

NICOTINIC AND MUSCARINIC CHOLINERGIC RECEPTORS IN HONEY BEE (*APIS MELLIFERA*) BRAIN

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Abstract—1. Honey bee head homogenates contained particulate components capable of binding the nicotinic receptor antagonist α -bungarotoxin (BGT) and the muscarinic receptor antagonist quinuclidinyl benzilate (QNB). Specific binding of [125 I]BGT (defined by nicotine) and [3 H]QNB (defined by atropine) was heat sensitive, linear with tissue concentration, and saturable. B_{\max} values were 204 fmol mg protein $^{-1}$ for [125 I]BGT and 57 fmol mg protein $^{-1}$ for [3 H]QNB yielding a binding site ratio of 3.6:1. Hill coefficients were 1.0 for each radioligand.

2. Binding by both radioligands was rapid and reversible. Association (k_{+1}) and dissociation (k_{-1}) rates were $1.38 \times 10^6 \text{ s}^{-1} \text{ M}^{-1}$ and $6.2 \times 10^{-4} \text{ s}^{-1}$ for [125 I]BGT and $3.27 \times 10^6 \text{ s}^{-1} \text{ M}^{-1}$ and $9.4 \times 10^{-5} \text{ s}^{-1}$ for [3 H]QNB. The dissociation rate constants (K_D) were 450 pM (k_{-1}/k_{+1}) and 743 pM (saturation) for [125 I]BGT and 30 pM (k_{-1}/k_{+1}) and 96 pM (saturation) for [3 H]QNB.

3. Pharmacological profiles were nicotinic for [125 I]BGT with nicotine (K_i , $2.6 \times 10^{-7} \text{ M}$), D-tubocurarine (K_i , $1.0 \times 10^{-6} \text{ M}$), and ACh + dichlorvos (K_i , $4.5 \times 10^{-6} \text{ M}$) being the most potent inhibitors and muscarinic for [3 H]QNB with (\pm)-QNB (K_i , $2.2 \times 10^{-12} \text{ M}$), S(+)-dextetimide (K_i , $2.9 \times 10^{-11} \text{ M}$), atropine (K_i , $6.9 \times 10^{-10} \text{ M}$), and scopolamine (K_i , $4.1 \times 10^{-10} \text{ M}$) being most potent.

4. It appeared that [125 I]BGT and [3 H]QNB were binding with high affinity to honey bee brain populations of nicotinic and muscarinic cholinergic receptors, respectively.

INTRODUCTION

Although the cholinergic system in insects generally has been extensively studied, there have been relatively few investigations of its components in honey bees, *Apis mellifera* L., with the exception of AChE. Using biochemical and histochemical approaches, the properties and localization of honey bee head AChE have been determined (Richards and Cutkomp, 1945; Casida, 1955; Metcalf *et al.*, 1955; Wolfe and Smallman, 1956; Kunkee and Zweig, 1963; Krysan and Kruckeberg, 1970; Booth and Metcalf, 1972; Kral and Schneider, 1981; Belzunces *et al.*, 1988; Kreissl and Bicker, 1989). Honey bee brain homogenates also were found to contain ACh at a concentration of 2.56 pmol μg protein $^{-1}$ (Fuchs *et al.*, 1989). With respect to cholinergic receptors, Kreissl and Bicker (1989) demonstrated binding of locust nicotinic ACh receptor antiserum to certain honey bee brain components. They concluded that the antiserum cross-reacted with honey bee ACh receptors, an interpretation supported by the fact that BGT binding sites were present in some areas of strong immunoreactivity (Kreissl and Bicker, 1989). Sherby *et al.* (1986), using cation exchange chromatography, observed specific binding of radioactive BGT to honey

bee head membranes. In this paper we used biochemical and pharmacological approaches to gain further insight into honey bee brain ACh receptors.

MATERIALS AND METHODS

Insects

Worker bees from colonies maintained by the Department of Entomology were shaken from combs directly onto dry ice and kept in a freezer at -88°C until used.

Drugs and other chemicals

[125 I]BGT (specific activity 18.6 $\mu\text{Ci g}^{-1}$ or 148.8 Ci mmol $^{-1}$) was obtained from Dupont NEN (Boston, MA). It was diluted with distilled water, divided into aliquots ranging from 2 to 10 μCi each, and kept in glass tubes at -20°C until required. L-[Benzilic-4,4'- ^3H (N)]QNB (specific activity 45.7 Ci mmol $^{-1}$) also was purchased from NEN. The following drugs were obtained from Research Biochemicals, Inc. (Natick, MA): S(+)-QNB, (\pm)-QNB, (+)-*cis*-dioxolane, S(+)-dextetimide HCl, R(-)-levetimid HCl, and oxotremorine sesquifumarate. Atropine sulfate, (-)-scopolamine HCl, decamethonium bromide, (-)-nicotine hydrogen tartrate, D-tubocurarine chloride, hexamethonium bromide, ACh iodide, and eserine sulfate were purchased from Sigma Chemical Co. (St Louis, MO). Other chemicals included amitraz, SN 49844, chlordimeform, demethylchlordimeform, and formetanate hydrochloride from NOR-AM Chemical Co. (Wilmington, DE); azinphosphmethyl from Mobay Chemical Corp. (Kansas City, MO); dichlorvos from Shell Development Co. (Modesto, CA), and Tetram from ICI (Bracknell, U.K.).

Tissue preparation

For BGT studies, whole bee heads were homogenized in phosphate buffer (50 mM, pH 7.2) containing 1.0 mM ethylenediaminetetra acetic acid (Sigma) and 1.0 mM

Abbreviations: AChE, acetylcholinesterase; ACh, acetylcholine; BGT, α -bungarotoxin; QNB, quinuclidinyl benzilate; demethylchlordimeform, *N'*-(4-chloro-*o*-tolyl)-*N*-methylformamidinium HCl; SN 49844, *N'*-(2,4-xylyl)-*N*-methylformamidinium HCl; Tetram, *O,O*-diethyl-*S*-2-diethylaminoethyl phosphorothiolate hydrogen oxalate. This paper is a contribution from the Missouri Agricultural Experiment Station, Journal Series No. 11,182.

phenylmethylsulfonyl fluoride (Sigma) with a Tissumizer (model SDT-1810, Tekmar Co., Cincinnati, OH) at power setting 100 for 8–10 s. In some experiments the homogenizing medium also contained Triton X-100 (Sigma) at a final concentration of 0.5 or 1.0%. The homogenate was filtered through eight layers of Nylon cloth and centrifuged (1000 g, 10 min, 4°C). The 1000 g supernatant was centrifuged (40,000 g, 10 min), and the pellet was resuspended and centrifuged (40,000 g, 10 min) again. The 40,000 g pellet was resuspended with buffer usually to a concentration equivalent to one head ml⁻¹. Typical particulate preparations with out Triton contained 0.34 ± 0.02 mg protein ml⁻¹ (n = 4) and those with the detergent (1.0%) contained 1.74 ± 0.03 mg protein ml⁻¹ (n = 10) as determined by the Lowry method with bovine serum albumin (Sigma) as standard (Lowry *et al.*, 1951; Wang and Smith, 1975).

For QNB studies, bee heads were homogenized in phosphate buffer (50 mM, pH 7.8), diluted with buffer to a concentration of one head ml⁻¹, and filtered through eight layers of Nylon cloth. The filtrate was centrifuged (40,000 g, 20 min), and the pellet was resuspended to a concentration of 0.5 heads ml⁻¹ and centrifuged (40,000 g, 20 min). The 40,000 g pellet was resuspended with buffer usually to a concentration equivalent to one head ml⁻¹. Typical preparations yielded 0.46 ± 0.01 mg protein ml⁻¹ (n = 6).

Radioligand binding assays

For saturation experiments, aliquots of bee preparation were added to 10 ml glass tubes followed by radioligand. Nonspecific binding was determined by adding nicotine in studies with BGT or atropine in studies with QNB. Specific binding was calculated as the difference between the total binding and nonspecific binding. Tube contents were incubated for 40 min at room temperature.

After incubation, the tube contents were poured into the wells of a manifold (model FH224, Hoefer Scientific Instruments, San Francisco, CA) containing wet glass-fiber filters (Whatman GF/B) and vacuum filtered (12–16 mm Hg). In studies with BGT, the filters were presoaked at least 40 min in 0.05% polyethylenimine (Sigma). Filters were washed four times with 2 ml aliquots of phosphate buffer and transferred to glass scintillation vials. Ten ml of Scintiverse BD (Fisher Scientific Co., St Louis, MO) were added, and the radioactivity was measured on a Beckman LS 7500 liquid scintillation spectrometer at counting efficiencies of 59% and 41% for radioactive BGT and QNB, respectively.

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).

The kinetics of association were studied by incubating an aliquot of bee head homogenate prepared as described above with [¹²⁵I]BGT or [³H]QNB. Another aliquot of homogenate was incubated with [¹²⁵I]BGT plus nicotine or [³H]QNB plus atropine. At selected time intervals, aliquots were analyzed by filtration and radioassay. The specific binding data obtained were fitted through a nonlinear regression program in SAS to the following equation to compute $K_{observed}$:

$$B = B_e \cdot (1 - e^{-K_{obs}t})$$

where B is the bound counts min⁻¹ at time t and B_e is the BGT bound in counts min⁻¹ at equilibrium. The association rate (k_{+1}) was calculated according to the following equation:

$$k_{+1} = \frac{K_{ob} - k_{-1}}{[Free radioligand]}$$

The kinetics of dissociation were studied by incubating bee head homogenate with [¹²⁵I]BGT or [³H]QNB until equilibrium was reached. Nicotine (for BGT) or atropine (for

QNB) was added, and aliquots were analyzed by filtration and radioassay at selected time intervals. The dissociation rate (k_{-1}) was determined by nonlinear regression according to the following equation:

$$B = B_0 \cdot e^{-k_{-1}t}$$

where B is the bound counts min⁻¹ at time t , and B_0 is the bound counts min⁻¹ at time zero.

For inhibition studies, ligands were dissolved and diluted in 5.0 mM hydrochloric acid (Bylund, 1987), except for (+)-QNB (methanol), (±)-QNB (methanol), azinphosmethyl (acetone), and amitraz (acetone). Aliquots of bee head homogenate were incubated with ligand and radioligand for 40 min prior to filtration and radioassay. In the case of ACh, bee head homogenate was preincubated for 15 min with dichlorvos or eserine (to inhibit AChE activity) prior to addition of ACh and radioligand. IC_{50} values were determined 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).

RESULTS

In preliminary experiments, we found that greater than 97% of the [¹²⁵I]BGT binding in bee head homogenates was associated with the 40,000 g particulate fraction. Moreover, the [¹²⁵I]BGT binding occurred only with brain tissue because no difference in binding of this radioligand was observed between particulate fractions of bee head and bee brain preparations when tested at a homogenate concentration equivalent to one head or brain ml⁻¹. Total and nonspecific binding values (counts min⁻¹) for head preparations were 1215 ± 36 and 193 ± 1, respectively, and for brain preparations were 1262 ± 31 and 178 ± 32, respectively. No [¹²⁵I]BGT binding was observed with homogenates of hypopharyngeal gland or head salivary glands. The addition of Triton X-100 to bee head homogenates did not affect [¹²⁵I]BGT binding. A bee head homogenate used after filtration through Nylon cloth and tested without centrifugation yielded total and nonspecific binding values (counts min⁻¹) of 1211 ± 44 and 243 ± 54, respectively.

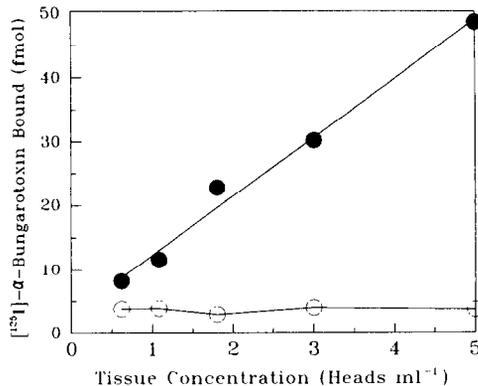


Fig. 1. Effect of varying the concentration of honey bee head tissue preparation on [¹²⁵I]BGT binding. Legend: ● = specific binding, ○ = nonspecific binding. Aliquots of resuspended 40,000 g pellet to give indicated final tissue equivalents were incubated for 40 min with [¹²⁵I]BGT (0.44 nM), and the specific (defined by 0.1 mM nicotine) and nonspecific binding was measured by radioassay after filtration. Each point is the mean of two replicates.

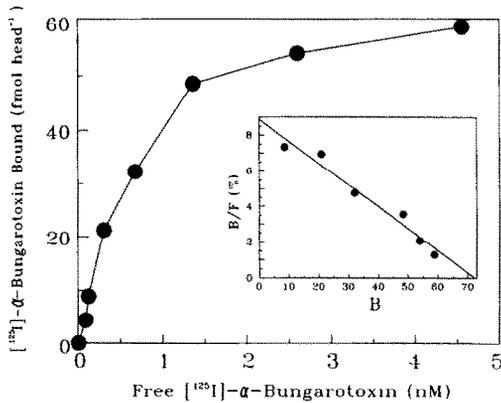


Fig. 2. Representative saturation binding curve and Rosenthal plot (inset) of specific [¹²⁵I]BGT binding to components in honey bee head homogenates. Aliquots (970 μ l) of resuspended 40,000 g pellet to give a final concentration equivalent to 1 head ml^{-1} were incubated for 40 min with indicated concentrations of [¹²⁵I]BGT, and the specific binding (defined by 0.1 mM nicotine) was determined by radioassay after filtration. Each point on the saturation curve is the mean of two replicates. Linear estimates for K_D and B_{max} from these data were 814.4 pM and 72.4 fmol head⁻¹, respectively. Mean nonlinear estimates for K_D and B_{max} values from three separate experiments were 742.8 ± 95.0 pM and 69.3 ± 0.7 fmol head⁻¹, respectively.

ively, whereas those values from a homogenate prepared with Triton X-100 (1.0%) were 1300 ± 122 and 197 ± 8 , respectively. [¹²⁵I]BGT binding activity by the detergent-treated bee head preparation was similar over the pH range 6.5–8.5 and was stable for two to three days at 4°C. A bee head homogenate heated for 10 min at 100°C gave a total [¹²⁵I]BGT binding value of 215 ± 24 counts min^{-1} .

The effect of varying the concentration of bee head preparation on [¹²⁵I]BGT binding is shown in Fig. 1.

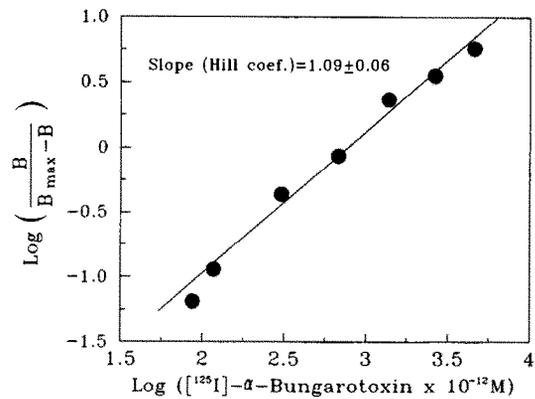


Fig. 3. Hill plot of [¹²⁵I]BGT saturation data from Fig. 2.

The specific binding was proportional to tissue concentration in the range of 0.5–5 heads ml^{-1} . Depending upon tissue concentration, specific binding ranged from 68 to 93% of total binding. It averaged about 85% of total binding at the concentration of 1 head ml^{-1} . [¹²⁵I]BGT specific binding was saturable at radioligand concentrations of 2.7 nM and higher (Fig. 2). Mean K_D and B_{max} values were 742.8 ± 95.0 pM and 69.3 ± 0.7 fmol head⁻¹ (203.8 fmol mg protein⁻¹); those values in presence of Triton X-100 (0.5%) (graphs not shown) were 732.9 ± 103.6 pM and 45.9 ± 12.8 fmol head⁻¹. The differences between the respective values obtained by the two methods of tissue preparation were not significantly different (*t*-test, $t_{2,0.05} = 2.59$, $P > 0.5$). A Hill plot of the saturation data in Fig. 2 yielded a coefficient of 1.09 ± 0.06 (Fig. 3).

[¹²⁵I]BGT binding to bee head preparations was rapid and reached equilibrium in about 40 min with an association rate (k_{+1}) of $1.38 \times 10^6 \text{ s}^{-1} \text{ M}^{-1}$ (Fig. 4). It also was reversible with a dissociation rate

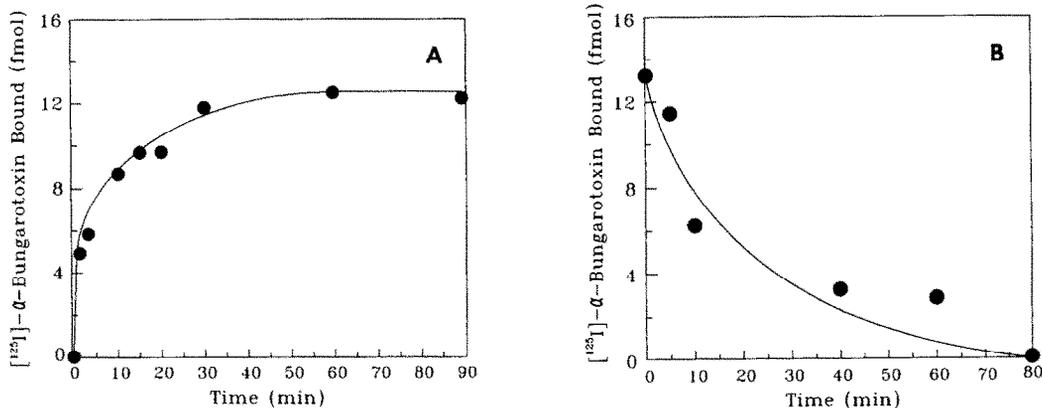


Fig. 4. Specific binding of [¹²⁵I]BGT to honey bee head preparations as a function of time. The association curve (A) was obtained by incubating 20 ml of the resuspended 40,000 g pellet from a Triton X-100-treated homogenate to give a final concentration equivalent to 1 head ml^{-1} with 100 μ l of [¹²⁵I]BGT (0.66 nM). Another 20 ml of homogenate at the same concentration was incubated with [¹²⁵I]BGT and nicotine (0.1 mM). At selected time intervals, duplicate 1 ml aliquots from each treatment were taken for analysis by filtration and radioassay. The association rate was calculated to be $1.38 \times 10^6 \text{ s}^{-1} \text{ M}^{-1}$. The dissociation curve (B) was obtained by incubating 20 ml of homogenate at the same concentration with [¹²⁵I]BGT (1.2 nM) until equilibrium was attained (45 min); nicotine (1.0 mM) was added, and duplicate 1 ml aliquots were taken at selected time intervals for analysis of specific binding by filtration and radioassay. The dissociation rate (k_{-1}) was calculated to be $6.2 \times 10^{-4} \text{ s}^{-1}$, and the dissociation rate constant (k_{-1}/k_{+1}) was 450 pM.

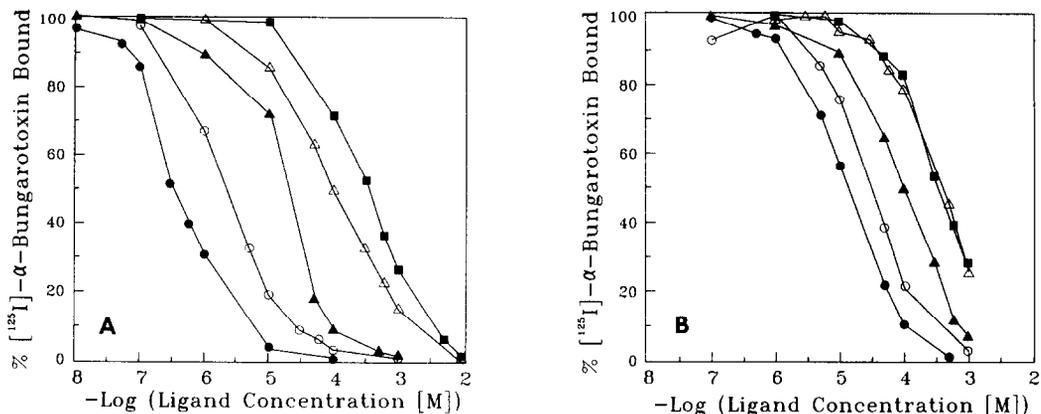


Fig. 5. Effect of cholinergic ligands on the binding of [125 I]BGT to honey bee head preparations. Nicotinic ligands (A): ● = nicotine; ○ = D-tubocurarine; ▲ = ACh + dichlorvos; △ = decamethonium; and ■ = hexamethonium. Muscarinic ligands (B): ● = atropine; ○ = S(+)-dextetimide; ▲ = (±)-QNB; △ = (+)-*cis*-dioxolane; and ■ = oxotremorine. Aliquots (800 μ l) of Triton X-100-treated homogenate at a final concentration equivalent to 1 head ml^{-1} were added to 10 ml tubes followed by ligand (100 μ l) and [125 I]BGT (100 μ l, 0.6–0.8 nM). Filtration and radioassay were conducted after a 40-min incubation. In the case of ACh + dichlorvos, the organophosphate (100 μ l, 1.0 mM) was preincubated for 15 min with the tissue preparation prior to addition of ACh and radioligand. Data are means of duplicate analyses from one representative experiment.

(k_{-1}) of $6.2 \times 10^{-4} \text{ s}^{-1}$. The dissociation rate constant calculated from the kinetic data (k_{-1}/k_{+1}) was 450 pM (Fig. 4).

The effects of cholinergic ligands on [125 I]BGT binding to bee head preparations is shown in Fig. 5. Of the five nicotinic ligands tested, nicotine (K_i $2.6 \times 10^{-7} \text{ M}$), D-tubocurarine (K_i $1.0 \times 10^{-6} \text{ M}$) and ACh + dichlorvos (K_i $4.5 \times 10^{-6} \text{ M}$), were the most potent inhibitors of [125 I]BGT binding. The other two nicotinic ligands, decamethonium (K_i $5.2 \times 10^{-5} \text{ M}$) and hexamethonium (K_i $1.4 \times 10^{-4} \text{ M}$), also inhibited [125 I]BGT binding, but they were not as potent as atropine (K_i $7.5 \times 10^{-6} \text{ M}$) and S(+)-dextetimide (K_i $1.7 \times 10^{-5} \text{ M}$), two muscarinic ligands. The other muscarinic ligands, (±)-QNB ($9.7 \times 10^{-5} \text{ M}$), oxotremorine (K_i $1.8 \times 10^{-4} \text{ M}$) and (±)-*cis*-dioxolane (K_i $2.0 \times 10^{-4} \text{ M}$), and the insecticides Tetram (K_i $2.5 \times 10^{-4} \text{ M}$), chlordimeform (K_i $6.2 \times 10^{-4} \text{ M}$), demethylchlordimeform (K_i $7.2 \times 10^{-4} \text{ M}$), SN 49844

(K_i $7.4 \times 10^{-4} \text{ M}$), formetanate ($K_i > 5 \times 10^{-4} \text{ M}$), azinphosmethyl ($K_i > 5 \times 10^{-4} \text{ M}$), amitraz ($K_i > 5 \times 10^{-4} \text{ M}$), and dichlorvos ($K_i > 5 \times 10^{-4} \text{ M}$) were not potent inhibitors of [125 I]BGT binding.

Greater than 98% of the [^3H]QNB binding in bee head homogenates was found in the 40,000 g pellet. The effect of varying the concentration of bee head preparation on [^3H]QNB binding is shown in Fig. 6. Specific binding was linear over the range of 0.5–2.5 heads ml^{-1} . The specific binding varied from 70 to 98% of the total binding, depending upon the homogenate concentration. Specific binding was greater than 90% of total binding when homogenate and [^3H]QNB concentrations were 1 head ml^{-1} and about 0.5 nM, respectively. The specific [^3H]QNB binding was destroyed by heat.

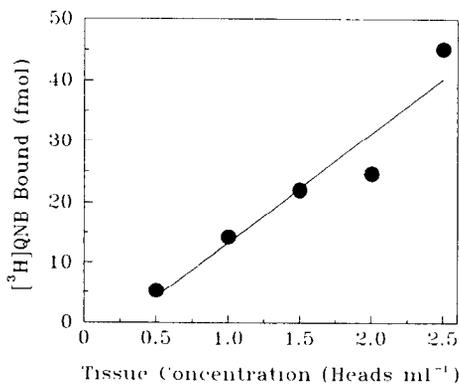


Fig. 6. Effect of varying the concentration of honey bee tissue preparation on [^3H]QNB binding. Aliquots of resuspended 40,000 g pellet to give indicated final tissue equivalents were incubated for 40 min with [^3H]QNB (1.08 nM), and the specific binding (defined by 0.1 mM atropine) was measured by filtration and radioassay.

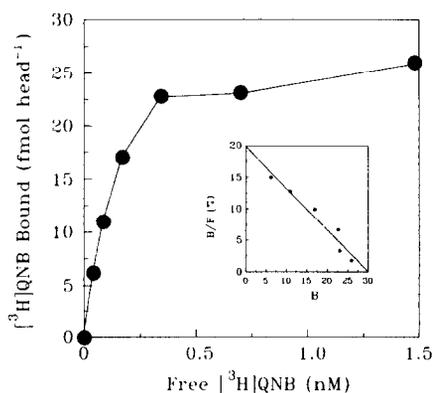


Fig. 7. Representative saturation binding curve and Rosenthal plot (inset) of specific [^3H]QNB binding to components in honey bee head homogenates. Protocol similar to that in Fig. 2, except specific binding was defined by atropine (0.1 mM). Linear estimates for K_D and B_{max} from these data were 151.3 pM and 30.0 fmol head $^{-1}$, respectively. Mean nonlinear estimates for K_D and B_{max} values from three independent experiments were $96.0 \pm 21.0 \text{ pM}$ and $26.0 \pm 1.2 \text{ fmol head}^{-1}$, respectively.

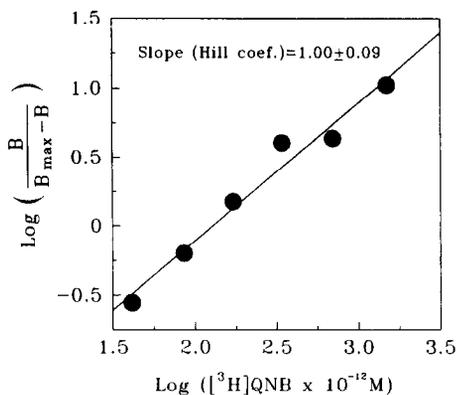


Fig. 8. Hill plot of $[^3\text{H}]\text{QNB}$ saturation data from Fig. 7.

$[^3\text{H}]\text{QNB}$ specific binding was saturable at radioligand concentrations of 0.75 nM and higher (Fig. 7). Mean K_D and B_{max} values were 96.0 ± 21.0 pM and 26.0 ± 1.2 fmol head $^{-1}$ (56.5 fmol mg protein $^{-1}$). The Hill coefficient for $[^3\text{H}]\text{QNB}$ binding to bee head preparations was calculated to be 1.00 ± 0.09 (Fig. 8).

Specific $[^3\text{H}]\text{QNB}$ binding was rapid and reversible reaching equilibrium in about 40 min (Fig. 9). Association (k_{+1}) and dissociation (k_{-1}) rates were found to be 3.27×10^6 s $^{-1}$ M $^{-1}$ and 9.4×10^{-5} s $^{-1}$, respectively, and the dissociation rate constant calculated from these data was 30 pM.

Figure 10 shows the effects of cholinergic ligands on $[^3\text{H}]\text{QNB}$ binding to bee head preparations. The muscarinic ligands (\pm)-QNB (K_i 2.2×10^{-12} M), S(+)-dextetimide (K_i 2.9×10^{-11} M), scopolamine (K_i 4.1×10^{-10} M), atropine (K_i 6.9×10^{-10} M), and (+)-QNB (K_i 4.5×10^{-8} M) clearly were more potent

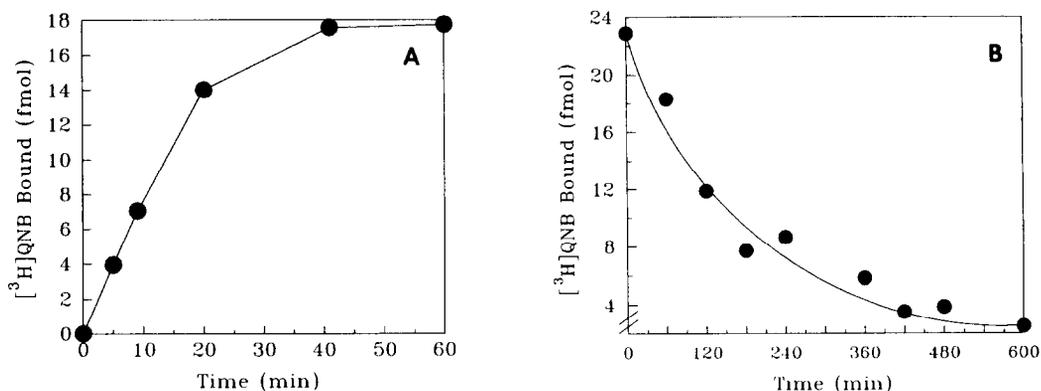


Fig. 9. Specific binding of $[^3\text{H}]\text{QNB}$ to honey bee head preparations as a function of time. Protocol similar to that in Fig. 4. For the association curve (A), the final concentrations of $[^3\text{H}]\text{QNB}$ and atropine were 0.29 nM and 0.1 mM, respectively. The association rate (k_{+1}) was calculated to be 3.27×10^6 s $^{-1}$ M $^{-1}$. In another experiment using 1.53 nM $[^3\text{H}]\text{QNB}$, the association rate was found to be 3.31×10^6 s $^{-1}$ M $^{-1}$. For the dissociation curve (B), the final concentrations of $[^3\text{H}]\text{QNB}$ and atropine were 0.68 nM and 0.1 mM, respectively. The dissociation rate (k_{-1}) from these data yielded a value of 1.1×10^{-4} s $^{-1}$; the mean rate ($n = 2$) was 9.4×10^{-5} s $^{-1}$. The dissociation rate constant (k_{-1}/k_{+1}) was 30 pM.

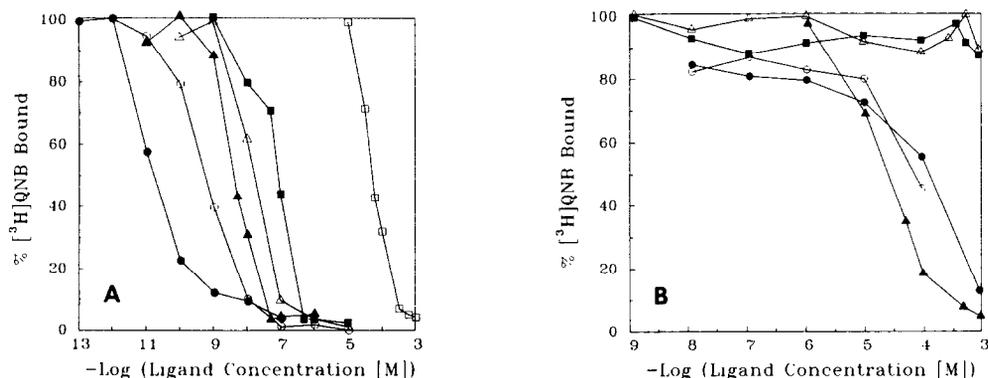


Fig. 10. Effect of cholinergic ligands on the binding of $[^3\text{H}]\text{QNB}$ to honey bee head preparations. Muscarinic ligands (A): \bullet = (\pm)-QNB; \circ = S(+)-dextetimide; \blacktriangle = scopolamine; \triangle = atropine; \blacksquare = (+)-QNB; and \square = R(-)-levetimidate. Nicotinic ligands (B): \bullet = ACh + eserine; \circ = ACh + dichlorvos; \blacktriangle = D-tubocurarine; \triangle = hexamethonium; and \blacksquare = nicotine. Aliquots (800 μl) of 40,000 g resuspended pellet to give final concentration equivalent to 1 head ml $^{-1}$ were added to 10 ml tubes followed by ligand (100 μl) and $[^3\text{H}]\text{QNB}$ (100 μl , 0.5–0.6 mM). Filtration and radioassay were conducted after a 40-min incubation. In the case of ACh + eserine or dichlorvos, the anticholinesterase agent (100 μl , 1.0 mM) was preincubated for 15 min with the tissue preparation prior to addition of ACh and radioligand. Data are means of duplicate analyses from one representative experiment.

inhibitors of [^3H]QNB binding than were D-tubocurarine (K_i 2.0×10^{-6} M), ACh + eserine (K_i 1.0×10^{-6} M), ACh + dichlorvos (K_i 1.1×10^{-5} M), nicotine (K_i $> 5 \times 10^{-5}$ M), hexamethonium (K_i $> 5 \times 10^{-5}$ M), dichlorvos (K_i $> 5 \times 10^{-5}$ M), and eserine (K_i $> 5 \times 10^{-5}$ M).

DISCUSSION

Kinetic studies revealed that binding of both [^{125}I]BGT and [^3H]QNB to bee head preparations was reversible and that association and dissociation rates were comparable to those reported for these radioligands in other organisms (Sattelle, 1980; Lummis and Sattelle, 1985; Huang and Knowles, 1990). The K_D values calculated from kinetic and saturation data revealed that the affinity of [^{125}I]BGT for bee head components was somewhat higher than that reported for this radioligand with other insect preparations (Sattelle, 1980; Lummis and Sattelle, 1985). The K_d of 743 pM from the BGT saturation data was about 4.3 times higher than that of 3 nM determined for this radioligand with honey bee brain preparation by a different technique (Sherby *et al.*, 1986). The affinity of [^3H]QNB for bee head preparations was considerably higher than that reported for most other insects (Sattelle, 1980; Shaker and Eldefrawi, 1981; Lummis and Sattelle, 1985) and bulb mites, *Rhizoglyphus echinopus* (Fumouze and Robin) (Huang and Knowles, 1990) and was similar to that reported from vertebrates (Lummis and Sattelle, 1985).

A comparison of B_{max} values for the two radioligands revealed that bee head preparations contained about 3.6 times more [^{125}I]BGT binding sites (204 fmol mg protein $^{-1}$) than [^3H]QNB binding sites (57 fmol mg protein $^{-1}$). Studies with other insects also have revealed the presence of more BGT than QNB binding sites (Sattelle, 1980; Lummis and Sattelle, 1985). However, the reverse situation has been found with some mammals (Sattelle, 1980) and possibly with bulb mites (Huang and Knowles, 1990; Knowles and Huang, unpublished results). In the other investigation of BGT binding to honey bee brain membranes (Sherby *et al.*, 1986), the B_{max} was found to be about 1.0 pmol mg protein $^{-1}$, a value about five times greater than that in the present study.

The pharmacological profiles for [^{125}I]BGT and [^3H]QNB binding to bee head preparations were clearly nicotinic and muscarinic, respectively. Interestingly, decamethonium and hexamethonium, two nicotinic antagonists, were not as potent as the muscarinic antagonist atropine as inhibitors of [^{125}I]BGT binding to bee head preparations. A similar relationship with these ligands and BGT binding has been reported in other insects (Schmidt-Nielsen *et al.*, 1977; Dudai, 1978; Hall, 1980; Lummis and Sattelle, 1985). Binding of [^3H]QNB to bee head preparations was stereoselective because a comparison of K_i values revealed that (+)-QNB was 20,450 times more potent than (+)-QNB and S(+)-dextetimide was 75,862 times more potent than R(-)-levetimidate as inhibitors of binding of this radioligand. Similar but less pronounced patterns of selectivity were demonstrated for these ligands when tested as inhibitors of [^3H]QNB binding to bulb mite homogenates (Huang and Knowles, 1990).

Our inhibition studies were performed with Triton X-100-treated bee head preparations. Because no significant differences were found in K_D and B_{max} values and in the K_i value of nicotine in presence or absence of this detergent, we assumed that the K_i values of the other ligands would not be affected by this treatment. Other more extensive studies comparing particulate and solubilized preparations found no appreciable differences in pharmacological profiles (Colquhoin and Rang, 1976; Schmidt-Nielsen *et al.*, 1977). Also, it seemed reasonable to conclude that treatment of the bee preparation with Triton X-100 did not solubilize the [^3H]BGT binding components because we were able to use successfully the filtration method with the detergent-treated homogenate, and solubilized receptors would not be expected to be retained by the glass-fiber filters.

None of the pesticides examined were potent inhibitors of [^{125}I]BGT binding to the bee preparations. However, some of these compounds were shown to possess activity as inhibitors of [^3H]QNB binding to bulb mite preparations (Huang and Knowles, 1990), and several of these compounds and other pesticides and related agents have been shown to inhibit binding of nicotinic or muscarinic radioligands to preparations from other organisms (Eldefrawi *et al.*, 1971, 1972; Schmidt-Nielsen *et al.*, 1977; Costa and Murphy, 1987; Bakry *et al.*, 1988; Costa *et al.*, 1988, 1989; Jett *et al.*, 1990; Katz and Marquis, 1990).

The kinetic and pharmacological properties of honey bee nicotinic and muscarinic cholinergic receptors appear similar to those reported from other insects. Moreover, it seems that some differences in properties of cholinergic receptors may exist between insects and mites. It might be possible to exploit these differences to control the parasitic mites of honey bees.

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