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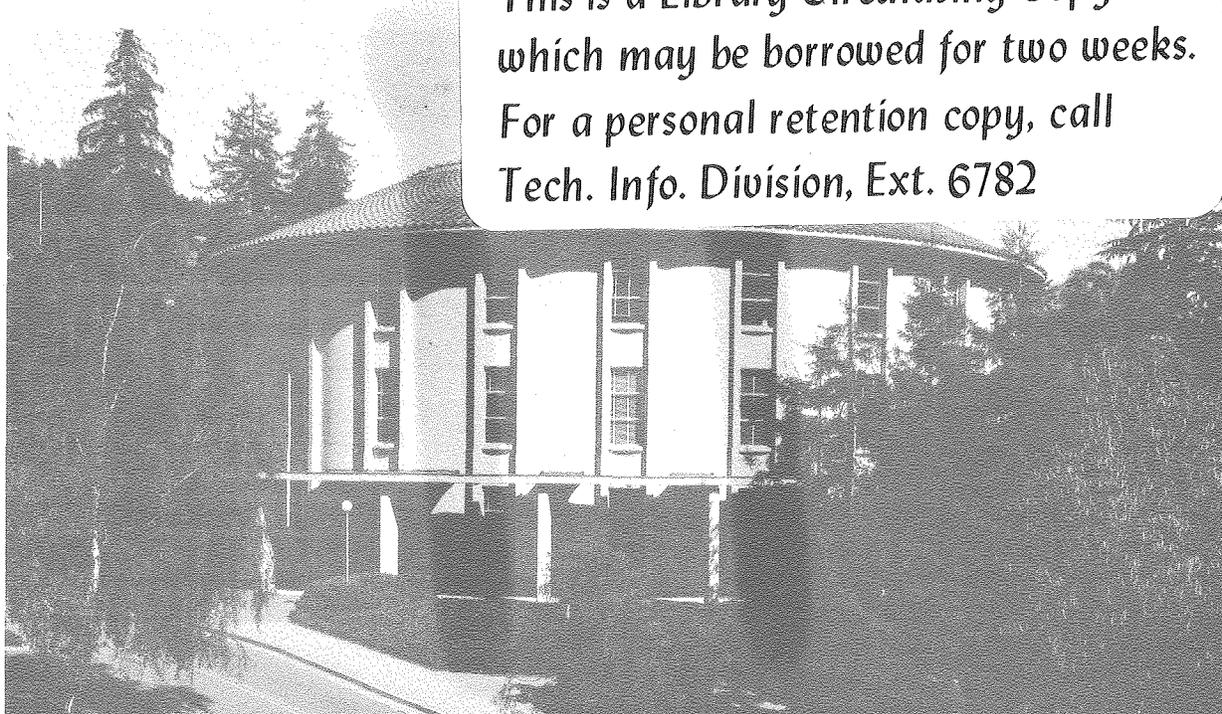
DISCRIMINATION BETWEEN CHOLINERGIC RECEPTORS IN RAT
BRAIN AND TORPEDO CALIFORNICA ELECTROPLAX BY BINDING
OF SNAKE VENOM TOXINS

Ronald J. Lukas, Michael R. Hanley, Hiromi Morimoto,
and Edward L. Bennett

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Running title:

Discrimination by Snake Toxins between Cholinergic Receptors

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SUMMARY. Toxins 4.7.3 and 4.9.3 from Dendroaspis viridis venom can antagonize nicotinic responses in frog spinal cord, while α -bungarotoxin has no demonstrable physiological action at that locus. Thus, sequence differences in homologous toxins may affect receptor-blocking activities on nicotinic sites in different tissues. We have compared the binding of ^{125}I -labeled dendrotoxins and ^3H -labeled α -bungarotoxin to rat brain membranes and Torpedo californica electric tissue. There are two [^{125}I]-dendrotoxin sites for every [^3H]- α -bungarotoxin site in both intact and solubilized rat brain membranes, but the toxin-binding site stoichiometry is one-to-one in intact and solubilized electroplax vesicles. All toxin-binding sites have similar nicotinic pharmacology, co-migrate on sucrose density gradients, and are co-enriched on solubilization. Unlabeled toxins block specific binding of radiolabeled toxins in each tissue. These observations indicate that electroplax and putative brain nicotinic receptors are not identical with respect to toxin binding characteristics, and suggest possible hypotheses as to why different toxins may be effective on tissue-specific receptor populations.

INTRODUCTION

The use of several snake venom neurotoxins for the isolation and assay of the nicotinic acetylcholine receptor of peripheral tissues is now well established (1-3). Several investigators have presented biochemical evidence that neurotoxins may also be used to characterize cholinergic receptors in the central nervous system of both vertebrates (4-6) and invertebrates (7,8). However, the significance of these results has been questioned because of inability of certain toxins to block as expected physiological responses at CNS nicotinic synapses (9-10). This contrasts sharply with pharmacological (4-8) and histological (11,12) evidence for their receptor specificity. Continued use of toxins to characterize brain nAChR therefore requires that their binding site be identified as authentic nAChR, or that their apparent electrophysical inactivity be explained.

Dendrotoxins 4.7.3 and 4.9.3 from the venom of Dendroaspis viridis (13), have been reported to antagonize cholinergic responses in an invertebrate ganglion (14) and central cholinergic neurotransmission of vertebrate motor-neurons (10). We have therefore radioactively labeled dendrotoxins 4.7.3 and 4.9.3 and compared their binding to nAChR from electric tissue and in rat brain to that of radioactively-labeled α -bungarotoxin. A preliminary report of these results has appeared (15).

MATERIALS AND METHODS

Dendrotoxin 4.7.3 was a generous gift of Dr. R.A. Shipolini. Dendrotoxin 4.9.3 (α -mambatoxin) was kindly provided by Dr. J. Patrick. Bungarus multicinctus crude venom was from Miami Serpentarium. Na-¹²⁵I (carrier free) was obtained from New England Nuclear. Liquid-nitrogen frozen Torpedo californica electric tissue was obtained from Pacific Bio-Marine Laboratories, Venice, CA

and stored at -70°C until use. Wag/Rig rats were from the Lawrence Berkeley Laboratory breeding colony.

Torpedo californica Ringer contained 250 mM NaCl, 4 mM CaCl_2 , 2 mM MgCl_2 , and 3 mM Na-Phosphate buffer (pH 7.2). Rat brain Ringer contained 115 mM NaCl, 5 mM KCl, 1.8 mM CaCl_2 , 1.3 mM MgSO_4 , and 33 mM Tris (pH 7.6). All binding and homogenization solutions contained 1 mg/ml bovine serum albumin, 0.02% NaN_3 , and 0.1 mM phenylmethylsulfonyl fluoride.

α -Bgt was purified, iodinated and tritiated as previously described (16-18), giving a biochemically and radiochemically pure final product (25 dpm/fmole specific activity). Ddt (Dendrotoxin 4.7.3) was radioactively labeled with ^{125}I by the protocol developed for α -Bgt (17), with several small modifications to improve yield and give pure [^{125}I]-Ddt. α -Mamba-toxin (Dendrotoxin 4.9.3) was labeled with ^{125}I at 55°C with an ICl_2 :toxin ratio of 2:1. Neither iodinated Ddt nor iodinated α -MT could be separated from native toxin by chromatography on CM-52. The specific activity of iodinated toxins, determined by direct counting on a Nuclear-Chicago γ -counter and conversion of the measured ultraviolet absorbance to protein concentration, was varied between 3 and 100 dpm per fmole. [$E_{0.1\%}^{280} = 1.32$ for α -Bgt (16), 1.70 for Ddt (based on absorbance of weighed sample), and 2.75 for α -MT (24)].

Torpedo californica nAChR-rich vesicles (0.3-0.8 nmol [^3H]- α -Bgt sites/mg protein) were prepared by the technique of Hazelbauer and Changeux (19). All binding assays were conducted at 21°C with continuous shaking. Binding of radioactively-labeled toxins to Torpedo vesicles was measured by a microfuge assay and the results confirmed by the filtration method of Weber and Changeux (20). Ten μl of the vesicle suspension (10 μg membrane protein/ml) were added to 230 μl of Torpedo Ringer in polypropylene microfuge tubes. For ligand and toxin competition studies, cholinergic

ligands or excess unlabeled toxins were added for 30 min prior to addition of radioactively-labeled toxins. All samples were then incubated for 60 min at 21°C, followed by a 30 min chase with added unlabeled α -Bgt (final concentration 1 μ M). Membranes were sedimented by centrifugation at 4°C for 6 min in a Beckman 152 microfuge, freed from the supernatants by aspiration, resuspended in 250 μ l of Ringer, sedimented again, and aspirated to a dry pellet. For [125 I]-labeled toxins, samples in γ -well tubes were counted directly in a γ -counter (41% efficiency). In the case of [3 H]- α -Bgt, pellets were quantitatively dispersed and transferred to scintillation vials either by solubilization in Protosol, by repeated extraction with trifluorethanol, or by exhaustive rinsing with Ringer. These three methods gave identical results. Samples were diluted with 16 ml of Aquasol 2 (Packard) and counted in a Packard Tri-Carb scintillation spectrometer (37-42% efficiency) after overnight storage in the dark to eliminate chemiluminescence.

The preparation of a crude mitochondrial fraction from rat brain and the centrifugal assay for toxin binding to brain membranes were as described elsewhere (21).

Toxin binding to solubilized receptor was determined by the DE81 filter disk method (22). When [3 H]- α -Bgt was tested, trapped radioactivity was desorbed from filters by overnight incubation in 1 ml of 1N NaOH containing 2 M NaCl, neutralized, and counted in 16 ml Aquasol 2.

Sucrose gradient centrifugation was performed as follows: From the brains of four rats, a crude particulate fraction containing synaptosomal and microsomal membranes was prepared by centrifugation of the supernatant remaining after removal of the crude nuclear pellet (21). The supernatant was spun at 100,000 x g for 30 min, the pellet was resuspended in 10

volumes of 5 mM sodium phosphate buffer (pH 7.4)/0.02% NaN₃/0.1 mM phenylmethylsulfonyl fluoride (hereafter termed "minimal phosphate buffer"), and pelleted again at 100,000 x g for 30 min. The pellets were resuspended and combined in rat brain Ringer to a concentration of 20 mg protein/ml and split into four 3 ml samples. Two samples were given ¹²⁵I- α -Bgt to a final saturation concentration of 10.4 nM and the other two were given ¹²⁵I-Ddt to a final saturation concentration of 14.6 nM. In one of the samples in each pair, excess α -Bgt was pre-equilibrated at a final concentration of 10 μ M for 30 min prior to the addition of radiolabeled toxins. These samples were used to define "non-specific binding". Radiolabeled toxins were incubated with all samples for 60 min at 21°C. Unlabeled α -Bgt (final concentration 10 μ M) was added to the second pair of samples (total binding) for 30 min additional incubation. Samples were freed from excess unbound toxin by centrifugation at 100,000 x g for 30 min. Each pellet containing bound radiolabeled toxin was resuspended in 3 ml of minimal phosphate buffer. Aliquots were checked for "specific binding" (total minus non-specific) and gave values of 21.3 fmoles/mg protein for [¹²⁵I]- α -Bgt and 46.6 fmoles/mg protein for [¹²⁵I]-Ddt. Each sample was then applied to a 36 ml 5-50% sucrose gradient prepared in minimal phosphate buffer and equilibrated to 4°C. Gradients were centrifuged in a Beckman SW27 rotor at 25,000 rpm for 16 hrs at 4°C. Each gradient was fractionated into 0.65 ml samples which were tested for protein content by the Lowry method and counted for radioactivity.

Additional details of procedures and data treatment are given in the figures and table legends.

RESULTS

For all preparations of [^{125}I]-Ddt, as for [^3H]- α -Bgt, direct specific activity determinations based on β - or γ -counting of radiolabeled toxin samples of known concentration agreed within 5% with biological specific activity measurements using Torpedo electroplaque binding site saturation assays with samples of accurately known nAChR concentration.

With separate experimental preparations of [^{125}I]-Ddt, pre-equilibrium saturation binding curves were obtained as in Fig. 1. Specific binding levels in brain were twice as high for [^{125}I]-Ddt as for [^3H]- α -Bgt, but were the same for saturation of binding to Torpedo vesicles. Analysis of Scatchard plots of a minimum of five independent saturation curves gave an average K_D for [^{125}I]-Ddt of 4.0 ± 0.6 nM in brain and 4.1 ± 1.5 nM in Torpedo. These values can be compared with 3.0 ± 0.5 nM in brain and 1.3 ± 0.4 nM in Torpedo for [^3H]- α -Bgt. The proportion of [^{125}I]-Ddt non-specifically bound increased with the degree of radioactive labeling of Ddt. Typically for [^{125}I]-Ddt preparations, specific binding at saturation was 20-40% of the total in rat brain and 50-80% of the total in Torpedo compared to 70% and 99% specific binding for [^3H]- α -Bgt in rat brain and Torpedo respectively. Scatchard and double reciprocal plots indicated that specific binding was apparently to a single homogeneous population of sites for both toxins in both tissues, although the higher non-specific binding of [^{125}I]-Ddt in brain makes this conclusion tentative. Nonetheless, the specific binding levels at saturation and the two-to-one ratio of Ddt to α -Bgt sites were independent of the degree of ^{125}I - incorporation or overall iodination.

Binding saturation stoichiometries were preserved after solubilization in deoxycholate (Fig. 2). Moreover, in both tissues, specific binding

activities of the two toxins was found to slightly co-enrich (1.1X in brain, 2.1X in Torpedo) with solubilization. Binding site stoichiometries were preserved for a variety of solubilization conditions despite variation in specific binding activities (Table II).

The nicotinic pharmacology of both toxin-binding sites was confirmed by the competition of cholinergic drugs toward toxin binding (Table 1). In each instance, membranes were preincubated with ligand before addition of radiolabeled toxin. Comparison of data for blockage of toxin binding to sites in each tissue showed that both carbachol and d-tubocurarine were markedly less effective in brain than in Torpedo, in agreement with other investigations (5,21). Muscarinic and non-nicotinic ligands were ineffective on the binding of either toxin.

Further evidence for the congruent physical localization of toxin sites was obtained by equilibrium sucrose gradient centrifugation of a heterogeneous brain particulate fraction (including both synaptosomal and microsomal membranes), as in Fig. 3. Maximum specific binding for both [125 I]-Ddt and [125 I]- α -Bgt, enriched 2.3-fold over the crude mitochondrial fraction, was evident at a buoyant density of 1.146 g/ml (compare to a density of 1.112 for a microsomal subfraction reportedly enriched in [125 I]- α -Bgt binding sites (23)). The radioactivity profiles show that the [125 I]-Ddt and [125 I]- α -Bgt binding sites follow the same distribution in the gradient, and do not follow total protein levels. Furthermore, the two-to-one [125 I]-Ddt:[125 I]- α -Bgt stoichiometry is preserved throughout the gradient.

Evidence that the molecular site of action for the two toxins is the same may be derived from competition assays. Data from experiments in which unlabeled and labeled toxins were coincubated with membrane-bound sites

indicated that unlabeled α -Bgt and Ddt quantitatively block specific binding of both the homologous and heterologous radiolabeled toxins in both tissues (Fig. 4). Apparent K_D 's calculated from the 50%-inhibition values of these plots gave good agreement with the values obtained from direct binding experiments (4.6 nM in brain and 5.9 nM in Torpedo) for Ddt, and 2.2 nM in brain and 1.2 nM in Torpedo for α -Bgt). In all cases, specific binding competition behavior again suggested a single population of sites for each ligand.

The ability of non-radiolabeled α -Bgt to quantitatively block [125 I]-Ddt binding to brain membranes, despite the apparent 2:1 Ddt: α -Bgt binding site stoichiometry, was unexpected. Appearance of another report (24) comparing receptor binding properties of [125 I]- α -Bgt and [125 I]-MT [mambatoxin, a toxin from Dendroaspis viridis venom corresponding to toxin 4.9.3 of Banks et al., (13)] prompted further investigation using [125 I]-MT. Patrick et al. (24) found 2:1 binding ratio of α -MT: α -Bgt in nerve and muscle cells in culture but detergent solubilized muscle or Torpedo extracts bound at 1:1. We have found the binding in rat brain membranes of α -MT was twice that of α -Bgt. Specific binding at saturation in rat membranes was 50% of total binding and $K_D^{app} = 2.0$ nM, as compared with 95% specific binding and apparent $K_D^{app} = 2.5$ nM in Torpedo. More than 80% of the α -MT binding was inhibited by 30 min preincubation with 1 mM tubocurarine or 1 μ M α -Bgt, or by pretreatment of the membranes with 100 μ M MBTA.

DISCUSSION

Dendrotoxin 4.7.3 and α -bungarotoxin apparently bind to the same population of rat brain receptors as judged by membrane cosolubilization, identical buoyant densities, and mutual site competition effectiveness. However, there are twice as many [125 I]-Ddt or [125 I]-MT as [3 H]- α -Bgt sites at saturation in rat brain. This is unlike the binding characteristics of the toxins in Torpedo californica vesicles wherein they exhibit identical pharmacological and saturation properties.

These results are to be compared with another report (24) describing binding of α -Bgt and α -MT to putative cholinergic receptors on BC₃H-1 muscle cell cultures and on PC12 cells (a pheochromocytoma cell line that can exhibit properties of sympathetic neurons). In that study, the ratio of [125 I]- α -MT to [125 I]- α -Bgt sites was 2:1 for both BC₃H-1 and PC12 cell cultures. In both cases, non-radiolabeled α -Bgt blocked 80-90% of specific [125 I]- α -MT binding (in accordance with our results). Interestingly, binding ratios were 1:1 when toxins were used to label detergent extracts of BC₃H-1 cells and purified Torpedo nAChR. In the present study, α -Bgt and α -Ddt binding stoichiometries were retained on solubilization by several different procedures.

An important consequence of these observations is that Torpedo and rat brain nAChR are distinguishable by the specific features of α -toxin recognition (see also 17). Thus, although Torpedo and rat brain nAChR appear similar in their physical behavior (5,25), their pharmacological responsiveness and site binding properties are different. There is a precedent for the proposition that toxin-binding can discriminate between evolutionary variants of nAChR in that reptilian neuromuscular nAChR from different species vary in their ability to bind α -toxins (26). There may

be other unique features of the brain receptor which can only be addressed by its direct study.

It is tempting to offer a possible explanation for the divergent antagonistic activities displayed by α -Bgt, Ddt or α -MT at vertebrate central cholinergic synapses. Such treatment must remain speculative given the unsettled nature of evidence regarding curaremimetic neurotoxin potency in central and autonomic nervous systems (9,10,14,27,28, see also 31-35), and the inherent danger in comparing biochemical and physiological results across species and nervous system loci.

Binding of both toxins can be prevented by pre-equilibration of the sites with both nicotinic agonists and antagonists. Thus, agonists must directly bind to both classes of toxin sites, or binding at one set of sites alters the properties of all sites so that toxin can no longer bind. Because α -Bgt is presumed to have no physiological effect (however, see 27,28), we have assumed that the α -Bgt sites are distinct from the agonist activation sites. Therefore, agonist inhibition of toxin binding should be non-competitive, and may come about because of agonist-induced changes in receptor state. Recent results (29) agree with these predictions. The ability of unlabeled Ddt to prevent the binding of α -Bgt while giving twice as many sites as α -Bgt would suggest that it binds at both the agonist and α -Bgt sites.

A severe complication that may compromise this analysis is the ability of α -Bgt to inhibit all the [125 I]-Ddt and [125 I]- α -MT binding, as α -Bgt is proposed to overlap with only one half of the Ddt agonist sites. One might propose that α -Bgt binding causes a change in the receptor state so as to block Ddt binding to the distal Ddt/agonist site, but has no direct effect on agonist binding. However, recent results from Wang et al. (30) are

interpreted as evidence for a single population of CNS sites binding both α -Bgt and agonists. Similarly, the concanavalin A competition results of Patrick *et al.* (24) mitigate against the distinct sites hypothesis. Furthermore, it would indeed be fortuitous that both Ddt sites display the same pattern of pharmacological profiles and homogeneous [^{125}I]-Ddt binding parameters and yet be so different with respect to α -Bgt binding.

Differences in binding of iodinated and tritiated toxins to brain nAChR may be manifest as a consequence of iodination (17). We have not yet excluded the possibility that use of [^{125}I]-Ddt rather than a tritium-labeled derivative might account for the 2:1 binding site stoichiometry in rat brain. However, 1:1 stoichiometry is observed for [^3H]- α -Bgt and [^{125}I]- α -Bgt binding sites (22).

That [^{125}I]-Ddt or [^{125}I]- α -MT may dimerize in solution would seem to be discounted (24). In tissues where the 2:1 binding stoichiometry is observed, there is the possibility that interaction of Ddt or α -MT with putative nicotinic receptors facilitates formation of a ternary toxin-receptor-toxin complex. Presumably, α -Bgt could neither promote nor participate in ternary complex formation, thus explaining its competition potency at the primary shared receptor toxin-binding site. While the cholinergic ligand competition data could be readily explained using this model, it is difficult to see how reactions of dendrotoxins with free receptor and with binary toxin-receptor complexes would be indistinguishable.

Thus, these results provide information on toxin-receptor interactions, and demonstrate the usefulness of multiple forms of receptor ligands in order to probe receptor structure and function.

ABBREVIATIONS

α -Bgt, α -bungarotoxin; [^3H]- α -Bgt; tritium-labeled α -bungarotoxin; [^{125}I]- α -Bgt, [^{125}I]- α -labeled (monoiodinated) α -bungarotoxin; BSA, bovine serum albumin; CNS, central nervous system; Ddt, dendrotoxin 4.7.3. [^{125}I]-Ddt, [^{125}I]-labeled dendrotoxin; nAChR, nicotinic acetylcholine receptor (from electroplax) or nicotinic-like acetylcholine receptor (from brain). α -MT, α -mambatoxin; ^{125}I - α -MT, ^{125}I -labeled (mono-iodinated) α -mambatoxin.

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FIGURE LEGENDS

Fig. 1. Specific binding saturation of [^3H]- α -Bgt (---○---○---) and [^{125}I]-Ddt (—●—●—) to rat brain and Torpedo californica membranes. Specific binding was determined as total binding minus non-specific binding (determined by pre-incubation of parallel samples with a large excess of unlabeled α -Bgt). The non-specific binding was linear with added radiolabeled toxins and was 70% (7440/10,800 cpm) for [^{125}I]-Ddt and 44% (340/780 cpm) for [^3H]- α -Bgt at 10 nM toxin in brain, and 32% (1594/5044 cpm) for [^{125}I]-Ddt and <1% (6/938 cpm) for [^3H]- α -Bgt 10.6 nM toxin in Torpedo.

Fig. 2. Specific binding saturation of [^3H]- α -Bgt (---○---○---) and [^{125}I]-Ddt (—●—●—) to deoxycholate solubilized nAChR from rat brain and Torpedo. Membrane proteins were solubilized for 90 min using 20% (w/w) suspensions of membrane fractions (see Methods). All detergents were made up in 5 mM sodium phosphate buffer (pH 7.4) + 0.02% NaN_3 + 0.1 mM phenylmethylsulfonyl fluoride. Samples were then clarified by centrifugation at 100,000 x g for 60 min. Under these conditions, 55% of the α -Bgt sites and 28% of the protein were solubilized in Torpedo and 22% of the α -Bgt sites and 20% of the protein were solubilized from rat brain crude mitochondrial fraction, giving relative enrichments in specific toxin binding activities of 2.1 X and 1.1 X, respectively. The nonspecific binding was linear with added radiolabeled toxins and was 78% for [^{125}I]-Ddt and 56% for [^3H]- α -Bgt at 14 nM toxin in brain, and 48% for [^{125}I]-Ddt and 12% for [^3H]- α -Bgt at 11 nM in Torpedo. Brain and Torpedo protein concentrations were 0.7 mg and 0.1 μg ,

respectively in 200 μ l assay volumes.

FIG. 3. Sucrose density gradient centrifugation of pre-labeled rat brain crude particulate fractions. Specific binding activity for [3 H]- α -Bgt (o---o---o) and [125 I]-Ddt (●---●---●) was determined as in Methods and corrected for the indicated total protein content (—□—□—) in each fraction. It is the variability between separately-run gradients that accounts for the small deviation from perfect 2:1 stoichiometry of toxin sites across the binding peak.

Fig. 4. Competition toward radiolabeled toxin specific binding by unlabeled Ddt (—○—○—) and α -Bgt (—X—X—). Due to the relatively slow association and dissociation rates of toxin binding (21), competition potencies were tested under non-equilibrium conditions by adding both labeled and unlabeled toxins together at time zero, and coincubation for 60 min at 21°C. Toxin chase and assay were as in Methods. Each data point is the mean of three separate determinations. Graph A and B are data for brain, graphs C and D are data for Torpedo. the concentration of [125 I]-Ddt was 14.2 nM in A, 17.6 nM in C; and the concentration of [3 H]- α -Bgt was 10.0 nM in B and 8.8 nM in D. Brain and Torpedo membrane protein concentrations were 3.4 mg and 0.2 μ g respectively in 250 μ l assay volume.

Table 1. Comparative pharmacology of [^{125}I]-Ddt and [^3H]- α -Bgt membrane bound binding sites in Torpedo californica electroplax and rat brain

Drug [‡]	Torpedo		Rat Brain	
	$K_D^{\text{app}*}$ [^{125}I]-Ddt	μM [^3H]- α -Bgt	$K_D^{\text{app}*}$ [^{125}I]-Ddt	μM [^3H]- α -Bgt
Acetylcholine	1.1 \pm 0.3 (2)	0.5 \pm 0.2 (2)	1.0 \pm 0.5 (5)	0.8 \pm 0.2 (3)
Carbachol	0.4 \pm 0.1 (2)	0.6 \pm 0.2 (2)	4.6 \pm 1.2 (3)	2.5 \pm 0.2 (3)
Nicotine	0.4 \pm 0.1 (2)	0.2 \pm 0.1 (2)	0.4 \pm 0.2 (4)	0.3 \pm 0.05 (3)
d-Tubocurarine	0.1 \pm 0.05 (2)	0.1 \pm .-.5 (2)	2.9 \pm 2.0 (5)	4.4 \pm 2.5 (3)
Atropine	> 100 (1)	> 100 (1)		> 100 (1)

* K_D^{app} was calculated assuming a strictly competitive interaction of the ligands at a single class of sites. We have preliminary evidence against these simplifying assumptions (28) but use this calculation as the simplest method of comparing data obtained versus two different quasi-reversible ligands and correcting for the differences in affinities and incubation concentrations of the radiolabeled toxins. All ligands were pre-incubated with samples for 30 min at 21°C before the addition of radiolabeled toxins and binding assays as in Methods.

[†]Preequilibrated in the presence of either 20 μM BS-284c51, 20 μM neostigmine or 100 μM eserine for 10 min before acetylcholine addition, these drugs quantitatively inhibit acetylcholine hydrolysis but have no effect on toxin binding.

[‡]Negative competition at 100 μM :dopamine, γ -amino butyrate (GABA), glutamate, glycine, histamine and noradrenaline.

TABLE II

Solubilization of α -toxin binding sites from rat brain crude mitochondrial fractions

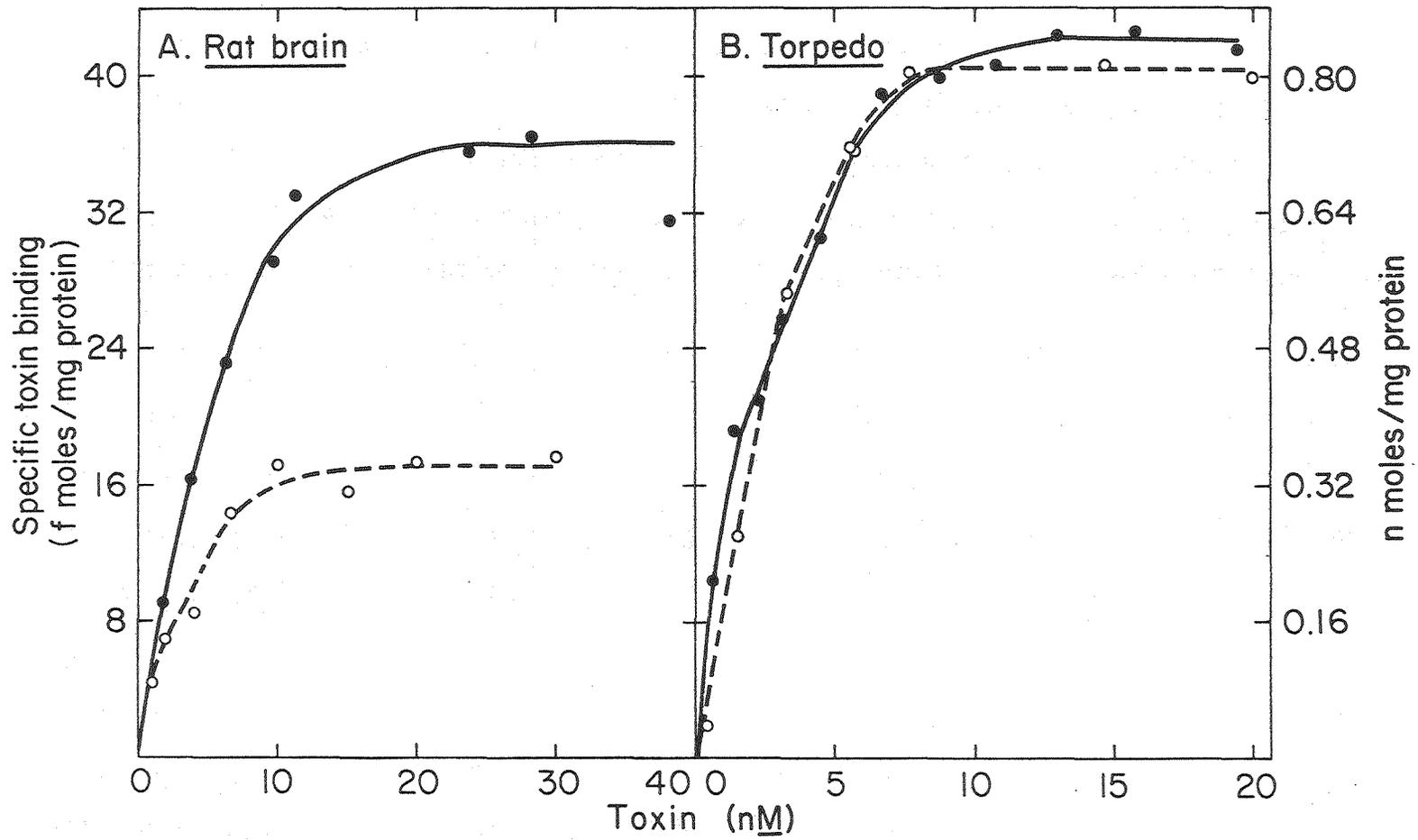
<u>Treatment*</u>	<u>% Protein†</u> <u>Solubilized</u>	<u>¹²⁵I-Ddt sites</u> <u>% Solubilized‡</u>	<u>SAE₁ §</u>	<u>[³H]-α-Bgt sites</u> <u>% Solubilized‡</u>	<u>SAE₂ §</u>	<u>SAE₁/SAE₂</u>
0.1% Sodium deoxycholate, 4°C	12	15	1.25	16	1.33	0.94
1% Sodium deoxycholate, 4°C	20	22	1.10	23	1.15	0.96
1% Sodium deoxycholate, 37°C	32	44	1.38	43	1.34	1.03
1% Sodium deoxycholate + 2M NaCl, 37°C	39	42	1.08	40	1.03	1.05
1% Emulphogene BC-720, 4°C	25	35	1.40	37	1.48	0.95
1% Emulphogene BC-720, 37°C	29	42	1.45	40	1.38	1.05
0.1% Triton X-100, 4°C	38	25	0.66	28	0.74	0.89
1% Triton X-100 4°C	53	40	0.75	44	0.83	0.90
1% Triton X-100 37°C	71	80	1.13	82	1.15	0.98

* All detergent extractions were done for 90 min using 10% (w/w) suspensions of crude mitochondrial fractions (see Methods). All detergents were prepared in 5mM sodium phosphate buffer (pH 7.4) containing 0.2% NaN₃ and 1mM phenylmethylsulfonyl fluoride. Samples were then clarified by centrifugation at 100,000 X g for 60 min.

† By Lowry determination with standards and controls treated under the same conditions. Where interference was pronounced, samples were checked against a fluorescamine-filter disk method.

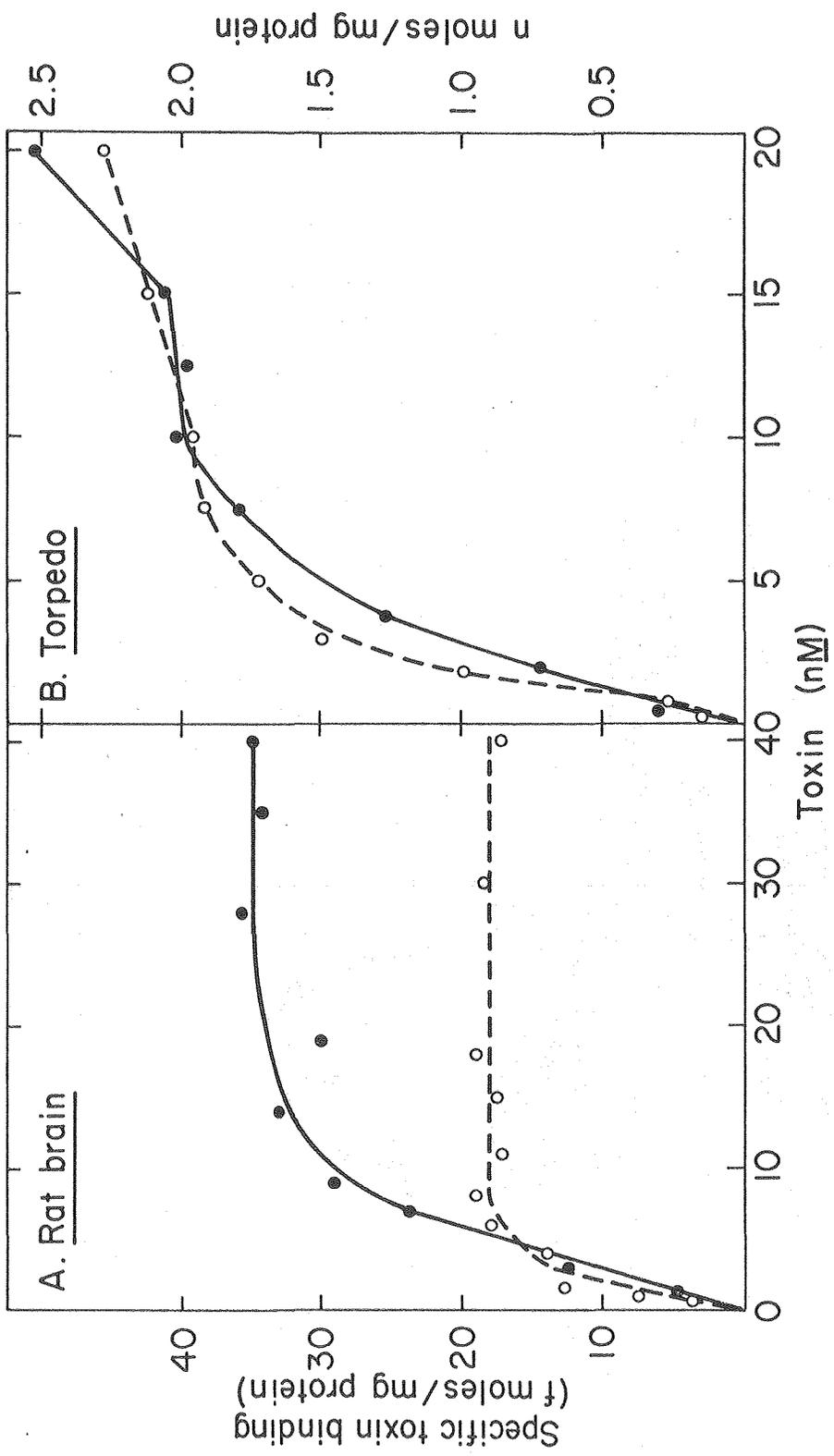
‡ Determined relative to aliquots taken from original crude mitochondrial fraction before extraction. Binding assays are given in Methods, using saturating concentrations of ¹²⁵I-Ddt (14.0nM) and [³H]- α -Bgt. (13.5nM).

§ Specific Activity Enrichment given by the ratio of the specific binding activity (fmoles α -toxin bound/mg protein) of detergent extracts versus original crude mitochondrial fraction homogenate.



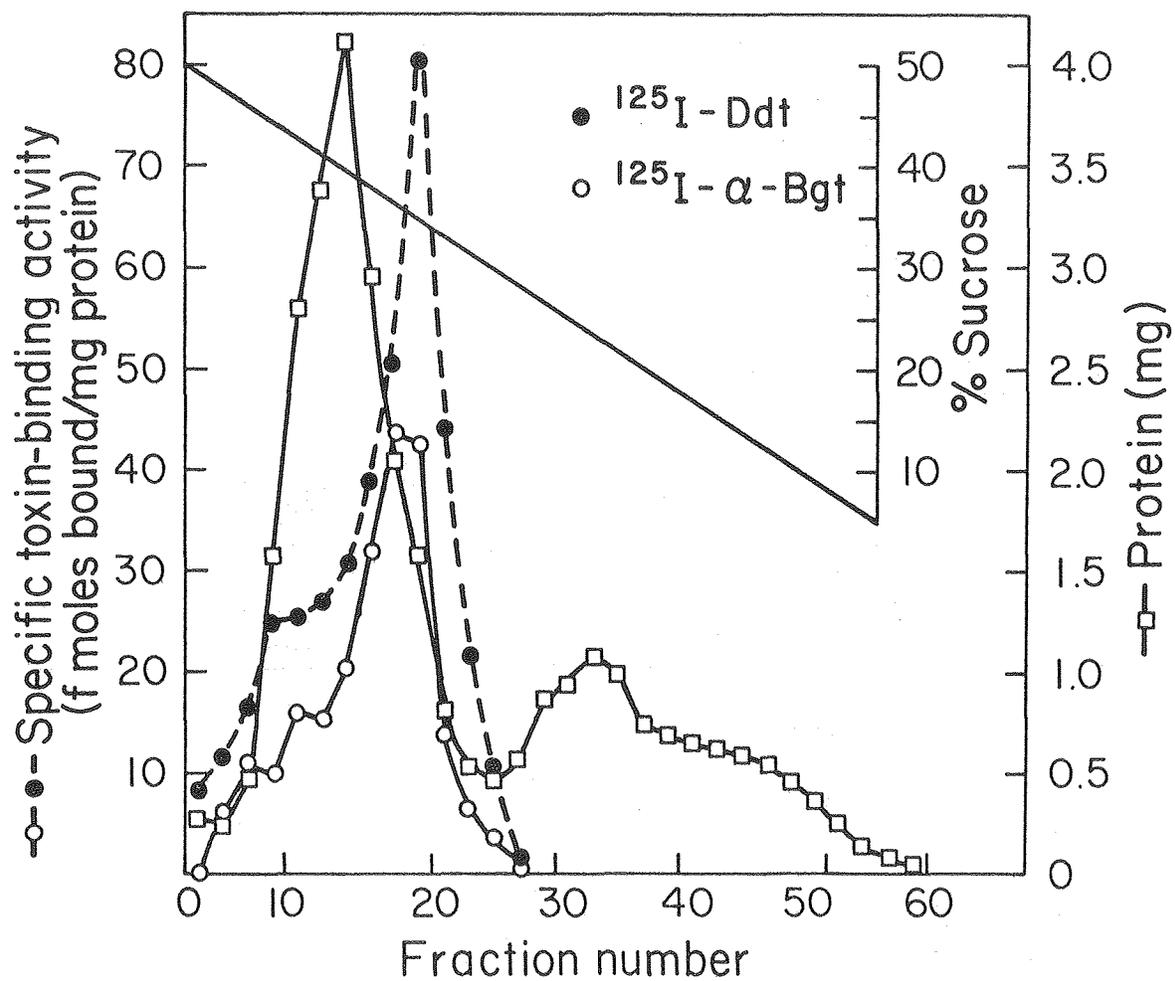
XBL 785-876

Fig. 1
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XBL 785-875

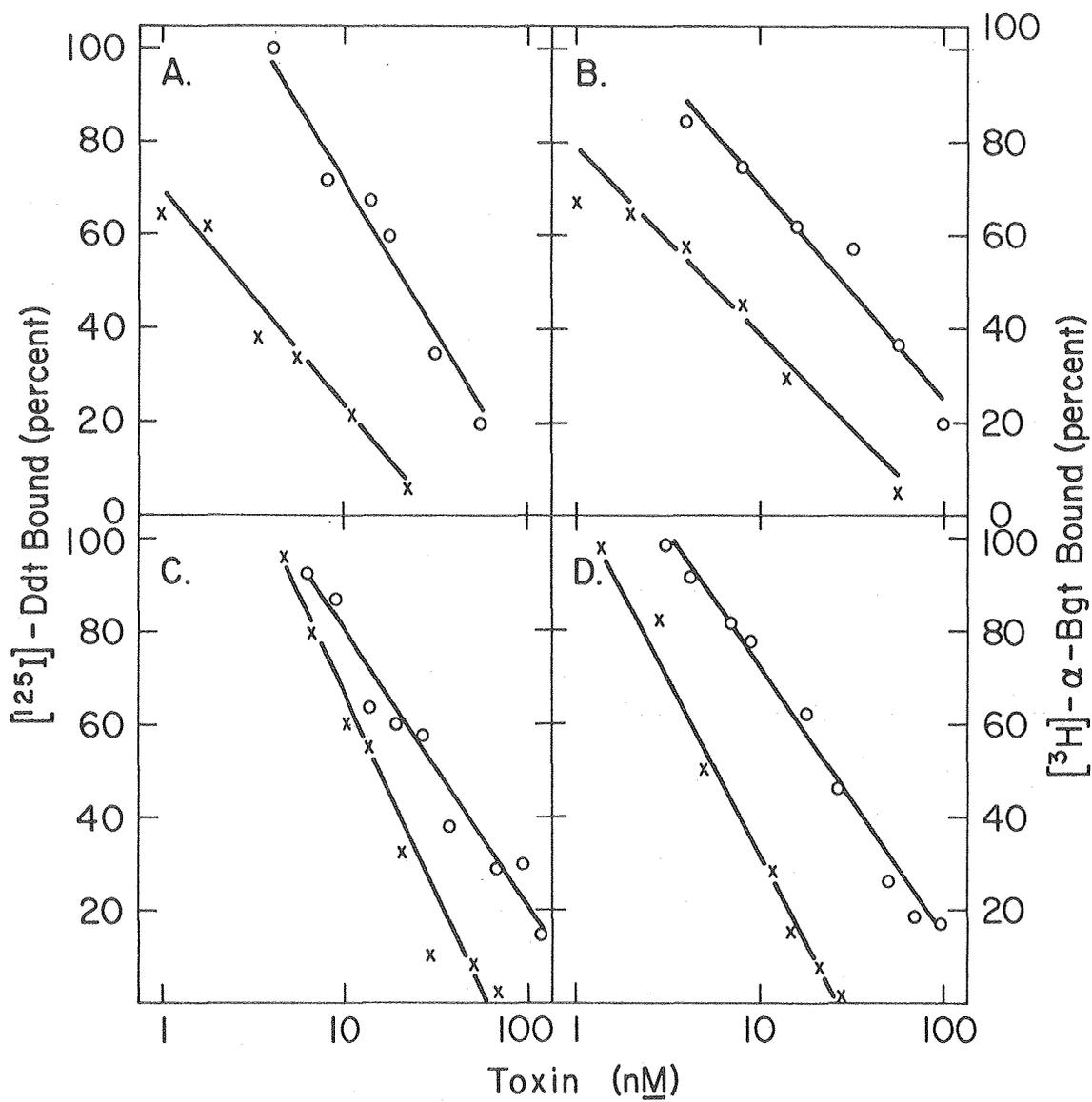
Fig. 2
Lukas et al



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Fig. 3

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Fig. 4
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