([^{3}H]-QMDP) and of [^{3}H]-mepyramine. Values of K_a (M^{-1}) against [^{3}H]-QMDP are either taken from Table 3 or are the weighted means from 2–3 independent determinations. Values of K_a against [^{3}H]-mepyramine were either measured in this study (Table 1 and: icotidine, $9.7 \pm 0.2 \times 10^6$ M^{-1}; temelastine, $1.6 \pm 0.1 \times 10^9$ M^{-1}; N-methylatropine, $5.1 \pm 0.2 \times 10^4$ M^{-1}) or taken from previous studies in this laboratory (Hill *et al.*, 1978; Hill & Young, 1978, 1981; Wallace & Young, 1983; Aceves *et al.*, 1985; Carswell & Young, 1986). The inhibitors are identified by the number against each point, which corresponds to the number in column 1 of Table 3. The line drawn has a slope of 1.0.

0.4 nM [^{3}H]-QMDP not inhibited by 0.4 μM mepyramine was high, approximately 85% (3 determinations) compared with 38% for 0.4 nM [^{3}H]-mepyramine (2 determinations), measured on the same membrane preparation. An attempt to reduce the level of non-specific binding by adopting the rigorous washing procedure used successfully in studies of the binding of [^{3}H]-histamine to rat cerebral cortex (Steinberg *et al.*, 1985) reduced the level of non-specific binding of [^{3}H]-QMDP to 73 $\pm$ 1% in one experiment with (+)-QMDP (Table 4), but in 2 other experiments failed to give any significant reduction. Determination of the K_a for mepyramine in rat cerebral cortex from inhibition of [^{3}H]-QMDP binding gave a value of 2.8 $\pm$ 1.4 $\times$ 10^8 M^{-1}, similar to that obtained from mepyramine inhibition of [^{3}H]-mepyramine binding, 3.5 $\pm$ 0.2 $\times$ 10^8 M^{-1}. Both values are in good agreement with the K_a of 3.5 $\times$ 10^8 M^{-1} reported previously (Aceves *et al.*, 1985).

Non-H_1-receptor binding of [^{3}H]-QMDP

The good accord in general between the best-fit values of the percentage of inhibitor-insensitive binding and the corresponding values for 0.4 μM mepyramine (Table 2) provides evidence that at low concentrations, 0.4–0.6 nM, [^{3}H]-QMDP binds predominantly to the H_1-receptor. However, when higher concentrations of inhibitors were employed some further inhibition of [^{3}H]-QMDP binding became apparent. The effect is illustrated by the inhibition by (+)-QMDP of the binding of 0.47 nM [^{3}H]-QMDP (Figure 5a). At concentrations above 0.4 μM (+)-QMDP the inhibition of [^{3}H]-QMDP binding is greater than that produced by 0.4 μM mepyramine. These points were omitted in fitting the curve, but even so the best-fit values of the level of (+)-QMDP-insensitive binding was numerically less than the percentage of binding insensitive to 0.4 μM mepyramine in 4 of 5 experiments in which direct comparison was made. The effect is particularly clear for the (−)-isomer of 4-methyldiphenhydramine (Table 3), for which it was necessary to use high concentrations to define the foot of the curve. The presence of secondary sites was demonstrated more directly by (+)-QMDP inhibition of 1.05 nM [^{3}H]-QMDP binding in the presence of 1 μM temelastine to block H_1-receptor binding (Figure 5b). Temelastine (SK&F 93944) (Brown *et al.*, 1986), a new H_1-antagonist structurally unrelated to diarylalkylamines such as QMDP or mepyramine, produced no apparent inhibition of secondary binding sites for [^{3}H]-QMDP at concentrations up to 1 μM (the highest tested) and may prove to be a more satisfactory agent for defining the H_1-receptor binding of [^{3}H]-QMDP. In the experiment shown, 0.4 μM

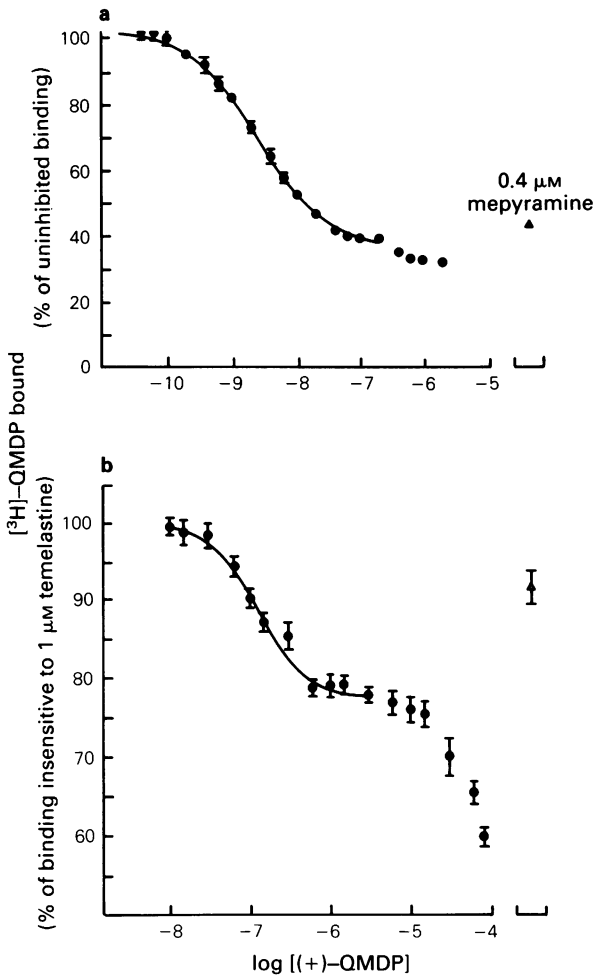

Figure 5 Inhibition of [^{3}H]-(+)-N-methyl-4-methyldiphenhydramine ([^{3}H]-QMDP) binding by (+)-QMDP. (a) Measurements of the binding of 0.47 nM [^{3}H]-QMDP were made as described under Methods with 0.3% PEI-treated filters. Points are the mean calculated from 5 replicate determinations at each inhibitor concentration and 20 replicates in the absence of inhibitor; approx. s.e. mean shown by vertical bars. Where no error bars are shown the error was within the size of the symbol. The last four points were not used in determining the best-fit curve drawn (K_a 4.2 $\pm$ 0.2 $\times$ 10^8 M^{-1}, Hill coefficient 0.93 $\pm$ 0.04). The level of inhibition given by 0.4 μM mepyramine (△) is shown for comparison. (b) Measurements made in the presence of 1 μM temelastine; 100% on the ordinate represents the temelastine-insensitive binding of 1.05 nM [^{3}H]-QMDP (42 $\pm$ 1% of the uninhibited binding of [^{3}H]-QMDP). Details otherwise as in (a). Only the points covered by the line were used in calculating the best-fit curve drawn. Note that the ordinate scale does not start at zero.

mepyramine gave a $9 \pm 2\%$ inhibition of the temelastine-insensitive binding of [^{3}H]-QMDP (corresponding to 4% inhibition of the total binding of 1.05 nM [^{3}H]-QMDP). An estimate of the affinity of [^{3}H]-QMDP for the principal secondary binding site (Figure 5b) was obtained by fitting the inhibition data between 10 nM and 4 μM (+)-QMDP and yielded a value of K_a of $1.2 \pm 0.1 \times 10^7$ M^{-1} (Hill coefficient 1.47 ± 0.18). A second similar experiment, but with 1.54 nM [^{3}H]-QMDP, gave an estimated K_a of $1.8 \pm 0.2 \times 10^7$ M^{-1} (Hill coefficient 1.06 ± 0.17), with the same proportion of temelastine-insensitive binding inhibited by 0.4 μM mepyramine ($9 \pm 2\%$). The capacity of this secondary site for [^{3}H]-QMDP estimated from these experiments was 2–3 nmol g^{-1} protein, i.e. an order of magnitude greater than the H_1-receptor density. High concentrations of (+)-QMDP (>1 μM) produced some inhibition of the binding of [^{3}H]-QMDP to glass fibre filters in the absence of cerebellar homogenate, but the effect was small and could at the most account for only *circa* 15% of the binding ascribed to the secondary site.

The identity of the secondary site remains unknown. Methylatropine (1 μM) produced only a $3 \pm 2\%$ inhibition of the temelastine-insensitive binding of [^{3}H]-QMDP. Nimodipine (1 μM), verapamil (1 μM), cimetidine (100 μM) and choline (0.1 mM) also failed to produce any significant inhibition.

Discussion

There can be little doubt from the evidence presented above that [^{3}H]-QMDP does bind selectively to the histamine H_1-receptor. This is clearly the most important property which any new radioligand must possess and encourages further investigation of [^{3}H]-QMDP as a quaternary ligand for the H_1-receptor in measurements on intact cells. However, in certain respects the binding properties of [^{3}H]-QMDP are something less than ideal. This applies in particular to levels of non-specific binding, species dependence and chemical stability.

The high level of non-specific binding of [^{3}H]-QMDP compared to that of [^{3}H]-mepyramine is disappointing. At least part of this non-specific component is due to binding to secondary, medium-affinity sites. This problem was anticipated in tissues with a high density of muscarinic receptors, where even a low fractional occupancy of [^{3}H]-QMDP (K_a for the muscarinic receptor, 1.1×10^7 M^{-1}) can lead to the labelling of a significant number of sites. Thus in guinea-pig cerebral cortex where the muscarinic receptor density determined from [^{3}H]-N-methylscopolamine binding is 13 fold higher than the density of H_1-receptors labelled by [^{3}H]-mepyramine (J.M. Young, unpublished observations), even at 1 nM [^{3}H]-QMDP 22% of the bound ligand will be to muscarinic receptors (neglecting non-H_1- and non-muscarinic receptor binding). In practice, the muscarinic binding of [^{3}H]-QMDP is not a major problem, since a selective muscarinic antagonist can be included in assay mixtures, although as a general principle the presence of additional pharmacological agents is better avoided. However, it is clear from the measurements of [^{3}H]-QMDP binding to cerebellar homogenates, where muscarinic binding is not a problem, since the receptor density is low (cf. Results), that [^{3}H]-QMDP also binds to other secondary sites. One site, presumed to be a single site on the evidence of a Hill coefficient near unity, with an affinity of approximately 10^7 M^{-1} is reasonably well defined and accounts for some 10% of the total binding of 1–1.5 nM [^{3}H]-QMDP. The remaining lower affinity non-receptor sites may well represent less well-defined interactions of an amphiphilic agent, although the relatively strong binding of the quaternary N-methyl derivative of the tricyclic H_1-antagonist deptropine to alpha-1 acid glycoprotein (Van der Sluijs & Meijer, 1985) should be noted.

The identity of the principal, non-muscarinic secondary site is unknown. There must be some suspicion that it could be an ion channel of some description, since diphenylalkylamines, structurally related to QMDP (although 3^y amines), are well known as a class of Ca^{2+} channel antagonists (Spedding, 1985) and compounds of the same type have appreciable affinities against [^{3}H]-batrachotoxinin binding, presumably to Na^+ channels, in rat brain (Grima *et al.*, 1986; Romey *et al.*, 1987). The presence of this secondary site raises the problem of the definition of the H_1-receptor binding of [^{3}H]-QMDP, for which most of the antagonists examined, including mepyramine, appeared to have some affinity. In the greater part of this study, non-receptor binding of [^{3}H]-QMDP has been defined as the binding insensitive to 0.4 μM mepyramine, but temelastine (SK&F 93944), which became available at a later stage, now looks likely to be a more satisfactory agent. At a concentration of 1 μM, temelastine gave no indication of inhibition of secondary binding sites for [^{3}H]-QMDP and consistently produced a lesser inhibition of [^{3}H]-QMDP binding than 0·4 μM mepyramine. The difference is small, approximately 4% of the total binding of 1–1.5 nM [^{3}H]-QMDP, but it will in theory introduce a small error into the determination of affinity from binding curves where the non-specific binding was determined with 0.4 μM mepyramine (e.g. Figure 3). Similarly the affinity determined for a number of the antagonists (Table 3), where the best-fit values of non-specific binding were similar to that for 0.4 μM mepyramine measured concurrently, may be marginally low. The error will be small, as indeed the good correlation with affin-

ities determined against [^{3}H]-mepyramine (Figure 4) indicates and many more measurements at high antagonist concentrations would be necessary to fit the data satisfactorily to a multi-site model.

The second disappointing property of [^{3}H]-QMDP binding is the marked species dependence, evidenced by the much lower affinity in rat brain, $1.6 \times 10^8\,M^{-1}$, than in guinea-pig brain, $1.0 \times 10^9\,M^{-1}$ (Table 4). Lower affinities for H_1-antagonists in the rat than in the guinea-pig appears to be the rule (Chang *et al.*, 1979; Hill & Young, 1980) and of the high affinity compound only doxepin has been shown to have near equal affinities in the two species (Chang *et al.*, 1979; Tran *et al.*, 1981; Aceves *et al.*, 1985). We have not made a wide study of the species variation in the affinity of [^{3}H]-QMDP for H_1-receptors, but the evidence of the marked fall in the rat does mean that the utility of [^{3}H]-QMDP in studies on binding to intact cells in culture is likely to be dependent on the species from which the cells were derived. In this respect [^{3}H]-QMDP shares the same disadvantage as [^{125}I]-iodobolpyramine, which shows great promise for autoradiographic studies of H_1-receptors in guinea-pig brain, but is seemingly ineffective in rat brain (Korner *et al.*, 1986). The one fortunate feature of this species variation is that antagonist affinities for H_1-receptors in human brain appear to be only a little lower than those in guinea-pig brain (Chang *et al.*, 1979).

The third property of [^{3}H]-QMDP which is less than ideal is the lability of the ether bond. This is not a serious problem in that the rate of hydrolysis under the usual storage conditions is slow, although occasionally preparations have seemed to breakdown more rapidly. However, it will need to be borne in mind that one of the hydrolysis products is [^{3}H]-choline, which is transported into certain cells, and this will make it necessary to check the purity of [^{3}H]-QMDP at frequent intervals. (+)-QMDP itself is only a weak inhibitor of choline uptake into human erythrocytes (J.M. Treherne and L. Widdowson, unpublished observations).

These undesirable features of the binding of [^{3}H]-QMDP raise the question of whether a better choice might have been made of a quaternary H_1-antagonist for radiolabelling. The binding of [^{3}H]-mepyramine to secondary sites has been described (Hill & Young, 1981; Hadfield *et al.*, 1983) and presumably the quaternary derivative of mepyramine would be very likely to show the same behaviour. The same applies to other diarylalkylamines, including the diphenhydramine series, of which (+)-QMDP appeared for other reasons to offer the best prospects (cf. under 'Choice of diphenhydramine derivative for labelling' in Results). Tricyclic compounds, such as doxepin, have the attraction of very high H_1-receptor affinity and lesser species variation, but the well-known ability of this group, as with phenothiazines, to bind to a wide variety of receptors allows no surprise that the binding of [^{3}H]-doxepin is complex (Tran *et al.*, 1981; Taylor & Richelson, 1982). Again it seems likely that quaternary derivatives would show the same problem of selectivity. Temelastine may well not bind to the same spectrum of secondary sites and its apparent lack of ability to penetrate the CNS (Brown *et al.*, 1986) could indicate that it will not easily cross cell membranes. No binding studies have been reported with high specific activity labelled derivatives, but the high proportion of [^{14}C]-temelastine bound to plasma proteins (Brown *et al.*, 1986) does not augur well. In addition, temelastine shows a particularly strong species dependence (Calcutt *et al.*, 1987).

Most of this discussion has concentrated on potential problems with [^{3}H]-QMDP in binding studies. However, the prime conclusion to be drawn from the results presented here is that in favourable circumstances [^{3}H]-QMDP is an acceptable radioligand for the histamine H_1-receptor. Further, it does have the advantage of having a relatively high specific activity (for a tritium-labelled compound), $83\,Ci\,mmol^{-1}$, which will ease its use with small amounts of tissue or at low receptor occupancy. The real test will be to use it to study binding to intact cells.

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