Alpha-bungarotoxin (α-Bgtx) and beta-bungarotoxin (β-Bgtx) binding activities in human cadaver brain

Suhail Rasool[1,2], Bansi L. Jailkhani[2], Mohd Irshad[2], Madhuri Behari[3] and Vinay Goyal[3]

[1]Department of Molecular Biology and Biochemistry, University of California, USA
[2]Department of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi, India
[3]Neurology Cardio Neuro Centre, All India Institute of Medical Sciences, New Delhi, India

Submitted: 10 March 2007
Accepted: 22 June 2007

Arch Med Sci 2007; 3, 2: 93-101
Copyright © 2007 Termedia & Banach

Corresponding author:
Dr. Suhail Rasool
C/O: Department
of Molecular Biology
and Biochemistry
3438 McGaugh Hall
University of California Irvine USA
92697
Phone: 714-791-2076
E-mail:
suhailrasool@hotmail.com

Abstract

Introduction: Using ^{125}Iα-Bgtx as a ligand, postsynaptic cholinergic nicotinic receptors has been identified in species. Presynaptic receptors can be defined as receptors at or near the nerve terminal that can positively or negatively modulate transmitter release, that directly or indirectly influence the probability of an action potential resulting in exocytosis. These receptors are identified by utilizing β-Bgtx. Our study involves the identification of these receptors in different regions of brain.

Material and methods: The activity of postsynaptic AChR and presynaptic membrane receptor in membrane preparation and triton extract of muscle and brain tissue was determined by virtue of its high affinity for α and β-Bgtx by rapid filtration method in case of membrane preparations and ammonium sulphate precipitation method in triton extracts i.e; solubilized receptor.

Results: Specific α-Bgtx binding as fmoles/mg tissue was observed in occipital lobe (6.1±0.1) parietal lobe (6±0.6), temporal lobe (5.2±1.4), cerebral cortex (6.2±0.3), hippocampus(5.6±0.2), hypothalamus (4.3±0.4) and brain stem (4.8±0.2). No specific binding of α-Bgtx was found in cerebellum and frontal lobe. β-Bgtx binding activity was present in all the above regions of brain and was in range of 4.2-4.9 fmoles/mg tissue. Presynaptic receptors also shows immunoreactivity with myasthenic sera.

Conclusions: In addition to muscle, β-Bgtx binding proteins are present in each regions of the brain, while as α-Bgtx binding proteins are absent in some regions. β-Bgtx binding sites could play an important role in the pathogenesis of myasthenia gravis.

Key words: α-bungarotoxin, β-bungarotoxin, regional distribution and cadaver brain.

Introduction

The presence of nicotinic cholinergic receptors in mammalian brain has been suggested by a number of investigators with the application of nicotinic ligands [1, 2]. α-Bgtx is a selective antagonist of postsynaptic nicotinic acetylcholine receptors in vertebrate and insects. By the use of potent nicotinic antagonist ^{125}Iα-Bgtx as a ligand, cholinergic nicotinic receptors has been identified in rat brain [3] and honey bee brain [4]. Three radio ligands have been commonly used to label putative nicotinic cholinoreceptors in the mammalian central nervous system: the agonists ^{3}H nicotine and ^{3}H acetylcholine in the presence of atropine to block muscranic receptors and snake venom extract ^{125}Iα-Bgtx. Binding studies

employing brain homogenates indicate that the regional distribution of both ^{3}H nicotine and ^{3}H ACh differ from that of ^{125}I-Bgtx [2]. It was generally agreed that in rat brain there is only one class of sites for α-Bgtx, but there is a difference in properties and in the distribution of α-Bgtx and high affinity nicotine binding sites [18] suggest that these ligands label separate receptor population.

β-Bgtx a snake venom toxin which acts solely on the presynaptic membrane of cholinergic nerves [5, 6] and blocks neuronal A-type K^+-channels [7]. β-Bgtx produces an irreversible blockade of neurotransmission by acting preferentially on nerve terminals and inhibiting the release of transmitters [8]. Studies on the distribution of the β-Bgtx receptors indicate their predominance in nerve terminal rich areas of rat brain [9]. Release of acetylcholine and choline by the action of β-Bgtx has been studied in various species [10].

^{125}Iβ-Bgtx binding sites were identified in synaptic membrane fraction of chick brain [11], synaptosomes of torpedo electric organ by autoradiography [12], in CNS of rat [13].

High affinity acceptors for ^{125}Iβ-Bgtx have been identified on the cryostat sections of the rat's brain and mapped quantitatively using ^{3}H-sensitive sheet film autoradiography [13]. β-Bgtx affects the release of several neurotransmitters including acetylcholine [22-24] and binds specifically to a single class of non interacting sites of high affinity on the rat's cerebral synaptosomes [9]. Using ^{125}Iβ-Bgtx as a ligand β-Bgtx binding proteins were identified in synaptic fraction of the chick's brain with an affinity constant of 0.47±0.14 nM [11]. The present study aims to identify the localization of α and β-Bgtx binding proteins in different regions of human cadaver brain.

Table I. Details of cadavers

Cad. No	Age/sex	P. No Reason	Time PM (hrs)	Tissue
1	20 y/M	2094/01 accident	23	muscle
2	18 y/M	874/01 accident	17	muscle
3	56 y/M	893/01 accident	23	muscle
4	17 y/M	1049/01 accident	5	muscle brain
5	29 y/F	277/02 sucide	16	muscle
6	70 y/M	522/02 accident	16	muscle
7	58 y/M	679/02 accident	24	muscle brain
8	55 y/M	896/02 accident	6	muscle
9	25 y/M	1317/02 sucide	6	muscle
10	26 y/M	9/03 accident	11	muscle brain
11	27 y/F	189/03	8	muscle brain
12	45 y/M	588/03	17	muscle

Material and methods

Alpha bungarotoxin (α-Bgtx), beta bungarotoxin (β-Bgtx), Tween-20, bovine serum albumin (BSA) were from Sigma Chemical co., St. Louis Radio-Iodine ^{125}I from Saxson laboratories, GFC filter discs from Whatman. Other reagents were of analytical grade.

Receptor solubilization

Different cadaver tissues including brain regions were obtained from mortuary of AIIMS. The details are given in Table I. All the tissues were from non smoker fellows. The membranes and their triton extracts were prepared as described earlier [14]. Briefly the tissue was homogenised in four volumes of chilled homogenising buffer (HB) (0.01 phosphate buffer, pH 7.4) containing 1 M NaCl, 0.02% NaN_3, 0.001 M EDTA, 0.1 M benzethonium chloride, 0.002 M benzemidene hydrochloride, and 0.0001 M phenylmethyl sulphonyl fluoride and 0.5 mg/ml bacitracin. The homogenate was centrifuged at 20,000 g for 60 min at 4°C. The supernatant was collected (soluble supernatant) and pellet was suspended in homogenizing buffer and stored in aliquots at –20°C as membranes or extracted for 3 hrs at 4°C in two volumes of HB containing 2% (v/v) Triton X-100. The supernatant (triton extract) obtained on centrifugation at 20,000 g for 60 min at 4°C was filtered through glass wool, aliquoted and stored as solubilised receptor (Triton Extract) at –20°C.

Radio-iodination of toxin (Alpha-Bgtx and Beta-Bgtx)

Radio-iodination of α and β-Bgtx by Iodogen (1,3,4,6-tetrachloro-3α6α-diphenylglycouril) method was performed [15]. Dichloromethane solubilized iodogen was introduced into glass tubes and evaporated with gentle swirling by passing nitrogen gas to form a thin film. To the precoated Iodogen tube, phosphate buffer + 2-10 μg α or β-Bgtx + 0.5 mCi of ^{125}I Na, were added in sequence and the resultant volume was made to 100 μl. The mixture was incubated for 15 min at RT followed by addition of 20 μl of 2% KI. The contents were gently mixed and transferred onto Sephadex G-25 column for separating toxin from free Iodine. Elution was done at RT with 0.01 M phosphate Buffer containing 0.1% BSA. The void volume peaks containing radio-iodinated toxins were pooled and stored aliquoted at –20°C.

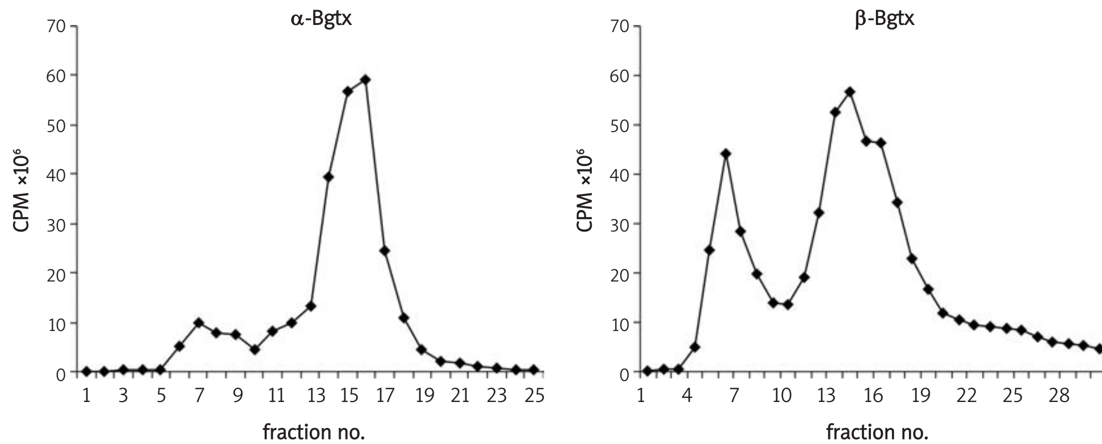

Figure 1. Sephadex G-25 profile of Iodinated Toxin

To the precoated Iodogen tube, phosphate buffer, 2 µg α or β-Bgtx and (^{125}I) Na of 0.5 mCi were added in sequence and the resultant volume was made to 100 µl. The mixture was incubated for 15 minutes at RT followed by addition of 20 µl of 2% KI. The contents were gently mixed and transferred onto Sephadex G-25 column for separating toxin from free Iodine. Elution was done at RT with 0.01 M phosphate buffer containing 0.1% BSA. 1 ml of each fraction was collected and counted in a gamma counter. The radio-iodinated peak fractions were pooled and specific activity was calculated. The experiments was done thrice and specific activity of iodinated of α and β-Bgtx was $1.38 \times 10^8 \pm 0.2$ and $5 \times 10^8 \pm 2.6$ respectively

Binding assay for receptor activity

The activity of postsynaptic AChR and presynaptic membrane receptor in membrane preparation and triton extract of muscle and brain tissue was determined by virtue of its high affinity for α and β-Bgtx. Radio-iodinated α or β-Bgtx was used as a ligand. The free and bound ligand was separated by rapid filtration method in case of membrane preparations [16] and ammonium sulphate precipitation method in triton extracts i.e; solubilized receptor [17].

Rapid filtration method

The membrane suspension was incubated with ^{125}Iα or β-Bgtx in 0.01 M phosphate buffer pH 7.4 containing 0.1% BSA for 30 minutes at room temperature. Incubation was followed by addition of 5 ml of ice cold 0.01 M phosphate buffer pH 7.4. The mixture was rapidly filtered through glass filter disc (2.5 cm diameter). The disc was washed 3 times with ice cold 0.01 M phosphate buffer (5 ml each) and counted for radioactivity in a γ-counter [15, 16].

Ammonium sulphate precipitation method (for solubilized receptor)

^{125}Iα or β-Bgtx was incubated with triton extract for 60 minutes at 37°C. 60% saturated solution of ammonium sulphate was added and allowed to stand for 16 hrs at 4°C. The precipitate was then filtered on GFC glass filter discs. The disc was washed 3 times with 30% ammonium sulphate solution and the radioactivity was counted on a γ-counter [17]. To determine the non specific binding the samples was incubated in the presence of 100 molar excess of non radioactive toxin. The difference in total binding and non-specific binding counts represents the specific binding of the receptors.

Results

Binding assays and specificity

α-Bgtx and β-Bgtx were iodinated by the iodogen method as described in the methodology [15]. The elution profile is shown in Figure 1. The peak fractions (radio-iodinated toxins) were pooled and counted in a gamma counter and their specific activity was calculated. Specific activities of three preparations of radio-iodinated α and β-Bgtx are given in Table II.

Table II. Specific activities for radio-iodinated α and β-Bgtx prepared by iodogen method

α-Bgtx

No.	Toxin	^{125}I	Pool volum (ml)	Specific activity (cpm/nmole)
1	2 µg/10 µl	0.5 mci	3	1.46×10^8
2	2 µg/10 µl	0.5 mci	2.5	1.34×10^8
3	2 µg/10 µl	0.5 mci	3	1.38×10^8

Mean ±SD= $1.38 \times 10^8 \pm 0.02$

β-Bgtx

No.	Toxin	^{125}I	Pool volum (ml)	Specific activity (cpm/nmole)
1	2 µg/10 µl	0.5 mci	2.8	3.8×10^8
2	2 µg/10 µl	0.5 mci	4	8×10^8
3	2 µg/10 µl	0.5 mci	3	3×10^8

Mean ±SD= $5 \times 10^8 \pm 2.6$

The mean specific activities of ^{125}Iα-Bgtx and ^{125}Iβ-Bgtx were $1.38\pm0.02\times10^8$ cpm/nmole and $5\pm2.6\times10^8$ cpm/nmole, respectively. After collecting the tissues from mortuary we wash it cold PBS and then proceesed for making membranes and solubilized receptor. The specific binding of α-Bgtx to membranes and triton extract of fresh muscle is 19.8±0.2 and 10.2±0.12 fmoles/mg tissue respectively. While in cadaver muscle, the specific biding is 19.4±0.15 and 9.8±0.16 fmoles/mg tissue in membranes and triton extract. For β-Bgtx, the specific binding with membranes and triton extract of fresh muscle is 10.2±0.15 and 7.2±0.12 fmoles/mg tissue. Similarly the specific binding of β-Bgtx with membranes and triton extract of cadaver muscle is 9.6±0.14 and 7.1±0.12 fmoles/mg tissue. The extent of non specific binding was calculated by adding 100 M excess of non radioactive toxin. Figure 2 shows that specific binding of radio-iodinated α and β-Bgtx to membranes/triton extract of fresh and cadaver muscle is identical. The binding is saturable and concentration dependent. Binding of radio-iodinated toxin (α or β) to triton extract of parietal lobe and cerebellum was done by ammonium sulphate precipitation method as described in methodology. Specific α and β-Bgtx binding activity was observed in parietal lobe, while as in cerebellum shows only β-Bgtx activity was observed as shown in Figure 3. In addition to parietal lobe and cerebellum, specific α and β-Bgtx binding was observed in liver and different regions of brain. Specific binding in terms of fmoles/mg tissue was studied in parietal lobe (6±0.6) occipital lobe (6.1±0.1) temporal lobe (5.2±1.4), cerebral cortex (6.2±0.3), hippocampus (5.6±0.2), hypothalamus (4.3±0.4) and brain stem (4.8±0.2). No specific binding of alpha bungarotoxin was found in cerebellum and frontal lobe. Beta bungarotoxin binding activity was present in all the above regions of brain and was in range of 4.2-4.9 fmoles/mg tissue (Figure 4). Both α and β-Bgtx was absent in liver.

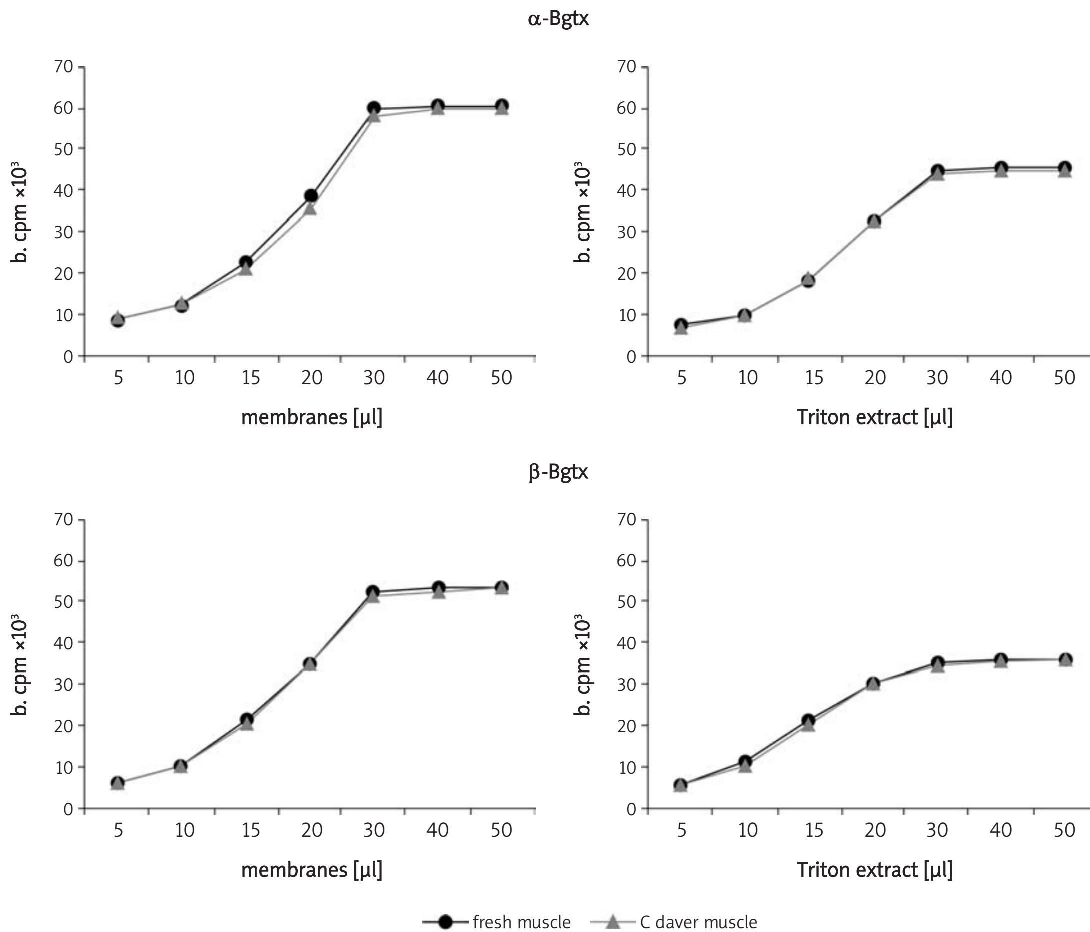

Figure 2. Binding of radiolabelled α and β-Bgtx to membranes and triton extract of fresh and cadaver muscle of human

Indicated volumes of membranes and triton extract preparations from fresh and cadaver skeletal muscle were incubated with 1.5×10^5 cpm of ^{125}I-αBgtx and ^{125}I-βBgtx separately for 60 minutes in a total reaction volume of 100 μl and after incubation filtered on GFC discs and washed thrice with phosphate buffer (in case of membranes) and 30% ammonium sulphate (in case of triton extract).The disc was then counted in a gamma counter. The extent of nonspecific binding was assessed by including 100 times molar excess of non radioactive toxin. The conc. curve of specific binding is shown

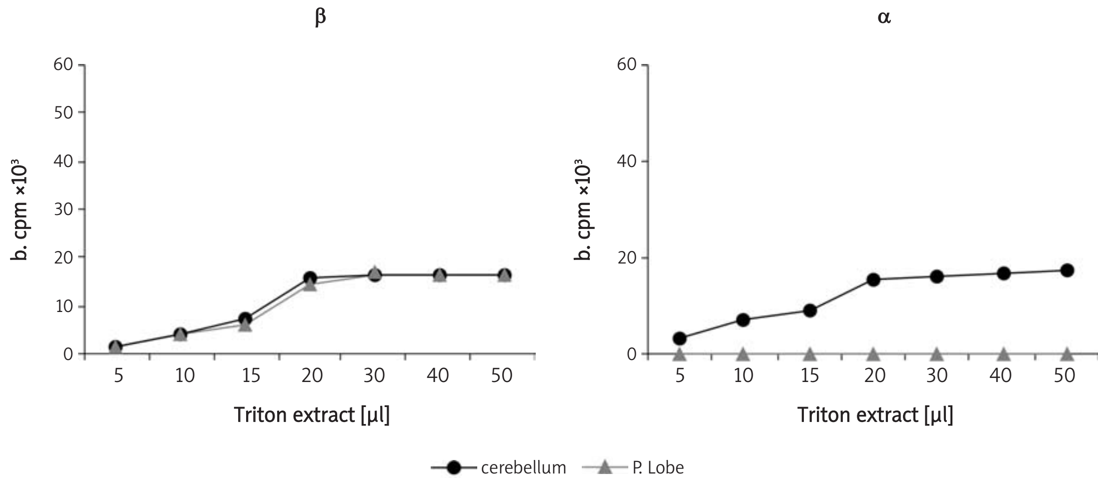

Figure 3. Binding of radio-iodinated toxin α and β-Bgtx to triton extract of two different regions of cadaver brain cerebellum and parietal lobe

Indicated volumes of triton extract preparations from cerebellum and parietal region of cadaver brain were incubated with 1.5×10^5 cpm of ^{125}I-βBgtx and ^{125}I-αBgtx separately for 60 minutes in a total reaction volume of 100 ml. After adding 50 ml of 60% saturated ammonium sulphate, reaction mixture was incubated at 4°C overnight. After precipitation, reaction mixture was filtered on GFC discs and washed thrice with 30% ammonium sulphate. The filter disc was then counted in a gamma counter. The extent of nonspecific binding was assessed by including 100 times molar excess of non radioactive toxin

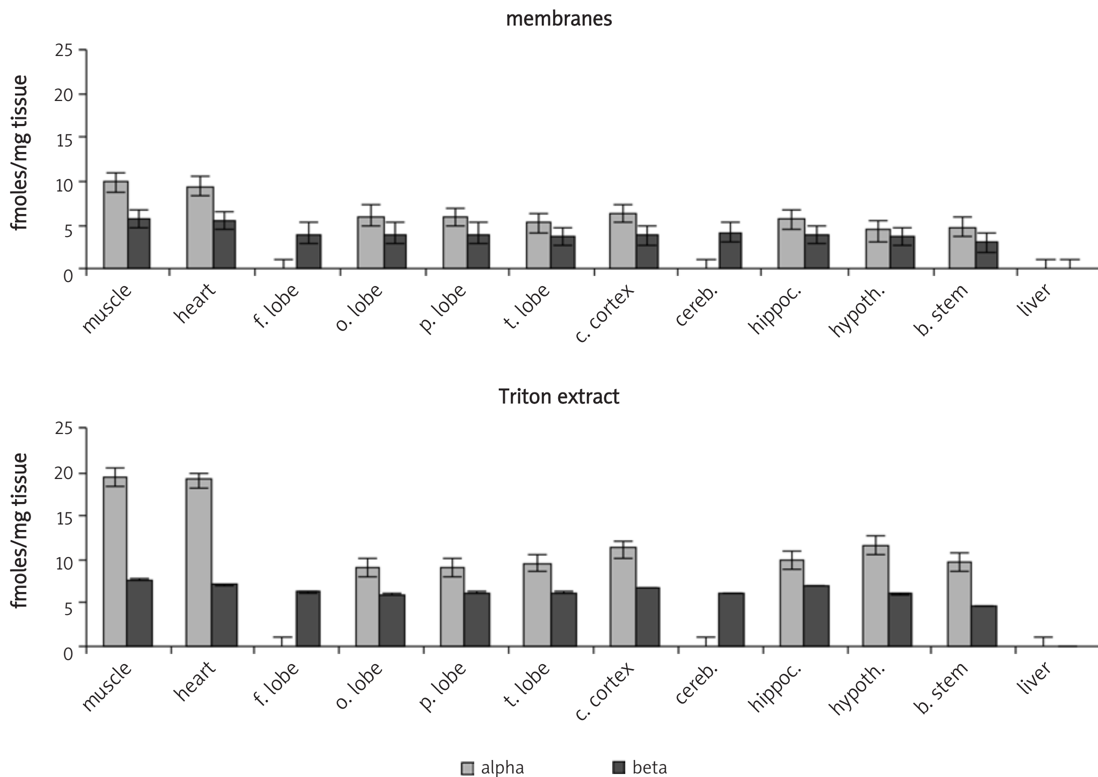

Figure 4. Binding of radio-iodinated α and β-Bgtx to membranes and triton extract of different tissues of human cadaver

50 µl of membranes and triton of extract of different tissues were incubated with 1.5×10^5 cpm of ^{125}I-αBgtx or ^{125}Iβ-Bgtx for 60 minutes in a total reaction volume of 100 ml. In case of membrane binding reaction mixture was filtered on GFC discs, washed thrice with phosphate buffer and counted in a gamma counter. In case of binding to triton extract, 50 ml of 60% saturated ammonium sulphate was added to the reaction mixture and incubated at 4°C overnight. After precipitation, reaction mixture was filtered on GFC discs and washed thrice 30% ammonium sulphate. The disc was then counted in a gamma counter. The extent of nonspecific binding was assessed by including 100 times molar excess of non radioactive toxin

Specificity

Using competition ELISA (Figure 5), it was observed there was a consistent decrease in bound cpm with α-cold toxin but not with β-cold toxin, when triton extract of skeletal muscle was incubated with ^{125}Iα-Bgtx and competed with both α and β-cold toxin. Similarly triton extract of cadaver skeletal muscle was incubated with 1.5×10^5 cpm of ^{125}Iβ-Bgtx in the presence of different concentration of cold toxin (α or β). There was decrease in bound cpm with α cold toxin, but no competition was observed with β (Figure 5). Hence indicating that ^{125}I-α Bgtx and ^{125}I-β Bgtx are specific for the α-Bgtx and β-Bgtx binding proteins respectively.

The binding of radiolabelled α-Bgtx was found in membranes and triton extracts of human cadaver tissue. No specific binding was observed in frontal lobe, cerebellum and liver membranes and triton extract hence indicating that α-Bgtx binding proteins are absent in these tissues. Radio-labelled β-Bgtx binding sites was also observed in the membranes and triton extract of human cadaver tissue. No specific binding was observed in liver membranes and triton extract (Figure 4). The binding was described in terms of fmoles/mg tissue.

Different tissues of human cadaver were collected on different postmortem time. The details are given in Table III. Specific binding of radiolabelled α and β-Bgtx were studied in membranes and triton extract of different tissues. It was observed there is not a significant decline in specific binding to membranes and triton extract of muscle with increase in post mortem time (5-24) (Figure 6 and Table IV). There is a decline (11-24 hrs) in specific binding of radiolabelled α and β-Bgtx to membranes and triton extract of different brain regions (Figure 7). This means that α and β-Bgtx binding proteins in muscle are more stable.

Discussion

Regional distribution of ^{125}Iα-Bgtx binding protein in rat brain has been identified by autoradiography and revealed high binding in hippocampus, hypothalamus and other regions, while as cerebellum was devoid of specific binding [3]. Using micropunched tissue homogenates ^{125}Iα-Bgtx binding was observed in hypothalamus followed by hippocampus,

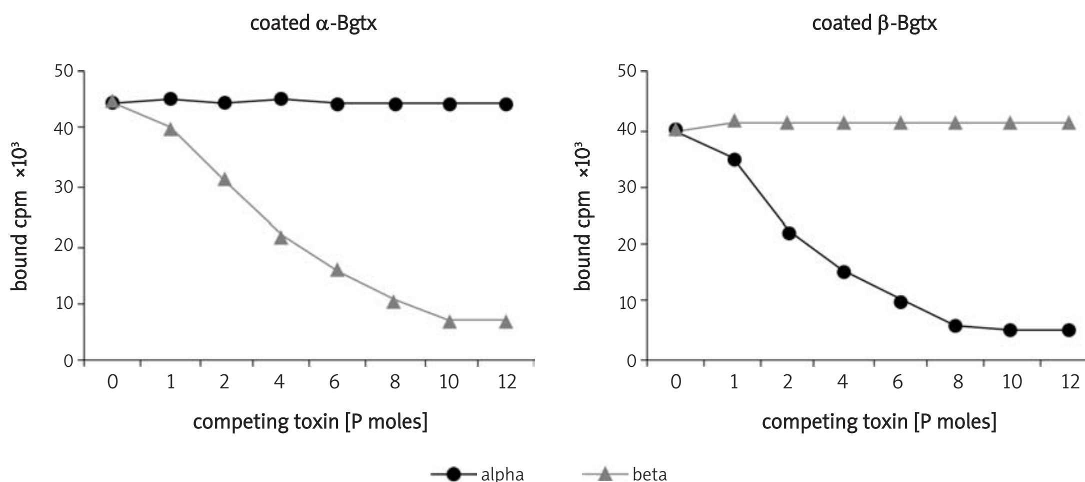

Figure 5. Specificity of radiolabelled α and β-Bgtx

Indicated volumes of triton extract preparation from cadaver skeletal muscle were incubated with 1.5×10^5 cpm of ^{125}Iα-Bgtx in the presence of inceased concentration of competing non radio-active toxin both α and β. Similarly triton extract of muscle was incubated with 1.5×10^5 cpm of ^{125}Iβ-Bgtx in the presence of increased concentration of non radioactive toxin both a and b for 60 minutes in a total reaction volume of 100 ml and after adding 50 ml of 60% saturated ammonium sulphate, reaction mixture was incubated at 4°C overnight. After precipitation, reaction mixture was filtered on GFC discs and washed thrice with 30% ammonium sulphate. The filter disc was then counted in a gamma counter. The extent of nonspecific binding was assessed by including 100 times molar excess of non radioactive toxin

Table III. Effect of postmortem time on toxin binding

Postmortem time (hrs)	Membrane fmoles/mg tissue		Triton extract fmoles/mg tissue	
	α	β	α	β
Group I (0-8 hrs) (n=4)	19.75±0.68	9.26±0.5	9.85±0.25	6.55±0.4
Group II (8-16 hrs) (n=3)	18.49±0.52	8.34±0.1	9.1±0.06	5.98±0.1
Group III (16-24 hrs) (n=5)	18.3±0.04	7.83±0.2	8.6±0.12	5.44±0.1

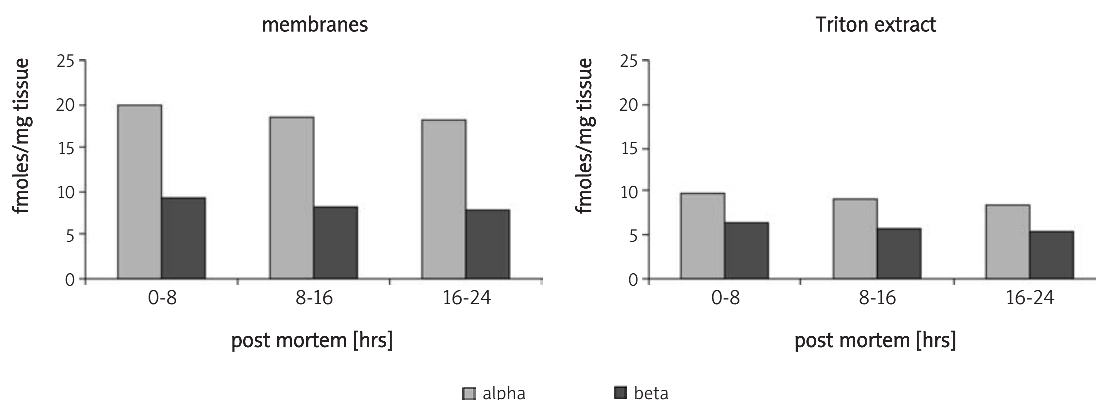

Figure 6. Effect of post mortem time on toxin binding in human cadaver skeletal muscle

The effect of postmortem time on toxin binding in skeletal muscle of different cadavers was studied (group I 0-8 hrs, II 8-16 hrs and III 16-24 hrs). Binding of radiolabelled toxins (both alpha and beta) to membranes and triton extract of these cadaver muscle was determined as given in the methodology

Table IV. Toxin binding profile in parietal lobe and occipital I lobe of different human cadavers

Parietal lobe

PM (hrs)	α-Bgtx		β-Bgtx	
	membranes (fmoles/mg tissue)	Triton extract (fmoles/mg tissue)	membranes (fmoles/mg tissue)	Triton extract (fmoles/mg tissue)
5	10.2±0.2	6.4±0.2	7.6±0.15	5.1±0.2
8	10±0.15	6±0.16	7.4±0.12	4.7±0.15
11	8.4±0.12	5.4±0.15	7±0.2	4.2±0.2
24	7±0.2	3.8±0.12	5.4±0.15	3.8±0.2

Occipital lobe

PM (hrs)	α-Bgtx		β-Bgtx	
	membranes (fmoles/mg tissue)	Triton extract (fmoles/mg tissue)	membranes (fmoles/mg tissue)	Triton extract (fmoles/mg tissue)
5	10.5±0.2	6.5±0.2	7.5±0.2	4.3±0.2
8	10.2±0.15	6.2±0.17	7.1±0.15	4±0.2
11	8±0.14	6±0.11	6.5±0.2	3.8±0.15
24	6.2±0.15	4±0.2	5.2±0.2	3±0.2

cortex, globus pallidus, nucleus caudatus and nucleus accumbens of rat brain [1]. Invitro autoradiography reveals the presence of ^{125}Iα-Bgtx binding sites, localized in the subesophageal ganglion, the optic tubercles, antennal lobes of honey bee brain [4]. Three radio ligands have been commonly used to lable putative nicotinic cholinoreceptor in the mammalian central nervous system:the agnosists ^{3}H nicotine and ^{3}H acetylcholine in the presence of atropine to block muscranic receptors and snake venom extract ^{125}Iα-Bgtx. Binding studies employing brain homogenates indicate that the regional distribution of both ^{3}H nicotine and ^{3}H acetylcholine ACh differ from that of ^{125}Iα-Bgtx [2]. It is generally agreed that in rat brain there is only one class of sites for α-Bgtx [7] but difference in properties and distribution of α-Bgtx and high affinity nicotine binding sites [18].

β-Bgtx blocks cholinergic transmission by presynaptic action [19]. High affinity β-Bgtx binding sites were identified in CNS of rat [9, 11, 20, 21], torpedo [12] chick [7]. Enriched areas of grey mater and synaptic regions of central nervous system of rat brain, particularly binds with ^{125}Iβ-Bgtx. Using ^{3}H sensitive sheet film autoradiography, it has been demonstrated that β-Bgtx acceptors are voltage sensitive K channels [13]. Electron microscopic autoradiography was used for evaluation of ^{125}Iβ-Bgtx in synaptosomes of torpedo electric organ, which inhibits the release of transmitter on plasma

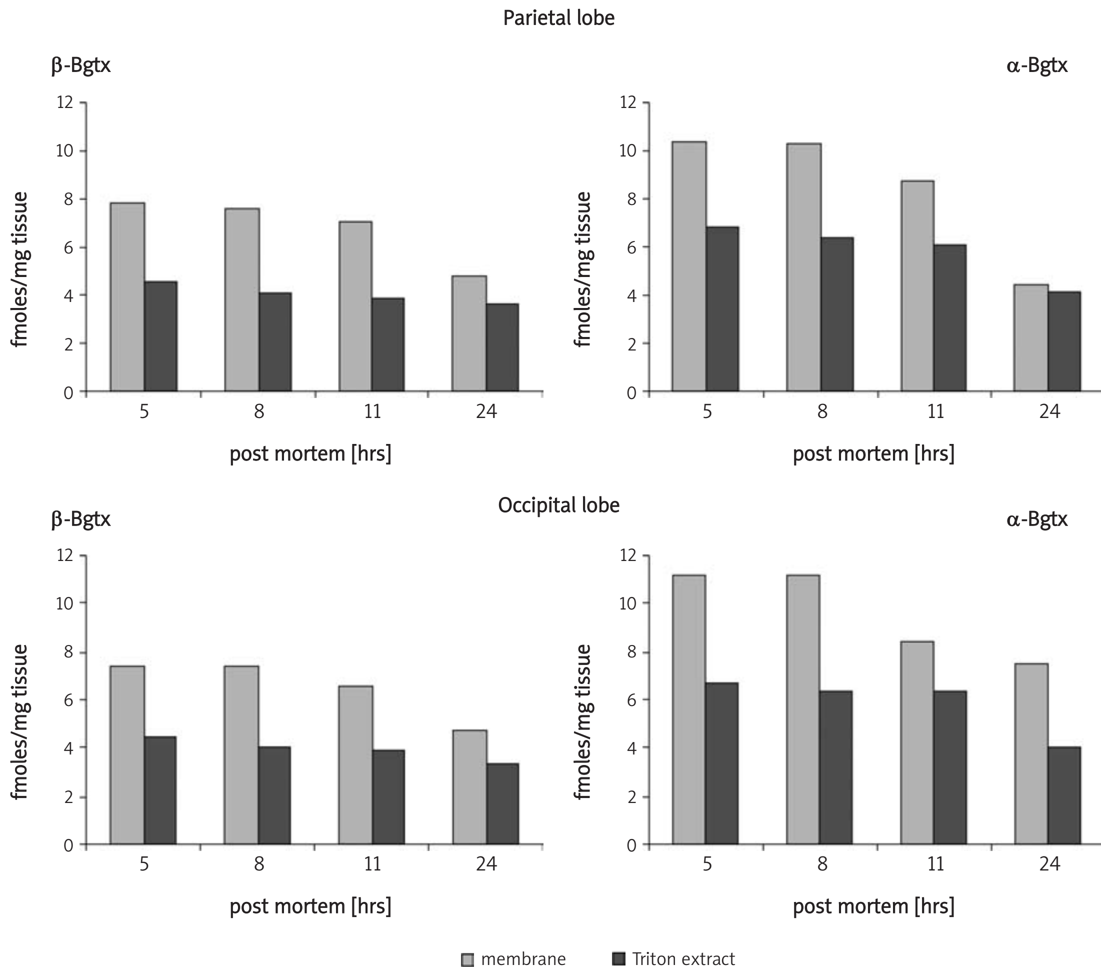

Figure 7. Effect of post mortem time on toxin binding in parietal lobe and occipital lobe of human cadaver brain

Different regions of cadaver brain (parietal lobe, occipital lobe) were collected on different postmortem time (5-24 hrs). Binding of radiolabelled toxins (both α and β-Bgtx) to membranes and triton extract of these cadaver brain was determined as given in the methodology

membrane [12]. Using these radiolabelled toxins (α or β-Bgtx) as a specific ligand, we explored the regional distribution of α-Bgtx and β-Bgtx binding sites in human cadaver muscle and in different regions of brain. In most brain regions ^{125}Iα-Bgtx binding sites are localized to neuronal cell bodies (Breese et al., 1997). Specific binding of radio-iodinated α and β-Bgtx was carried out in membranes (rapid filtration method) and triton extract (ammonium sulphate precipitation method) of fresh and cadaver muscle. The binding was saturable and concentration dependent (Figure 2). α and β-Bgtx binding proteins have been identified in the membranes and triton extract of different tissues of human cadaver (Figure 5). α-Bgtx binding proteins were absent in the cerebellum, frontal lobe and liver tissues. Radio-labelled β-Bgtx binding sites were also observed in the membranes and triton extract of human cadaver tissue. All the brain regions, skeletal muscle and heart (except liver) contain presynaptic receptor (β-Bgtx binding protein) (Figure 5).

The cadaver tissues used for this study differed in postmortem as well as the age of the cadavers. There was no significant decline in specific binding to membranes and triton extract of muscle with increase in post mortem time (5-24 h), but there is a little decline (11-24 h) in specific binding of radiolabelled α and β-Bgtx to membranes and triton extract of the different brain regions (Figures 6-7). It has earlier been reported that postmortem delays (up to 96 h at 4°C) did not produce any significant changes in the density and binding properties of the nAChR [25].

We also observed that in addition to postsynaptic receptors (α-Bgtx binding proteins), presynaptic receptors (β-Bgtx binding proteins) also shows immunoreactivity with Myasthenic sera.

Conclusions

In conclusions, MG is not only due to the damage of the postsynaptic membrane, but it could concurrently be a consequence of antibody

mediated damage of the presynaptic membrane, resulting particularly the damage of its β-Bgtx binding sites and could play an important role in the pathogenesis of myasthenia gravis.

Acknowledgement

We thank to Indian Council of Medical Research for providing grant.

References:

1. Kamiya H, Takano Y, Kusunoki T, et al. Studies on 125I-alpha bungarotoxin binding sites in rat brain. Brain Res Bull 1982; 8: 431-433.
2. Clarke PB, Schwartz RD, Paul SM, Pert CB, Pert A.. Nicotinic binding in rat brain: autoradiographic comparison of [3H] acetylcholine, [3H] nicotine and [125I] alpha-bungarotoxin. J Neurosci 1985; 15: 1307-1315.
3. Segal M, Dudai Y, Amsterdam A. Distribution of an Alpha-bungarotoxin-binding cholinergic nicotinic receptors in rat brain. Brain Res 1978; 148: 105-119.
4. Scheidler A, Kaulen P, Bruning G, Erber J. Quantitative autoradiographic localization of [125I] alpha-bungarotoxin binding sites in the honeybee brain. Brain Res 1990; 534: 332-335.
5. Anderson AJ, Harvey AL, Rowan EG. Facilitation of nurotransmitter release by snake of chick brain. J Biol Chem 1987; 10: 10015-10022.
6. Kato AC, Pinto JE, Glavinovic M, Collier B. Action of a beta--bungarotoxin on autonomic ganglia and adrenergic transmission. Can J Physiol Pharmacol 1977; 55: 574-584.
7. Schmidt RR, Betz H. The beta-bungarotoxin binding protein from chick brain: binding sites for different neuronal K+ channel ligands co-fractionate upon partial purification. FEBS Lett 1988; 240: 65-70.
8. Halliwell JV, Dolly JO. Preferential action of beta-bungarotoxin at nerve terminal regions in the hippocampus. Neurosci Lett 1982; 30: 321-327.
9. Othman IB, Wilkin GP, Dolly JO. Synaptic binding sites in brain for 3H-fl-bungarotoxin a specific probe that perturbs transmitter release. Neurochem Int 1983; 5: 487-496.
10. Newton MW, Gundersen CB, Jenden DJ. The effect of beta-bungarotoxin on acetylcholine and choline content of vertebrate tissues. Toxicon 1983; 21: 825-832.
11. Rehm H, Henrich B. Binding of beta-bungarotoxin to synaptic fraction of chick brain. J Biol Chem 1982; 10: 10015-10022.
12. Esquerda JE, Solsona C, Marsal J. Binding of beta-bungarotoxin to Torpedo electric organ synaptosomes. A high resolution autoradiographic study. Neuroscience 1982; 751-758.
13. Pelchen-Mathews A, Oliver JD. Distribution of acceptors for? beta-bungarotoxin in the CNS of rat. Brain Research 1988; 44: 127-138.
14. Jailkhani BL, Asthana D, Jaffery NF, Kumar R, Ahuja GK. A simplified ELISA for anti-receptor antibodies in myasthenia gravis. J Immunol Methods 1986; 86: 115-118.
15. Jailkhani BL, Asthana D, Jaffery NF, Subbalaxmi B. alpha-bungarotoxin aggregates on radioiodination with chloramine-T but not with iodogen. J Neuroimmunol 1984; 6: 337-345.
16. Schmidt J, Raftery MA. A simple assay for the study of solubilized acetylcholine receptors. Anal Biochem 1973; 52: 349-54.
17. Meunier JC, Olsen RW, Changeux JP. Studies on the cholinergic receptor protein from Electrophorus electricus. Effect of detergents on some hydrodynamic properties of the receptor protein in solution. FEBS Lett 1972; 24: 63-68.
18. Marks MJ, Collins AC. Characterization of nicotine binding in mouse brain and comparison with the binding of alpha-bungarotoxin and quinuclidinyl benzilate. Mol Pharmacol 1982; 22: 554-564.
19. Strong PN, Goerke J, Oberg SG, Kelly RB. beta-bungarotoxin, a pre-synaptic toxin with enzymatic activity. Proc Nat Acad Sci USA 1976; 73: 178-182.
20. Rugolo M, Dolly JO, Nicholls DG. The mechanism of action of beta-bungarotoxin at the presynaptic plasma membrane. Biochem J 1986; 233: 519-523.
21. Pelchen-Mathews A, Dolly JO. Distribution of acceptors for beta-bungarotoxin in the central nervous system of the rat. Brain Research 1988; 441: 127-138.
22. Spokes JW, Dolly JO. Complete purification of beta-bungarotoxin. Characterization of its action and that of tityustoxin on synaptosomal accumulation and release of acetylcholine. Biochim Biophys Acta 1980; 596: 81-93.
23. Tse CK, Dolly JO, Diniz CR. Effects of beta-bungarotoxin and tityustoxin on accumulation of putative amino acid neurotransmitters by rat cortex synaptosomes. Neuroscience 1980; 5: 135-143.
24. Sen I, Grantham PA, Cooper JR. Mechanism of action of beta-bungarotoxin on synaptosomal preparations. Proc Natl Acad Sci U S A 1976; 73: 2664-2668.
25. Anderson AJ, Harvey AI, Rowan EG. Facilitation of nurotransmitter release by snake toxin. Synaptic transmitters and Receptors Wiley, Chichester and Academia Prague 1987; 392-398.