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PROPERTIES OF A QUINUCLIDINYL BENZILATE BINDING COMPONENT IN THE BULB MITE

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Abstract—1. About 95% of the specific binding (defined by atropine) of the muscarinic ACh receptor antagonist [^{3}H]-quinuclidinyl benzilate occurred in the low speed pellet (5500 *g*, 10 min) of whole bulb mite homogenates and was heat sensitive, linear with tissue concentration and saturable. Rosenthal analysis indicated that [^{3}H]QNB was binding with high affinity (K_d 474 pM) to a single class of low density (B_{max} 202 fmol g^{-1}) binding sites; the Hill coefficient was 1.0.

2. Kinetic studies revealed that binding was rapid and reversible, with association (k_{+1}) and dissociation (k_{-1}) rate constants of 7.8×10^5 sec^{-1} M^{-1} and 3.1×10^{-4} sec^{-1}, respectively. The K_d from kinetic data (k_{-1}/k_{+1}) was 414 pM.

3. Muscarinic drugs generally were much more potent than were nicotinic drugs as inhibitors of [^{3}H]QNB binding, and muscarinic antagonists generally were more potent than were agonists.

4. Binding was stereoselective because $R(-)$QNB was about 10,000 times more potent than $S(+)$-QNB, and dexetimide was about 1000 times more potent than its enantiomer levetimide as inhibitors of [^{3}H]QNB binding.

5. Pirenzepine, a selective antagonist of mammalian M-1 muscarinic receptors was very effective in protecting against [^{3}H]QNB binding, whereas methoctramine, a selective antagonist of M-2 muscarinic receptors, was much less effective.

6. Altogether these data suggest that the specific binding of [^{3}H]QNB is most likely to a popoulation of putative muscarinic ACh receptors, probably of the M-1 subtype.

INTRODUCTION

With few exceptions, studies of mite neurochemistry have been confined to the cholinergic system. ChE was the initial component of this system to be found in a mite, and it also has been the one most thoroughly examined. Casida (1955) showed that whole homogenates of the grain mite, *Acarus siro* L., hydrolyzed ACh; however, ACh deacetylation was not especially sensitive to inhibition by the organophosphate tetraethylpyrophosphate or the carbamate eserine. Most of the subsequent research on mite ChE's has been conducted with spider mites, particularly the twospotted spider mite, *Tetranychus urticae* Koch. Voss (1959, 1960) and Dauterman and Mehrotra (1963) demonstrated the presence of ChE in twospotted spider mite homogenates, and McEnroe (1963), using a histochemical technique, showed that the acetylthiocholine-hydrolyzing activity was restricted to the spider mite nervous system, with the stain being deposited in the synaptic area of the brain, along the surface of the nerves, and in local areas on the surface of the rostrum. Other research has extended our knowledge of the properties of mite ChE's (Smissaert, 1964; Voss and Matsumura, 1965; Motoyama and Saito, 1968; Smissaert *et al.*, 1970; Zahavi and Tahori, 1970; Zahavi *et al.*, 1971; Aziz and Knowles, 1974; Blank and Osborne, 1979).

ACh was the next cholinergic system component to be found in mites. Mehrotra (1961) demonstrated the presence of this compound in twospotted spider mites at a concentration of 25.0 μg/g of whole mites using paper chromatography, paper electrophoresis and pharmacology bioassay. Mehrotra (1963) also showed that twospotted spider mite homogenates synthesized ACh when fortified with radioactive choline and acetyl donor, providing evidence for the presence in these organisms of ChAT. Errampalli and Knowles (unpublished) found that the bulb mite, *Rhizoglyphus echinopus* (Fumouze and Robin), also possessed ChAT activity because homogenates synthesized ACh from choline and radioactive acetyl coenzyme A.

The presence in mites of three of the essential components of a cholinergic system therefore has been demonstrated. However, there apparently have been no published reports on mite ACh receptors or on any other neuroreceptors in these organisms. In the present study, we used radioactive QNB to label constituents in bulb mite homogenates that behaved kinetically and pharmacologically as putative muscarinic ACh receptors.

Abbreviations: ChE, cholinesterase; ACh, acetylcholine; ChAT, choline acetyltransferase; QNB, quinuclidinyl benzilate; demethylchlordimeform, *N'*-(4-chloro-*o*-tolyl)-*N*-methylformamidine HCl; SN 49844, *N'*-methylformamidine HCl; Tetram, *O,O*-diethyl-*S*-2-diethylaminoethyl phosphorothiolate hydrogen oxalate; DFP, di*iso*propyl phosphorofluoridate; R 16661, 2-(*O,S*-dimethylthiophosphorylimino)-3-ethyl-*S*-methyl-1,3-oxazolidine.

MATERIALS AND METHODS

Tissue preparation

Four grams of bulb mites from a laboratory colony maintained on a wheat germ-based artificial medium (Bot and Meyer, 1967) were subdivided into four lots of about 1 g

each. One lot was placed in a homogenizing tube containing about 10 ml of phosphate buffer (50 mM, pH 6.8) and homogenized with a Tissumizer (Model SDT-1810, Tekmar Company, Cincinnati, OH) at power setting 100 for 8–10 sec. The homogenate was filtered through eight layers of nylon cloth into a 250 ml beaker kept in ice. This procedure was repeated with the other three lots, the homogenates being filtered through the same nylon cloth into the beaker. The residue on the nylon cloth was rinsed several times with phosphate buffer, and the rinses were added to the filtrate. Buffer was added to the beaker contents to give a total volume (160 ml) of 40 times the mite weight. Equal aliquots of this preparation were added to four 50-ml centrifuge tubes and centrifuged (5500 *g*, 10 min, 4°C). The supernatants were discarded, and the pellets were resuspended in buffer to a volume of 30 times the mite weight. After centrifugation as above, the supernatants were discarded, and each pellet was resuspended in a small volume of buffer. The four preparations were combined, and buffer was added to give a final concentration of 100 mg of mites (wet weight) per ml equivalent to about 750 mites per ml. One ml contained an average of 2.63 mg of protein as determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. This preparation was kept on ice and used in binding experiments within 30 min.

Drugs and other chemicals

L-[Benzilic-4,4′-^{3}H(N)]-QNB (specific activity 44 Ci/mmol) was obtained from DuPont NEN® (Boston, MA). The following drugs were purchased from Research Biochemicals, Inc. (Natick, MA): R(−)-QNB, S(+)-QNB, ±QNB, (+)-*cis*-dioxolane, S(+)-dexetimide HCl, R(−)-levetimide HCl, oxotremorine sesquifumarate, methoctramine HCl, and pirenzepine diHCl. Atropine sulfate, (−)-scopolamine HCl, decamethonium bromide, carbamylcholine chloride, (−)-nicotine hydrogen tartrate, D-tubocurarine chloride, hexamethonium bromide, and ACh iodide were obtained from Sigma Chemical Co. (St Louis, MO). Other chemicals and sources included: SN49844 and formetanate hydrochloride from NOR-AM Chemical Co. (Wilmington, DE); azinphosmethyl from Mobay Chemical Corp. (Kansas City, MO); dichlorvos from Shell Development Co. (Modesto, CA); and Tetram from ICI (Bracknell, England).

Radioligand binding assays

For saturation experiments, 970 μl aliquots of tissue preparation were added to 1.5-ml plastic microcentrifuge tubes followed by 20 μl of [^{3}H]QNB; final concentrations of QNB ranged from 0.01 to 2.8 nM. Nonspecific binding was determined by adding 10 μl of 10 mM atropine sulfate (0.1 mM final concentration). Specific binding was calculated as the difference between the total binding (no atropine) and nonspecific binding. The tube contents were incubated for 30 min at room temperature, and binding was assessed by centrifugation and vacuum filtration methods.

In the centrifugation method, tube contents after incubation were centrifuged (11,000 *g*, 4 min), and the supernatants were carefully removed. After the pellet was rinsed with 1.5 ml of phosphate buffer, 500 μl of 1 N sodium hydroxide were added, and the tube contents were vortexed vigorously for about 10 sec. Following dissolution of the pellet, the tube was placed in a 20-ml glass scintillation vial, and 10 ml of Scintiverse II (Fisher Scientific Co., St Louis, MO) were added. After dark adaptation, the radioactivity was measured on a Beckman LS 7500 liquid scintillation counter at a counting efficiency of 41%.

For the filtration studies, saturation experiments were carried out as described above except in 10 ml glass tubes. After incubation, the tube contents were poured into the wells of a ten place manifold (Model FH 224, Hoefer Scientific Instruments, San Francisco, CA) containing wet prewashed glass fiber filters (Whatman GF/C) and vacuum filtered (12–16 mmHg). The filters were washed with about 3 ml of phosphate buffer and placed in glass scintillation vials. Scintillation fluid was added and the vial contents were shaken for about 1 hr and radioassayed as described above.

Duplicates were analyzed in each experiment, and were representative of at least three separate experiments. The apparent dissociation constant (K_d) and the maximum number of binding sites (B_{max}) were determined by a SAS program using nonlinear regression (SAS, 1982a,b). The Hill coefficient (n_H) was calculated from the saturation data using linear regression (Bennett, 1978).

Because it was necessary to use high tissue concentrations, filters frequently became clogged; therefore, the centrifugation method was used in subsequent experiments. To study on-rate kinetics, 970 μl of tissue preparation were incubated with 20 μl of [^{3}H]QNB (1.2 nM final concentration) for six time intervals ranging from 1 to 30 min prior to analysis by centrifugation, and the association rate constant (k_{+1}) was determined as described by Bennett (1978). For off-rate kinetics, 970 μl of tissue preparation were incubated with [^{3}H]QNB (1.2 nM final concentration) for 25 min when equilibrium was reached. Atropine (1.0 μM final concentration) was added, and specific binding was assessed by centrifugation at 10 different time intervals ranging from 1 to 60 min. The dissociation rate constant (k_{-1}) was determined as described by Bennett (1978).

For inhibition studies, drugs were dissolved and diluted in 5 mM hydrochloric acid (Bylund, 1987), except for azinphosmethyl which was dissolved in acetone. Tissue preparation (970 μl) was added to microcentrifuge tubes followed by inhibitor (10 μl) and [^{3}H]QNB (1.2–1.5 nM final concentration), and centrifugation assay was conducted after a 30 min incubation. Initially, potential inhibitors were evaluated at a final concentration of 10 μM. IC_{50} values, using 7–12 different final concentrations ranging from 1.0 mM to 0.1 pM, were determined for active inhibitors using a nonlinear least squares parametric curve fitting program (CDATA; EMF software, Knoxville, TN). IC_{50} values were converted to K_i values as described by Cheng and Prusoff (1973). Kinetic and inhibition data are means of duplicate analyses in at least two separate experiments.

RESULTS

The 5500 *g* pellet of bulb mite homogenates contained about 95% of [^{3}H]QNB specific binding, which was defined as total binding minus binding in presence of 0.1 mM atropine. When measured by the centrifugation method, specific [^{3}H]QNB binding, which could be abolished by heat and which was about 15% of total binding, was linear in the range of 0.025–0.25 g/ml (Fig. 1) and was saturable at radioligand concentrations of 1.5 nM and higher (Fig. 2). Mean (±SEM) B_{max} and K_d values calculated from the saturation curves from centrifugation analysis were 202.3 (23.0) fmol/g and 474.1 (16.2) pM ($N = 5$) (Table 1), respectively, whereas B_{max} and K_d values from filtration analysis were 209.7 (72.0) and 1037.2 (632.2) ($N = 2$), respectively. The Hill coefficient was 1.0 indicating noncooperativity (Fig. 3).

The specific binding of [^{3}H]QNB was fast and reached saturation in about 20 min with an association rate constant (k_{+1}) of 7.8×10^5 sec^{-1} M^{-1}; the dissociation rate constant (k_{-1}) was 3.2×10^{-4} sec^{-1} (Fig. 4, Table 1). The K_d calculated from the kinetic data (k_{-1}/k_{+1}) was 414 pM and was in close agreement with the value of 474 pM obtained from the saturation binding data (Table 1).

The pharmacological profile of [^{3}H]QNB binding to mite preparations is given in Table 2. It is apparent that muscarinic drugs like QNB, dioxolane,

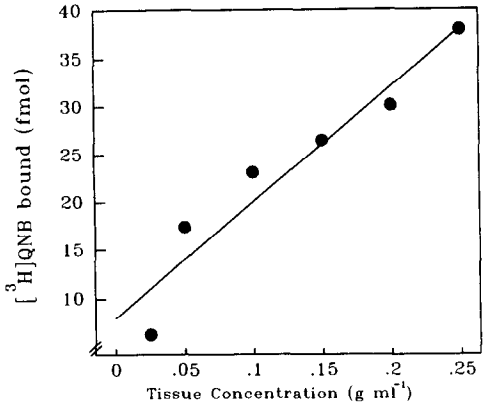

Fig. 1. Effect of varying the concentration of bulb mite tissue preparation on specific [^{3}H]QNB binding. Whole bulb mite homogenate was centrifuged (5500 *g*), and aliquots of resuspended pellet equivalent to 0.025 to 0.25 mg/ml were incubated with [^{3}H]QNB (1.2 nM). After 30 min, specific binding, as defined by 0.1 mM atropine, was measured by radioassay of the pellet obtained after centrifugation (11,000 *g*).

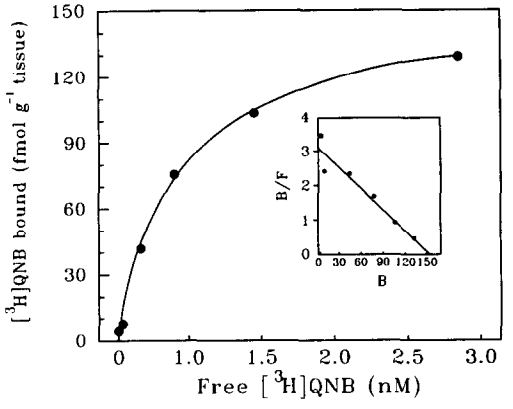

Fig. 2. Saturation binding curve and Rosenthal plot (inset) of specific [^{3}H]QNB binding to component in bulb mite tissue preparation. Aliquots (970 μl) of resuspended 5000 *g* pellet of bulb mite whole homogenate (100 mg/ml) were incubated with the various indicated concentrations of [^{3}H]QNB for 30 min, and specific binding, as defined by 0.1 mM, was measured by radioassay of the pellet obtained after centrifugation (11,000 *g*). Each point on the saturation curve is the mean of two replicates; the range was smaller than the size of the symbol. From the Rosenthal plot of this representative saturation curve, an apparent dissociation constant (K_D) of 491 pM and maximum number of binding sites (B_{max}) of 153 fmol/g of tissue (wet weight) were calculated.

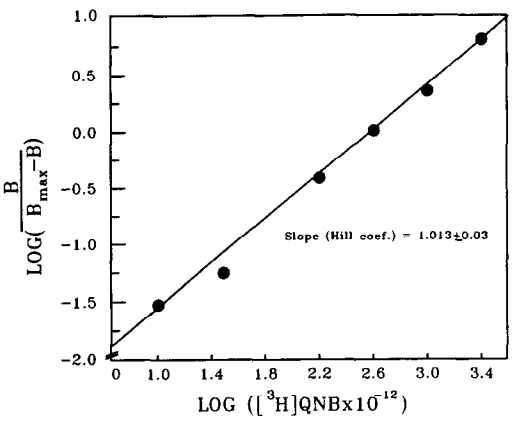

Fig. 3. Hill plot of saturation data from Fig. 2. Hill coefficient (n_H) of unity indicated noncooperativity.

dexetimide, scopolamine, atropine, pirenzepine and oxotremorine were strong inhibitors of QNB binding and were much more potent than were nicotinic drugs like decamethonium, nicotine, tubocurarine and hexamethonium. The two muscarinic antagonists, levetimide and methoctramine, were not potent inhibitors of [^{3}H]QNB binding and were exceptions to this generalization. Further, muscarinic antagonists like QNB, dexetimide, scopolamine, atropine and pirenzepine were more potent than were muscarinic agonists like oxotremorine, ACh and carbamylcholine, except for the agonist dioxolane. [^{3}H]QNB binding also was stereoselective because R(−)-QNB was about 10,000 times more potent than S(+)-QNB, and S(+)-dexetimide was about 1000 times more potent than its enantiomer R(−)-levetimide. Another interesting relationship was the fact that pirenzepine was about 1000 times more potent than methoctramine; these two antagonists can be used to differentiate muscarinic receptor subtypes in some organisms.

Some organophosphate (Tetram, azinphosmethyl), carbamate (formetanate), and formamidine (SN49844) acaricides also demonstrated some activity as inhibitors of [^{3}H]QNB binding.

DISCUSSION

Bulb mites are small organisms with the adults weighing about 133 ± 7.6 ng each. Because removal of nervous tissue was not practical, it was necessary to conduct binding studies with preparations from whole mite homogenates. Furthermore, the ratio of

Table 1. [^{3}H]QNB binding parameters from bulb mite and other organisms

Organism	K_d (equilibrium, nM)	k_{+1} (M^{-1} sec^{-1})	k_{-1} (sec^{-1})	K_d (k_{-1}/k_{+1}, nM)
Rhizoglyphus echinopus	0.47	7.8×10^5	3.2×10^{-4}	0.41
Periplaneta americana	8.0	1.3×10^5	2.4×10^{-4}	1.9
Locusta migratoria	0.8	2.2×10^6	9.9×10^{-4}	0.5
Musca domestica	2.4	4.2×10^4	1.2×10^{-4}	2.8
Drosophila melanogaster	0.7	2.0×10^6	3.0×10^{-4}	0.2
Guinea pig	0.4	6.7×10^6	2.0×10^{-4}	0.03
Chick	0.12	2.3×10^6	1.5×10^{-4}	0.06

Table modified after Lummis and Sattelle (1985) by addition of data from bulb mite and house fly. Tissue source and reference: *P. americana* nerve cords (Lummis and Sattelle, 1985); *L. migratoria* head ganglia (Breer, 1981); *M. domestica* heads (Shaker and Eldefrawi, 1981); *D. melanogaster* heads (Haim *et al.*, 1979); guinea pig ileum (Yamamura and Snyder, 1974); and chick brain (Siman and Klein, 1979).

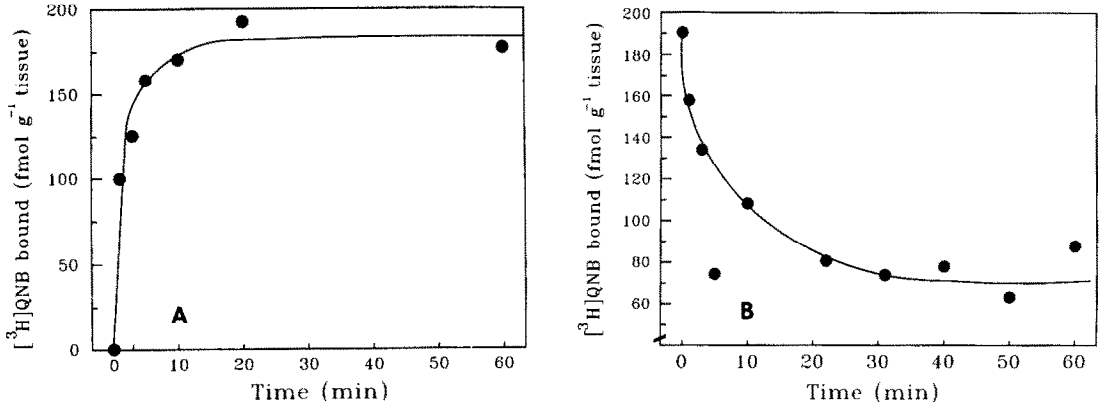

Fig. 4. Specific binding of [^{3}H]QNB to component in bulb mite tissue preparation as a function of time. The association curve (b) was determined by incubating aliquots (970 μl) of resuspended 5000 g pellet of bulb mite homogenate (100 mg/ml) with [^{3}H]QNB (1.2 nM) for indicated intervals of time, and specific binding, as defined by atropine (0.1 mM), was measured by radioassay of the pellets obtained after centrifugation (11,000 g). The dissociation curve (B) was determined by incubating aliquots (970 μl) of resuspended pellet with [^{3}H]QNB (1.2 nM) for 25 min (zero time on figure) to reach equilibrium. Atropine (1.0 μM) was added, and the specific binding of the radioligand was determined at indicated intervals of time by centrifugation and radioassay. The on rate constant (k_{+1}) was calculated to be 7.8×10^5 M^{-1} sec^{-1}, and the off rate constant (k_{-1}) was calculated to be 3.2×10^{-4} sec^{-1}; the dissociation rate constant (k_{-1}/k_{+1}) was calculated to be 414 pM.

nervous tissue to body weight is, apparently, quite low, and it was necessary to use many mites. This combination of whole homogenates of many mites resulted in preparations that had lower than desirable levels of specific QNB binding and contributed to decreased sensitivity with concommitant increased variability in some of the assays. Moreover, the preparations frequently clogged glass fiber filters necessitating use of the centrifugation assay even though the kinetics of QNB binding were such that the filtration assay otherwise would have been the technique of choice. The use of higher specific activity radioligands or purification of specific binding constituents or both could obviate some of these difficulties in future studies with other radioligands and mite preparations.

The above problems notwithstanding, we were able to demonstrate high affinity, saturable, rapid, reversible and stereoselective binding of the muscarinic ACh receptor antagonist [^{3}H]QNB to an apparent single class of binding sites in the low-speed pellet from whole bulb mite homogenates. Although the density (B_{max}) of the binding sites was rather low based on total body weight (202 fmol g^{-1} or 7.68 fmol mg protein^{-1}), it was within the same order of magnitude as that found in house fly, *Musca domestica* L., heads (800 fmol g^{-1} or 40 fmol mg protein^{-1}) by Shaker and Eldefrawi (1981) with the same radioligand. The kinetic parameters determined for [^{3}H]QNB binding to bulb mites were comparable on the whole to those determined for this radioligand and other organisms (Table 1).

Table 2. Pharmacological profile of bulb mite [^{3}H]QNB binding sites

Drug	K_i (+SEM), M
R(−)-QNB	$2.5\ (2.9) \times 10^{-11}$
(+)-*cis*-Dioxolane	$7.0\ (7.3) \times 10^{-11}$
(±)-QNB	$2.9\ (2.7) \times 10^{-10}$
S(+)-Dexetimide	$1.9\ (2.1) \times 10^{-9}$
Scopolamine	$1.0\ (2.7) \times 10^{-8}$
Atropine	$1.2\ (0.7) \times 10^{-8}$
Pirenzepine	$3.3\ (3.1) \times 10^{-8}$
S(+)-QNB	$1.1\ (1.3) \times 10^{-7}$
Oxotrmorine	$2.6\ (0.2) \times 10^{-7}$
Decamethonium	$1.1\ (1.0) \times 10^{-6}$
R(−)-Levetimide	$2.1\ (0.3) \times 10^{-6}$
Tetram	$3.4\ (3.3) \times 10^{-6}$
ACh + Dichlorvos (1.0 mM)	$4.9\ (0.7) \times 10^{-6}$
Carbamylcholine	$9.4\ (8.7) \times 10^{-6}$
SN49844	$1.7\ (1.3) \times 10^{-3}$
Azinphosmethyl	$3.2\ (0.7) \times 10^{-5}$
Methoctramine	$3.4\ (0.9) \times 10^{-5}$
Nicotine	$4.6\ (1.2) \times 10^{-5}$
Formetanate	$5.9\ (3.7) \times 10^{-5}$
Tubocurarine	$>1 \times 10^{-4}$
Hexamethonium	$>1 \times 10^{-4}$
Dichlorvos	$>1 \times 10^{-4}$

[^{3}H]QNB binding was inhibited by both muscarinic and some nicotinic drugs; however, muscarinic drugs were generally much more potent than were nicotinic drugs, and muscarinic antagonists generally were much more potent than antagonists. There were three apparent exceptions to these generalizations. Dioxolane, a muscarinic agonist, was almost as potent as R(−)-QNB which was the most effective inhibitor of [^{3}H]QNB binding examined in this study. However, dioxolane is a 'super potent' muscarinic receptor agonist (Triggle and Belleau, 1962), thus its high activity was not unexpected. Levetimide and methoctramine, both muscarinic antagonists, possessed low inhibitory activity. Levetimide, the enantiomer of the active dexetimide, has been reported to be 'inactive' on muscarinic receptors (Janssen *et al.*, 1971). Methoctramine, although a muscarinic antagonist, is most active against the inhibitory M-2 receptor subtype (Melchiorre *et al.*, 1987). Its low activity against bulb mite preparations could indicate that muscarinic M-2 receptors were not present. In this context it should be mentioned that pirenzepine, a selective antagonist of the excitatory M-1 receptor subtype (Watson *et al.*, 1985; Roeske *et al.*, 1987), was very active against the binding of [^{3}H]QNB to bulb mite preparations. In mammals high densities of high affinity [^{3}H]pirenzepine binding sites (putative M-1

sites) have been found in the central nervous system and ganglia, whereas low affinity [^{3}H]pirenzepine binding sites (putative M-2 sites) have been observed in the central nervous system and in some peripheral tissues such as ileum and heart (Watson *et al.*, 1985). The fact that pirenzepine has high affinity for putative M-2 muscarinic receptors, which thus far have been found only in central nervous tissue, and that this drug was a potent inhibitor of [^{3}H]QNB binding in our study points toward a central nervous system localization of the bulb mite [^{3}H]QNB binding sites.

ACh in the presence of the organophosphate dichlorvos also inhibited [^{3}H]QNB binding as expected, and its level of activity compared favorably with that reported from other organisms (Satelle, 1980). It was necessary to use a ChE inhibitor such as dichlorvos in the assay of ACh because about 20% of the total ChE activity resides in this centrifugal fraction and because bulb mite ChE activity was completely inhibited by dichlorvos (1.0 mM) but was markedly insensitive to eserine (Errampalli and Knowles, unpublished). Dichlorvos alone did not block [^{3}H]QNB binding, providing evidence that the radioligand was not binding to the ChE.

The pharmacological profile of the [^{3}H]QNB binding sites in bulb mite preparations definitely was muscarinic. The most active nicotinic drug was decamethonium, and its K_i value was several orders of magnitude higher than the most active muscarinic ligands.

Bylund (1985) listed four criteria that, if satisfied, suggests that the observed binding of a radioligand is to a physiologic receptor. Binding should (1) be saturable and high affinity, (2) be rapid and reversible, (3) have the correct pharmacologic specificity, and (4) have a physiologically relevant distribution. The binding of [^{3}H]QNB to bulb mite preparations satisfied the first three of these criteria. The criterion of physiologically relevant distribution remains to be satisfied; however, the fact that specific [^{3}H]QNB binding resided in a low-speed centrifugal fraction suggested that the binding sites were associated with membranes. Moreover, as discussed above, the strong effect of pirenzepine provides a basis for suggesting that the [^{3}H]QNB is binding to mite central nervous system tissue. All of the evidence in this study is consistent with the identity of the bulb mite [^{3}H]QNB binding sites as putative muscarinic ACh receptors, probably of the M-1 subtype. However, additional research showing that the specific [^{3}H]QNB binding sites are localized in nervous tissue of the mite is needed.

Comments regarding the acaricidal activity of some of the ligands are appropriate. Bigg and Purvis (1976) reported that several muscarinic agonists including oxotremorine were toxic to ticks and mites; however, the mite species was not indicated, and toxicity data for the mite were not provided. It is possible that some of the muscarinic ligands used in the present study are toxic to mites. Two organophosphates (Tetram and azinphosmethyl) and a carbamate (formetanate) displayed some affinity for the [^{3}H]QNB binding sites. All three compounds are toxic to bulb mites (Knowles *et al.*, 1988). Interestingly, Tetram and formetanate are direct inhibitors of ChE and inhibit bulb mite ChE *in vitro* (Errampalli and Knowles, unpublished). However, azinphosmethyl is a phosphorodithioate and requires conversion to its phosphorothiolate analog (Guthoxon) prior to becoming a potent ChE inhibitor. This effect on [^{3}H]QNB binding by an organophosphate such as azinphosmethyl that is not a direct ChE inhibitor constitutes further evidence that the radioligand is not binding to this enzyme. Others have observed that some organophosphates and carbamates can compete with cholinergic radioligand binding to putative ACh receptors. For example, Eldefrawi *et al.* (1971) showed that the organophosphates DFP, Guthoxon, Tetram and R16661 at 0.1 mM blocked the binding of [^{3}H]nicotine and [^{3}H]decamethonium to preparations from house fly brain and the electroplaxes of *Torpedo* and *Electrophorus* and that Tetram reversibly blocked the binding of [^{3}H]muscarone and [^{3}H]ACh to *Torpedo electroplex*. Bakry *et al.* (1988) found that four organophosphate nerve agents (soman, sarin, tabun and VX) and one therapeutic organophosphate (echothiophate) were active on nicotinic ACh receptors from *Torpedo* electric organ and muscarinic ACh receptors from rat brain and N1E-115 neuroblastoma cultures. Of special interest was the potency of these compounds in blocking the binding of [^{3}H]-*cis*-methyldioxolane to a small population of muscarinic ACh receptors. Also, VX and echothiophate, but not sarin, soman and tabun, inhibited the binding of [^{3}H]QNB and [^{3}H]pirenzepine to muscarinic ACh receptors. The carbamates neostigmine and pyridostigmine were shown to inhibit the binding of [^{3}H]ACh to electroplax tissue (Eldefrawi *et al.*, 1972), and eserine and neostigmine were found to inhibit the binding of [^{125}I]-α-bungarotoxin to extracts of *Drosophila* (Schmidt-Nielsen *et al.*, 1977). It has been suggested that perturbation of ACh receptors by organophosphates could play a role in their insecticidal action (Eldefrawi *et al.*, 1971; Jones *et al.*, 1979). The results from the present study provide a basis for suggesting that the direct action of some organophosphates and carbamates with putative muscarinic ACh receptors might play a role in their acaricidal action.

Some formamidines like chlordimeform and its *N*-demethyl metabolite demethylchlordimeform are octopamine agonists in insects (Hollingworth and Lund, 1983) and α_2-adrenoceptor agonists in mice (Costa and Murphy, 1987), and they have been shown to have some inhibitory activity against [^{3}H]QNB binding to mouse forebrain preparations with IC_{50} values of 3.1×10^{-4} M and 1.6×10^{-4} M, respectively (Costa and Murphy, 1987). In the present study, we observed that SN 49844, a formamidine metabolite of the acaricide amitraz in mites (Franklin and Knowles, 1984), possessed some activity against [^{3}H]QNB binding. The mode of action of formamidines in mites presently is not clearly understood; however, it is possible that pertubation of muscarinic ACh receptors is involved in some cases.

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REFERENCES

Aziz S. A. and Knowles C. O. (1974) Hydrolysis of indophenyl acetate and thiocholine esters by twospotted spider mites (Acarina: Tetranychidae). *J. Kans. ent. Soc.* **47,** 239–243.

Bakry N. M. S., El-Rashidy A. H., Eldefrawi A. T. and Eldefrawi M. E. (1988) Direct actions of organophosphate anticholinesterases on nicotinic and muscarinic acetylcholine receptors. *J. Biochem. Toxicol.* **3,** 235–259.

Bennett J. P. Jr. (1978) Methods in binding studies. In *Neurotransmitter Receptor Binding* (Edited by Yamamura H. I., Enna S. J. and Kuhar M. J.), pp. 57–90. Raven Press, New York.

Bigg D. C. H. and Purvis S. R. (1976) Muscarinic agonists provide a new class of acaricide. *Nature* **262,** 220–222.

Blank R. H. and Osborne G. O. (1979) Studies on cholinesterase of the mites of *Sancassania berlesei* (Tyroglyphidae) and *Tetranychus urticae* (Tetranychidae): substrate-activity relationships and organophosphorus acaricide inhibition. *N.Z.J. agric Res.* **22,** 491–496.

Bot J. and Meyer M. K. P. (1967) An artificial rearing medium for acarid mites. *J. ent. Soc. sth Afr.* **29,** 199.

Breer H. (1981) Properties of putative nicotinic and muscarinic cholinergic receptors in the central nervous system of *Locusta migratoria*. *Neurochem. Int.* **3,** 43–52.

Bylund D. B. (1985) Neurotransmitters and their receptors. 4. Methodologic aspects. Society for Neuroscience, Washington, DC, U.S.A.

Bylund D. B. (1987) Biochemistry and pharmacology of the alpha-1 adrenergic receptor. In *The Alpha-1 Adrenergic Receptors* (Edited by Ruffolo R. R. Jr), pp. 19–69. The Humana Press, Clifton, New Jersey, U.S.A.

Casida J. E. (1955) Comparative enzymology of certain insect acetylesterases in relation to poisoning by organophosphorus insecticides. *Biochem. J.* **60,** 487–496.

Cheng Y. and Prusoff W. H. (1973) Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmac.* **22,** 3099–3108.

Costa L. G. and Murphy S. D. (1987) Interaction of the pesticide chlordimeform with adrenergic receptors in mouse brain: an *in vitro* study. *Arch. Toxic.* **59,** 323–327.

Dauterman W. C. and Mehrotra K. N. (1963) The *N*-alkyl group specificity of cholinesterase from the house fly, *Musca domestica* L., and the two-spotted spider mite, *Tetranychus telarius* L. *J. Insect Physiol.* **9,** 257–263.

Eldefrawi M. E., Britten A. G. and O'Brien R. D. (1971) Action of organophosphates on binding of cholinergic ligands. *Pestic. Biochem. Physiol.* **1,** 101–108.

Eldefrawi M. E., Eldefrawi A. T., Seifert S. and O'Brien R. D. (1972) Properties of a Lubrol-solubilized acetylcholine receptor from *Torpedo* electroplax. *Arch Biochem. Biophys.* **150,** 210–218.

Franklin E. J. and Knowles C. O. (1984) Degradation of xylyl formamidines by the twospotted spider mite (Acari: Tetranychidae). *J. econ. Ent.* **77,** 1405–1408.

Haim N., Nahum S. and Dudai Y. (1979) Properties of a putative muscarinic cholinergic receptor from *Drosophila melanogaster*. *J. Neurochem.* **32,** 543–552.

Hollingworth R. M. and Lund A. E. (1983) Behavioral and lethal action of amidines on invertebrates. In *Pesticide Chemistry: Human Welfare and the Environment* (Edited by Miyamoto J. and Kearney P. C.), pp. 15–24, Vol. 3, Pergamon Press, Oxford.

Janssen P. A. J., Niemegeers C. J. E., Schellekens K. H. L., Demoen P., Lenaerts F. M., van Nueten J. M., van Wijngaarden I. and Brugmans J. (1971) Benzetimide and its optical isomers. *Arzneim.-Forsch.* **21,** 1365–1373.

Jones S. W., Sudersham P. and O'Brien R. D. (1979) Interaction of insecticides with acetylcholine receptors. In *Neurotoxicology of Insecticides and Phermones* (Edited by Narahashi T.), pp. 259–275. Plenum Press, New York.

Knowles C. O., Errampalli D. D. and El-Sayed G. N. (1988) Comparative toxicities of selected pesticides to bulb mite (Acari: Acaridae) and twospotted spider mite (Acari: Tetranychidae). *J. econ. Ent.* **81,** 1586–1591.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193,** 265–275.

Lummis S. C. R. and Sattelle D. B. (1985) Binding of *N*-[propionyl-^{3}H]-propionylated α-bungarotoxin and L-[benzilic-4,4′-^{3}H]quinuclidinyl benzilate to CNS extracts of the cockroach *Periplaneta americana*. *Comp. Biochem. Physiol.* **80C,** 75–83.

McEnroe W. D. (1963) Esterases in the two-spotted spider mite. In *Adv. in Acarol.* Vol. 1 (Edited by Naegele J. A.), pp. 214–224. Comstock, Ithaca, New York.

Mehrotra K. N. (1961) The occurrence of acetylcholine in the two-spotted spider mite, *Tetranychus telarius* L. *J. Insect Physiol.* **6,** 180–184.

Mehrotra K. N. (1963) Biochemistry of nerve function in Acarina. In *Adv. in Acarol.* Vol. 1 (Edited by Neagele J. A.), pp. 209–213. Comstock, Ithaca, New York.

Melchiorre C., Cassinelli A. and Quaglia W. (1987) Differential blockade of muscarinic receptor subtypes by polymethylene tetramines. Novel class of selective antagonists of cardiac M-2 muscarinic receptors. *J. med. Chem.* **30,** 201–204.

Motoyama N. and Saito T. (1968) Substrate specificity of cholinesterase in mites. *Botyu-Kagaku* **33,** 77–80.

Roeske W. R., Wang J. X., Gulya K., Korc M., Serra M., Akerman M., Mei L., Vickroy T., Watson M. and Yamamura H. I. (1987) Biochemical, functional and anatomic evidence for putative muscarinic receptor subtypes using selective antagonists [^{3}H]AFDX-116 and [^{3}H]pirenzepine. In *Cell Mol. Basis Cholinergic Function* (Edited by Dowdall M. J. and Hawthorne J. N.), pp. 64–71. Horwood, Chichester, England.

SAS Institute (1982a) *User's Guide: Statistics*. SAS Institute, Cary, North Carolina.

SAS Institute (1982b) *User's Guide: Basic*. SAS Institute, Cary, North Carolina.

Sattelle D. B. (1980) Acetylcholine receptors of insects. In *Advances in Insect Physiol* (Edited by Berridge M. J., Treherne J. E. and Wigglesworth V. B.), pp. 215–315. Vol. 15. Academic Press, London.

Schmidt-Nielsen B. K., Gepner J. I., Teng N. N. H. and Hall L. M. (1977) Characterization of an α-bungarotoxin binding component from *Drosophila melanogaster*. *J. Neurochem.* **29,** 1013–1029.

Shaker N. and Eldefrawi A. (1981) Muscarinic receptor in housefly brain and its interaction with chlorobenzilate. *Pestic. Biochem. Physiol.* **15,** 14–20.

Siman R. G. and Klein W. L. (1979) Cholinergic activity regulates muscarinic receptors in central nervous system cultures. *Proc. natn. Acad. Sci. U.S.A.* **76,** 4141–4145.

Smissaert H. R. (1964) Cholinesterase inhibition in spider mites susceptible and resistant to organophosphates. *Science* **143,** 129–131.

Smissaert H. R., Voerman S., Oosterbrugge L. and Renooy N. (1970) Acetylcholinesterases of organophosphate-susceptible and resistant spider mites. *J. agric. Fd Chem.* **18,** 66–75.

Triggle D. J. and Belleau B. (1962) Studies on the chemical basis for cholinomimetic and cholinolytic activity. Part I. The synthesis and configuration of quaternary salts in the 1,3-dioxolane and oxazoline series. *Can. J. Chem.* **40,** 1201–1215.

Voss G. (1959) Esterasen bei der Spinnmilbe, *Tetranychus urticae* Koch (Acari, Trombidiformes, Tetranychidae). *Naturwiss.* **46,** 652.

Voss G. (1960) Esterasen bei der Spinnmilbe, *Tetranychus urticae* Koch (Acari, Trombidiformes, Tetranychidae). *Naturwiss.* **47,** 400–401.

Voss G. and Matsumura F. (1965) Biochemical studies on a modified and normal cholinesterase found in the Leverkusen strains of the twospotted spider mite. *Can. J. Biochem.* **43,** 63–72.

Watson M., Vickroy T. W., Roeske W. R. and Yamamura H. I. (1985) Functional and biochemical basis for multiple muscarinic acetylcholine receptors. *Prog. Neuro-Psychopharmac. Biol. Psychiat.* **9,** 569–584.

Yamamura H. I. and Snyder S. H. (1974) Muscarinic cholinergic receptor binding in the longitudinal muscle of the guinea pig ileum with [^{3}H]quinuclidinyl benzilate. *Molec. Pharmac.* **10,** 861–867.

Zahavi M. and Tahori A. S. (1970) Sensitivity of acetylcholinesterase in spider mites to organophosphorus compounds. *Biochem. Pharmac.* **19,** 219–225.

Zahavi M., Tahori A. S. and Klimer F. (1971) Insensitivity of acetylcholinesterases to organophosphorus compounds as related to size of esteratic site. *Molec. Pharmac.* **7,** 611–619.