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Letter to the Editor

Specific binding of [^{3}H]methylscopolamine to glass fibre filters

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The availability of radiolabelled ligands and the development of rapid filtration techniques have been the key to the success in measuring small amounts of drugs or neurotransmitter receptors, or both, in the brain and other tissues. One of the potential artifacts in radioligand-receptor binding studies is the procedure used to separate the bound from the free ligand (Bennet & Yamamura 1985). In particular, when a rapid and convenient filtration method is used, the problem could arise of the radioligand binding to the filter. Such binding will not only artificially increase the amount of non-specific binding but may become a relevant problem when the binding of the radioligand to the filters is decreased by a cold drug, i.e. when specific binding to the filters can be measured (Bielkiewicz & Cook 1985).

Here I report on the binding of [^{3}H]methylscopolamine (methylhyoscine) ([^{3}H]MS, Amersham, 72 Ci mmol^{-1}), a ligand increasingly used to label cholinergic muscarinic receptors, to glass fibre filters (Whatman GF/C). The radioligand was incubated with buffer (total volume 2 mL) at 27 °C for 1 h and the samples filtered under vacuum through the appropriate filter which was washed three times with 3 mL of ice-cold buffer. Filters were air-dried and counted for radioactivity in 10 mL of Liquiscint (National Diagnostic) in a TriCarb Scintillation Spectrometer at an efficiency of 40% for 10 min. At a concentration of 1 nM, approximately 0·5% of the total radioactivity was bound to GF/C filters. Initial experiments showed that the amount of binding was dependent upon the buffer used for the incubation; at a concentration of [^{3}H]MS 1 nM, the highest binding was observed with 50 mM Na/K phosphate buffer (589 ± 38 counts min^{-1}), lower binding was observed with phosphate buffer saline (PBS) buffer (383 ± 35 counts min^{-1}), and the lowest when Hank's buffer was used (251 ± 18 counts min^{-1}; for all data, n = 6; background counts of 30 counts min^{-1} have been subtracted). In all subsequent experiments Hank's buffer was used for incubation and washing.

In the presence of 10^{-5} M atropine, total binding was decreased by 50–60%. Specific binding could also be observed in the presence of three other muscarinic antagonists (scopolamine, methylscopolamine and methylatropine, all present at 10^{-5} M), and by three muscarinic agonists (carbachol, oxotremorine, acetylcholine, all present at 10^{-4} M) (Table 1). The highest specific binding (70% of total) was observed when methylatropine was used as a cold displacer. On the other hand, in the presence of the two non-muscarinic ligands GABA and noradrenaline (both at 10^{-3} M), no specific binding of [^{3}H]MS to GF/C filters could be detected, indicating the existence of a specificity for [^{3}H]MS binding to GF/C filters (Table 1).

Total binding of [^{3}H]MS to GF/C filters increased with increasing concentration of [^{3}H]MS, although at the maximal concentration used (10 nM), was only 0·1% of total radioactivity. Specific binding to GF/C filters was saturable (Fig. 1) and an affinity constant (Kd) of 1·2 nM could be calculated, which is similar to that reported for various tissues (Martos et al 1985; Malaisse et al 1985; Lee & El-Fakahany 1985).

If GF/C filters were presoaked in a 0·1% bovine serum albumin solution, total binding of [^{3}H]MS decreased by 45–60% and specific binding was also reduced to 15–30% (Table 1). On the other hand, pretreatment of filters with a 0·1% solution of poly-L-

Table 1. Binding of [^{3}H]methylscopolamine to glass fibre filters.

	[^{3}H]MS bound (counts min^{-1})	Specific binding (% of total)
GF/C filters		
No addition	240 ± 15	—
+ atropine 10^{-5} M	107 ± 9	55
+ methylatropine 10^{-5} M	72 ± 6	70
+ scopolamine 10^{-5} M	117 ± 9	51
+ methylscopolamine 10^{-5} M	84 ± 4	64
+ carbachol 10^{-4} M	104 ± 8	57
+ acetylcholine 10^{-4} M	112 ± 10	53
+ oxotremorine 10^{-4} M	91 ± 8	62
+ γ-aminobutyric acid 10^{-3} M	232 ± 16	3
+ noradrenaline 10^{-3} M	254 ± 21	0
GF/C filters presoaked in 0·1% bovine serum albumin		
No addition	132 ± 8	—
+ atropine 10^{-5} M	98 ± 6	26
GF/C filters presoaked in 0·1% poly-L-lysine		
No addition	45 ± 3	—
+ atropine 10^{-5} M	42 ± 2	7

[^{3}H]MS (1 nM) was incubated with 2 mL Hank's buffer for 1 h at 27 °C. Samples were filtered under vacuum and filters were washed three times with 3 mL of ice-cold buffer. The background counts were 30 ± 2 counts min^{-1} (n = 16), and were subtracted from each value. Each value represents the mean of (±s.e.m.) of 8–12 samples.

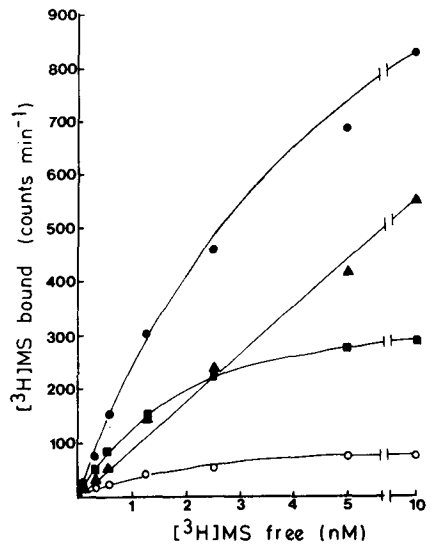

FIG. 1. Saturation binding of [^{3}H]methyl scopolamine ([^{3}H]MS) to Whatman GF/C filters. [^{3}H]MS was incubated for 1 h at 27 °C in Hank's buffer. Samples were filtered under vacuum and filters were washed three times with ice-cold buffer. Total binding is represented by the closed circles; non-specific binding (that observed in the presence of 10^{-5} M atropine) is indicated by the triangles. Squares represent specific binding. The open circles show the total binding of [^{3}H]MS to Whatman GF/C filter after they were presoaked in a 0·1% poly-L-lysine solution. Results are the mean of nine determinations.

lysine reduced the total binding of [^{3}H]MS to GF/C filters by more than 80% and virtually abolished specific binding (Table 1).

Binding of [^{3}H]MS to Whatman GF/B filters could also be detected. It was 30% higher than that to GF/C filters (315 ± 12 counts min^{-1} at a [^{3}H]MS concentration of 1 nM; n = 12) and 50% specific binding was observed in the presence of atropine (10^{-5} M) or carbachol (10^{-4} M). As previously observed, when GF/B filters were presoaked in 0·1% poly-L-lysine solution, binding of [^{3}H]MS was reduced by 75% (to 77 ± 4 counts min^{-1}; n = 12) and specific binding was negligible. When Millipore cellulose acetate filters (type EAWP 025) were used, binding of [^{3}H]MS was low (approximately 0·1% of total radioactivity; 53 ± 5 counts min^{-1}; n = 12) and specific binding (measured in the presence of 10^{-5} M atropine) was at most 4–6% of total binding, even at a higher concentration of [^{3}H]MS. For example, with a concentration of [^{3}H]MS of 5 nM, binding to the filter was 239 ± 20 and 227 ± 13 counts min^{-1} in the absence and presence of 10^{-5} M atropine, respectively.

These results appear to indicate the presence on glass fibre filters of a binding site for [^{3}H]MS, which has some of the pharmacological characteristics of muscarinic receptors, and reopen the problem of the binding of radioactive ligands to filters (see for example Cuatrecasas & Hollemberg 1975; Bielkiewicz & Cook 1985). With [^{3}H]MS, specific binding to filters is low in relation to the total amount of radioactivity, and may not play a relevant role when tissues with a high content of muscarinic receptors are being assayed. However, when the quantification of muscarinic receptors in tissues with low or very low receptor concentration, such as, for example certain cell types or in the gastropod mollusc *Aplysia* (Murray et al 1985), is attempted, filter binding could be a source of artifacts. While most studies of cholinergic muscarinic receptors have used [^{3}H]quinuclidinylbenzilate ([^{3}H]QNB) as a ligand (Yamamura & Snyder 1974), [^{3}H]MS has recently found increasing use. For example, [^{3}H]MS has been proved useful for binding studies in intact cells because of its lipophobicity (Gossuin et al 1984). Furthermore, it has been shown to have binding characteristics different from [^{3}H]QNB also in brain tissue (Lee & El-Fakahany 1985).

The results presented here indicate caution must be exercised when [^{3}H]MS is used as a muscarinic ligand and that measures to reduce filter binding (i.e. presoaking in poly-L-lysine or use of cellulose acetate filters) or alternative separation procedures (e.g. centrifugation) should be considered.

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