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Research Research

Investigating the Effect of Small Peptides from Neuropathogenic Bacteria on Potassium and Sodium Currents of Mice Hippocampal Neurons

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Abstract

Different cellular components of neuropathogenic bacteria have the capability to alter the homeostasis and normal function of Central Nervous System (CNS). *Clostridium tetani* (Ct), *Neisseria meningitides* (Nm), *Bacillus cereus* (Bc) and *Listeria monocytogenes* (Lm) are the principal pathogen of the CNS. The objective of present study was to examine the effects of a bacterial infection milieu on the electrophysiological properties of BALB/c mice hippocampal neurons by using the small peptides of neuropathogenic bacteria. Overnight separately grown bacterial culture in Nutrient Broth (NB), and human serum supplemented RPMI 1640 (RPMI), were used. Bacterial peptides were screened and purified from filter sterilized cell free broths (SCFBs), through high performance liquid chromatography with an electrochemical detection (HPLC-EC). Electrophysiological impact of peptides on voltage gated potassium (I_{dr}), and sodium (I_{Na}), channels currents were recorded at interval of 5 and 10 minutes using whole cell patch clamp technique. Prior to peptide exposure multiple readings of cultured neuronal cells were taken as control. The current profile indicated the peptides (0.5 mg) each of Ct, Bc and Lm isolated from both NB and RPMI significantly (P<0.05), inhibited I_{kv} currents. In contrast, $Bc_{(RPMI)}$ significantly (P<0.05), activated I_{Na} while peptides of Nm did not show any significant effect on both type of currents. Collectively these findings demonstrated the implication of bacterial peptides on the CNS by their intervention in electrophysiology of cells consequently initiated an array of other physiological functions associated in disease progression.

INTRODUCTION

Bacterial virulence factors being either cytosolic, membrane associated and secretory in nature, refer to the molecules that assist the bacterium colonizes the host at the cellular level [1]. A growing body of evidences has suggested that these molecules are being widely studied to explore new venues of development of central nervous system (CNS), infections [2-5]. These factors include bacterial cell wall components such as lipopolysaccharide (LPS), peptidoglycan (PG), and toxins of some bacteria which mainly cause infection in the CNS [2,6-10]. Bacteria may adopt one of the well-known mechanisms to directly invade the CNS: (i) directly penetrating the endothelial cells of the blood-brain barrier (ii) transportation of bacteria to the CNS within circulating leukocytes (iii) and through a neural route from peripheral tissues by intra-axonal transport [2,4]. On contrary, bacteria residing in blood or any other body sites also affect the CNS with the help of bacterial virulence factors [11]. Furthermore, it has been recently reported that bacterial virulence factors initiate brain inflammation itself or later to interacting with neuronal cells [8]. Consequently, several cellular and biochemical processes are being altered by bacterial virulence factors which in turn affect synaptic, immune, endocrine and behavioural responses controlled by the CNS thus, promisingly contribute in the progression of infection [2-5,12]. Thus, eliciting devastating and fatal condition which may leads to the permanent neurological dysfunction [11,12].

In view of the fact that CNS is equipped with many electrically excitable cells which possess a diverse and ubiquitous family of membrane proteins known as ion channels [13]. Therefore these cells serve as one of the target sites for bacterial virulence factors of neuropathogeinc bacteria [14]. Bacterial components, on the other hand may serve as a powerful stimulus for the voltage gated ion channels present at the level of plasma membrane [14,15]. There are two main voltage gated ion channels; potassium (I_{dr}) and sodium (I_{Na}) channels that play a key role in controlling repolarization and resting membrane potential and give rise to action potential upstroke in electrically excitable cell. Alterations in the membrane currents of these cells not only disturbs the normal functioning but may even leads to play a critical role in cellular signaling processes regulating neurotransmitter release, cell apoptosis and contribute in severity of infection [3,13-17].

Most of studies highlighted the role of bacterial LPS on ion channels of neurons. However, the effect of other components especially small peptides of neuropathogenic bacteria on ion channels is still unclear. Therefore, present study have been designed to investigate the effects these peptides on the electrophysiological properties of delayed rectifier (I_{dr}) and

transient-A type (I_A) K+ channels and sodium channels (I_{Na}) of hippocampal neurons of BALB/c mice.

MATERIALS AND METHODS

Chemicals

Unless otherwise stated, all the analytical grade chemicals and formulated bacteriological media used in present study were purchased from Merck (Frankfurter, Darmstadt, Germany) and Sigma (St. Louis, Missouri, USA). High performance liquid chromatography with electrochemical detection (HPLC-EC) system was acquired from Merck (Frankfurter, Darmstadt, Germany) with Chromeleon® 6.8.0 software.

Animals

Locally bred BALB/c male and female mice were allowed for matting and P2 mice pups were used for hippocampal neuron cell culture. Animals were housed individually with free access to standard rodent diet and water. All animal experiments were conducted in accordance with the policy and standard guidelines for animal experiments of the Institutional Ethics Committee. (Animal Study Protocol No: 2010-0518).

Bacteria

The study reported herein was carried out with neuropathogens of bacterial etiology i.e., *B. cereus* (Bc), *Cl. tetani* (Ct), *N. meningitides* (Nm), and *L. monocytogenes* (Lm) isolated and identified in our lab during different studies reported elsewhere [22,31].

Filter Sterilized Cell Free Cultural Broth (SCFB) Preparation

In order to prepare SCFB, Lm, Nm, Ct and Bc were grown separately in 100 mL of Nutrient broth (NB), and human serum supplemented RPMI 1640 media (10mL) for 24 hours at 37°C. Anaerobic conditions were maintained by providing 5% CO_2. After incubation each of the cultural broth was centrifuged at 5000 rpm for 10 minutes; supernatant was separately air dried overnight at ambient temperature. Dried SCFB was scrapped off by adding 2mL of distilled water (D/W) followed by filter sterilization using a 0.2 micron membrane Millipore filter. Blank broths of each of the above mentioned media were air dried and filter sterilized likewise, which served as control.

Extraction of Small Bioactive Peptides

Extraction of the bioactive peptides of neuropathogenic bacteria were carried out by injecting 25µL of each of the bacterial SCFB along with control to HPLC-EC system. HPLC-EC system was consisted of a Dionex ED50 electrochemical detector, a glassy carbon working electrode, 5µm octa decyl silane (ODS) reverse phase, 250 ×4.6 mm, C-18 column, and Chromeleon® 6.8.0 software. 0.1M KH_2PO_4 and Acetonirile (85:15) mobile phase was used. The flow rate of the pump (Dionex Ultimate 3000) was 1 mL/min. The sensitivity of the detector was 1.0 nA, and the potential of the working electrode was 0.6 V. Peak areas were quantified by comparison with external standards of amino acids. Peptides were identified based on their elution time for purification. Selected peaks were then purified by multiple rounds of HPLC-EC using 0.1M ammonium acetate and acetonitrile (85:15) buffer. Purity of the peptides was checked using the same buffer. These peptides (Table 1), were freeze dried and stored at -80°C till further use.

Cell Culture

To study the effects of bacterial peptides on the I_{dr} and I_{Na} channels of hippocampal neurons, brains of 1- 2 days old BALB/c mice were dissected. Brain was cut down into two half from mid-saggital plane and midbrain was removed from each hemisphere to expose hippocampus. Hippocampi were piched off with the help of curved forcep, chopped in Hank's Balanced Salt Solution (HBSS) and then transferred into a 15 mL falcon tube. Hippocampi were trypsinized for 20 minutes at 37°C in an atmosphere of 5% CO_2–95% air, then mechanically dissociated (≈ 20 times) by trituration with micropipette. Cells were then pelleted down by centrifugation at 1000 rpm for 10 minutes. Cells were suspended in Dulbecco's Modified Eagle Medium (DMEM) culture medium supplemented with 1% L-glutamate, 1% penicillin-streptomycin mixture and 2% B-27 and 10% Fetal Bovine Serum (FBS) and counted in a haemocytometer. Cover slips were pre coated with poly-L- Lysine (PLL) (50µg/mL). Approximately 20,000 cells were seeded on 22mm cover slips placed in a 6 well plate and cultured at 37°C in an atmosphere of 5% CO_2 – 95% air for 7 days. Medium was changed at an interval of 2 days.

Electrophysiological Recordings

Electrophysiological readings were carried out on 4-6 days old cultured mice hippocampal neurons at room temperature, using patch clamp technique in whole cell configuration. Cultured cells was perfused with extracellular fluid (ECF) containing (in mM) 140 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 glucose. The pH was adjusted to 7.4 with NaOH. After getting whole cells configuration, currents were recorded at holding potential of -60mV. Patch pipettes were made from borosilicate glass and had resistance of 5 MΩ. Pipette was filled with solution containing (in mM) 10 NaCl, 145 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES and 10 EGTA.

All signals were recorded with an HEKA-EPC-10 patch clamp amplifier operated in voltage clamp mode. Data acquisition was performed with a computer controlled interface using Patch Master Software version 2.3.

To activate voltage gated sodium and potassium channels, cells were stimulated from -60mV to +60mV for 100 msec.

Table 1: Description of the experimental challenges used to study I_{kv} and I_{Na}.

Challenge	Peptides Detail
Bacterial Peptides purified from spent Nutrient Broth	$Bc_{(NB)}$
	$Ct_{(NB)}$
	$Lm_{(NB)}$
	$Nm_{(NB)}$
	$Control_{(NB)}$
Bacterial Peptides purified from spent RPMI 1640 supplemented with human serum	$Bc_{(RPMI)}$
	$Ct_{(RPMI)}$
	$Lm_{(RPMI)}$
	$Nm_{(RPMI)}$
	$Control_{(RPMI)}$

followed by -110mV. To separate potassium currents, sodium channels were blocked by tetradotoxin (TTX, 1µM). Both voltage gated potassium and sodium currents were confirmed by their respective inhibitors i.e., tetraethylammonium (TEA) and TTX respectively.

Two pulses of similar protocol (from -60mV +60mV) were applied to the neurons for 200 msec but start from different holding potential of -110 mV and -50 mV respectively. The second pulse gives us only sustained currents (I_{dr}) because fast transient currents do not activate after -50 mV. To isolate the fast transient currents (I_A), traces of current recorded from -50 mV were digitally subtracted from the traces recorded at holding potential of -110 mV (Figure 1).

After recording control currents, each bacterial peptide (0.5 mg) was applied on same neuron and currents were recorded after 5 and 10 min. Both NB and RPMI 1640 media without any bacterial growth were used as negative control.

The peak currents (I), were measured at each potential normalized by membrane capacitance and plot against command potentials (V), to demonstrate IV relationship. Slow whole cells capacitance was consider as cells capacitance [30] and conductance was measure by using formula;

G = I/(Em-Erev)

where;

G = conductance

I = currents (pA)

Em = command voltage (mV)

Erev = reversal potential of ion

Only less than 10% changes in cell capacitance and series resistance was accepted for data analysis before and after incubation of peptides.

Statistical Analysis

Statistical data are expressed as mean ± SD. All data was evaluated by use of unpaired student's t-test using SPSS software version 21. P<0.05 was considered as significant.

RESULTS

Characterization of sodium and potassium currents of cultured hippocampal neurons

First we characterized both voltage gated ion channels in cultured hippocampal neurons. Figure 1A shows the potassium currents recorded from different starting holding potentials and resultant subtracted peaks of transient- A currents (I_A). Figure 1B represents pulse protocol of above currents. To isolate sodium currents, TEA was added in the ECF solution. Peak current density (300±50 pA/pF) of sodium channels was observed at -10mV and addition of TTX (1µM) in the bath solution inhibit these currents (Figure 1C).

Effects of Clostridium tetani Peptides on the voltage-sensitive Na+ and K+ Currents

This experiment was delineated to evaluate the effect purified small peptide of Cl. tetani (Ct) purified from spent media

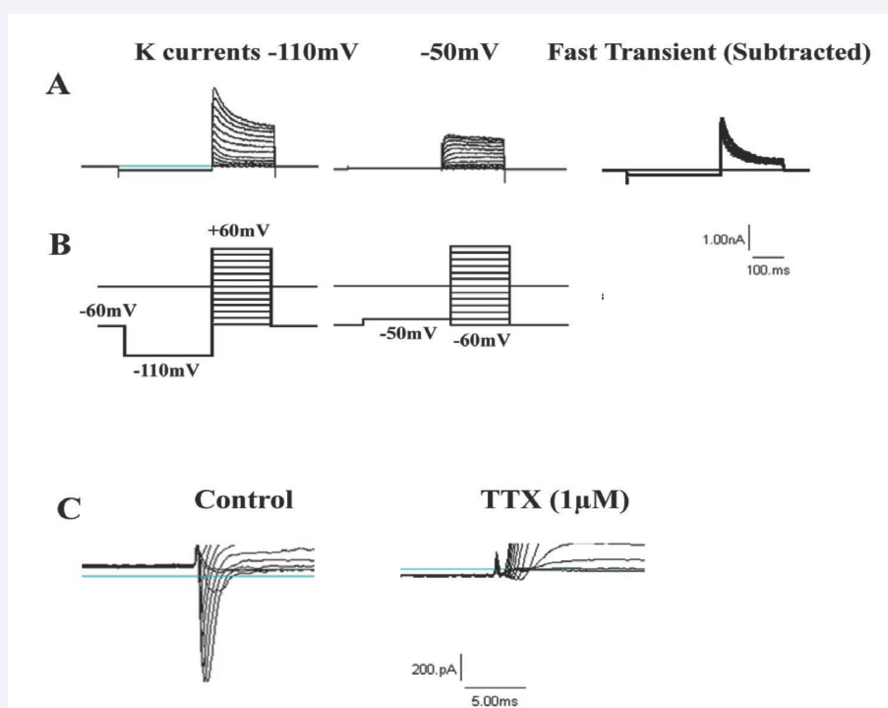

Figure 1 Isolation of Characterization of potassium and sodium currents of cultured hippocamal neurons. (A) Current traces of potassium channels on different holding potentials and subtracted traces of I_A; (B) showing pulse protocol of above represented currents; and (C) represent the TTX sensitive sodium currents. n=5.

on transient A-type (I_A) and delayed rectifier (I_{dr}) K+ and sodium channels (I_{Na}). Therefore, cultured BALB/c mice hippocampal neurons were exposed at the interval of 5 and 10 minutes with $Ct_{(NB)}$ and $Ct_{(RPMI)}$. The current families recorded for Idr channels (Figure 2A), significantly (P<0.05) showed that the Ct (NB) peptides depressed the Idr currents after 10 minutes (control 167.8 ± 34.3 pA vs Ct(NB) 110.9 ± 21 pA) of exposure while 5 minutes exposure did not show any prominent effect. Whereas Ct $_{(RPMI)}$ showed more quick response as compared to $Ct_{(NB)}$. It significantly (P<0.05) inhibited I_{dr} after both 5 and 10 minutes incubation (Control 206.1 ± 50.5 vs $Ct_{(RPMI)}$ 132.5 ± 55.8 pA for 5min and 114.22 ± 49.8pA for 10min). However, difference of response between 5 min and 10 min was not significant (Figure 2B). In contrast, no significant effect of this peptide was observed on Idr and INa (Figure 2C).

Effects of Neisseria meningitides Peptides on the voltage- sensitive Na+ and K+ Currents

Another set of experiment was conducted to evaluate the influence of Nm peptides i.e. $Nm_{(NB)}$ and $Nm_{(RPMI)}$ on cultured BALB/c mice hippocampal neurons. Both $Nm_{(NB)}$ and $Nm_{(RPMI)}$ did not show any significant effect on current amplitude of potassium (Control 118.9 ± 32.9 vs $Nm_{(NB)}$ 113.5 ± 36.3 for 5min and 104.5 ± 41.8) (Figure 3A) and (Control 101.3 ± 36 vs $Nm_{(RPMI)}$ 86.3±18.4 for 5min and 80.8 ± 17.7 for 10min) (Figure 3B). Similarly, there is no effect was observed on sodium currents (Figure 3C).

Effects of Bacillus cereus Peptides on the voltage-sensitive Na+ and K+ Currents

$Bc_{(NB)}$ and $Bc_{(RPMI)}$ peptides demonstrated different electrophysiological event, when applied on the cultured hippocampal neurons of BALB/c mice. It was significant to note that $Bc_{(NB)}$ did not changed potassium currents at any interval while $Bc_{(RPMI)}$ caused marked depression (P<0.05) of I_{dr} within 5 min of application (Control 161.7 ± 24.6 vs $Bc_{(RPMI)}$ 97.5 ± 20.5 pA). This depression was found to be increased progressively up to 10 minutes (Control 161.7 ± 24.6 vs $Bc_{(RPMI)}$ 76.2 ± 13.3 pA). One the other hand, no effects of Bc on I_A were recorded. Figure 4A and 4B exhibits the representative current traces of voltage-sensitive potassium channels with the treatment of $Bc_{(NB)}$ and $Bc_{(RPMI)}$ respectively.

Interestingly, only peptides extracted from RPMI media

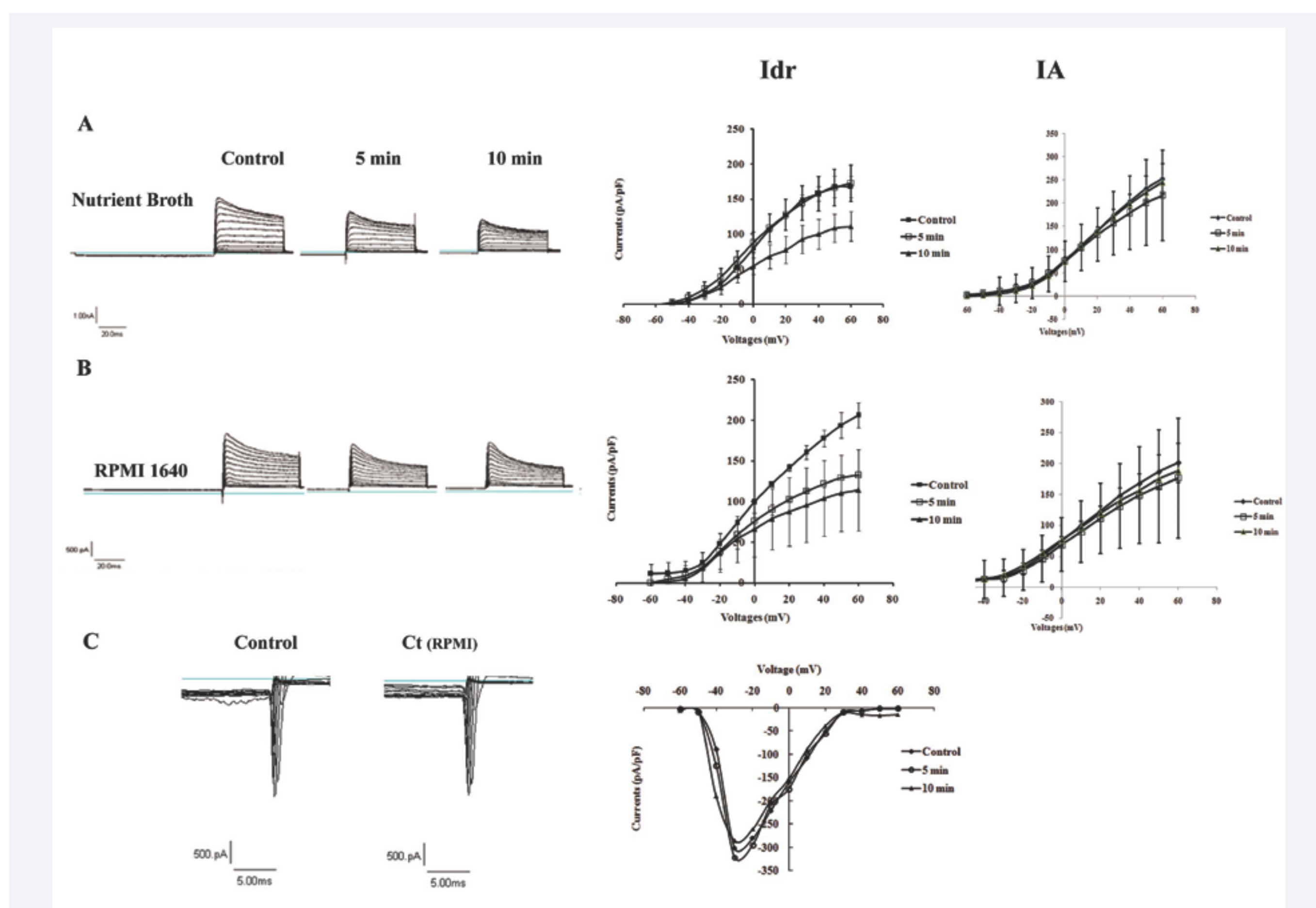

Figure 2 Effect of *Clostridium tetani* (Ct) peptides on potassium currents (I_{dr}) and sodium currents (I_{Na}) of cultured BALB/c mice hippocampal neurons. (A) Representative potassium current traces obtained from control neurons, following 5 min and 10 min incubation with $Ct_{(NB)}$ peptides; (B) representative potassium current traces obtained from control neurons, following 5 min and 10 min incubation with $Ct_{(RPMI)}$ peptides; and (C) representative sodium current traces of before and after Ct treatment. Current-voltage curves are present on the right side of each set of treatment. n=5 for each treatment.

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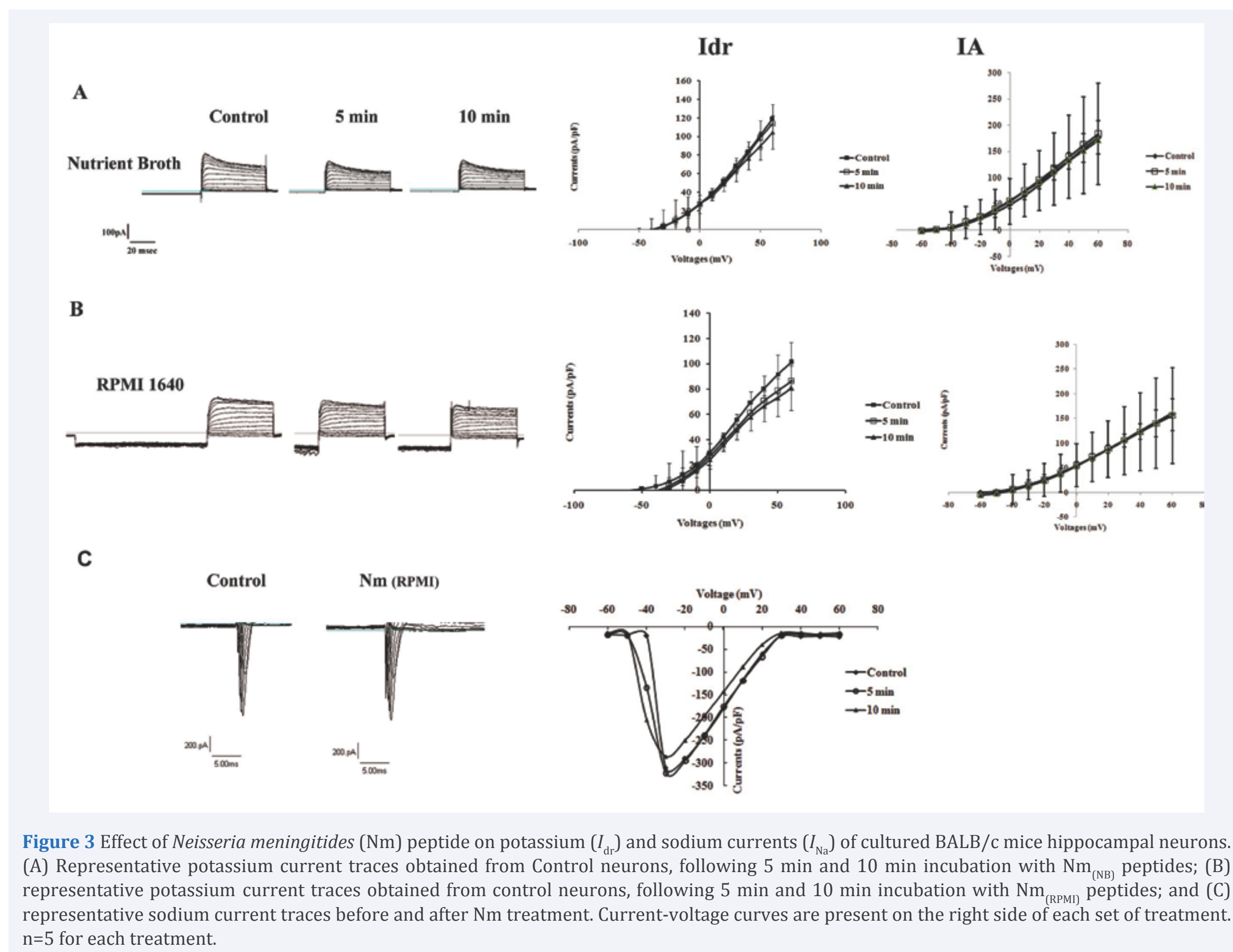

Figure 3 Effect of *Neisseria meningitides* (Nm) peptide on potassium (I_{dr}) and sodium currents (I_{Na}) of cultured BALB/c mice hippocampal neurons. (A) Representative potassium current traces obtained from Control neurons, following 5 min and 10 min incubation with $Nm_{(NB)}$ peptides; (B) representative potassium current traces obtained from control neurons, following 5 min and 10 min incubation with $Nm_{(RPMI)}$ peptides; and (C) representative sodium current traces before and after Nm treatment. Current-voltage curves are present on the right side of each set of treatment. n=5 for each treatment.

($Bc_{(RPMI)}$) showed a significant (P<0.05) decrease in sodium currents (Control -49pA vs $Bc_{(NB)}$ -21pA) (Figure 4C).

Effects of Listeria monocytogenes peptide on the voltage- sensitive Na+ and K+ Currents

Next, the evaluation of electrophysiological parameters of voltage- sensitive I_A and I_{dr} channels later to the exposure with $Lm_{(NB)}$ and $Lm_{(RPMI)}$ are represented in Figure 5. It is noteworthy to mention herein that Lm was found to be releasing the most potent peptides that significantly alter the normal homeostasis of aforementioned potassium and sodium channels. Compared with control currents, profound depression (P<0.05) was found in I_{dr} recorded after 10 min exposure of both $Lm_{(NB)}$ (Control 248.4 ± 15.4 vs $Lm_{(NB)}$ 104 ± 0.5pA) and $Lm_{(RPMI)}$ peptides (Control 169.26 ± 84.61 vs $Lm_{(RPMI)}$ 65.78 ± 12pA) (Figure 5A and 5B). Similar results were observed after 5min treatment of $Lm_{(RPMI)}$ peptides while $Lm_{(NB)}$ did not show any effect after 5 min. In addition to this, no inhibition of sodium currents was observed when treated with the $Lm_{(RPMI)}$ peptides.

Figure 6A, 6B, 6C and 6D defines the significant dependency in rate of conductance and voltage relationship obtained in response to Ct, Nm, Bc and Lm peptides which describes the effect on I_{dr} initiated at the range of -40 to -50mv.

DISCUSSION

Recent efforts to observe the interaction between pathogens peptides and neuron have discovered several atypical and direct contact points e.g. gated channels, Toll like receptors (TLRs) and downstream signalling pathway, Nitric Oxide (NO) receptor, neurotransmitters, behavioural changes in animal models and morphological changes in neurons, microglia and lymphocytes. The peptides of neuropathogenic bacteria used in present experimental study mimic the role of live bacteria, therefore to determine the influence of these peptides on the functionality of cells of central nervous system (CNS) and underlying electrophysiology of hippocampal neuronal cells in disease progression was elucidated by whole cell patch clamp technique.

It is significant to note that peptides from all four neuropathogenic bacteria showed profound negative effect on the outward conduction of K+ ion through tetraethylammonium (TEA), sensitive voltage gated potassium channels (I_{kv}) (Figure 2, 3, 4 and 5). Though, peptides of Lm reflected a drastic decline in efflux of K+ ions. Conversely, Na+ channels remain unaffected therefore Na+ influx remained continued to normal values as that of control (Figure 5A, 5B and 5C), except that of Bc peptide (Figure 4C). It is interesting to note that Bc peptides in fact enhanced

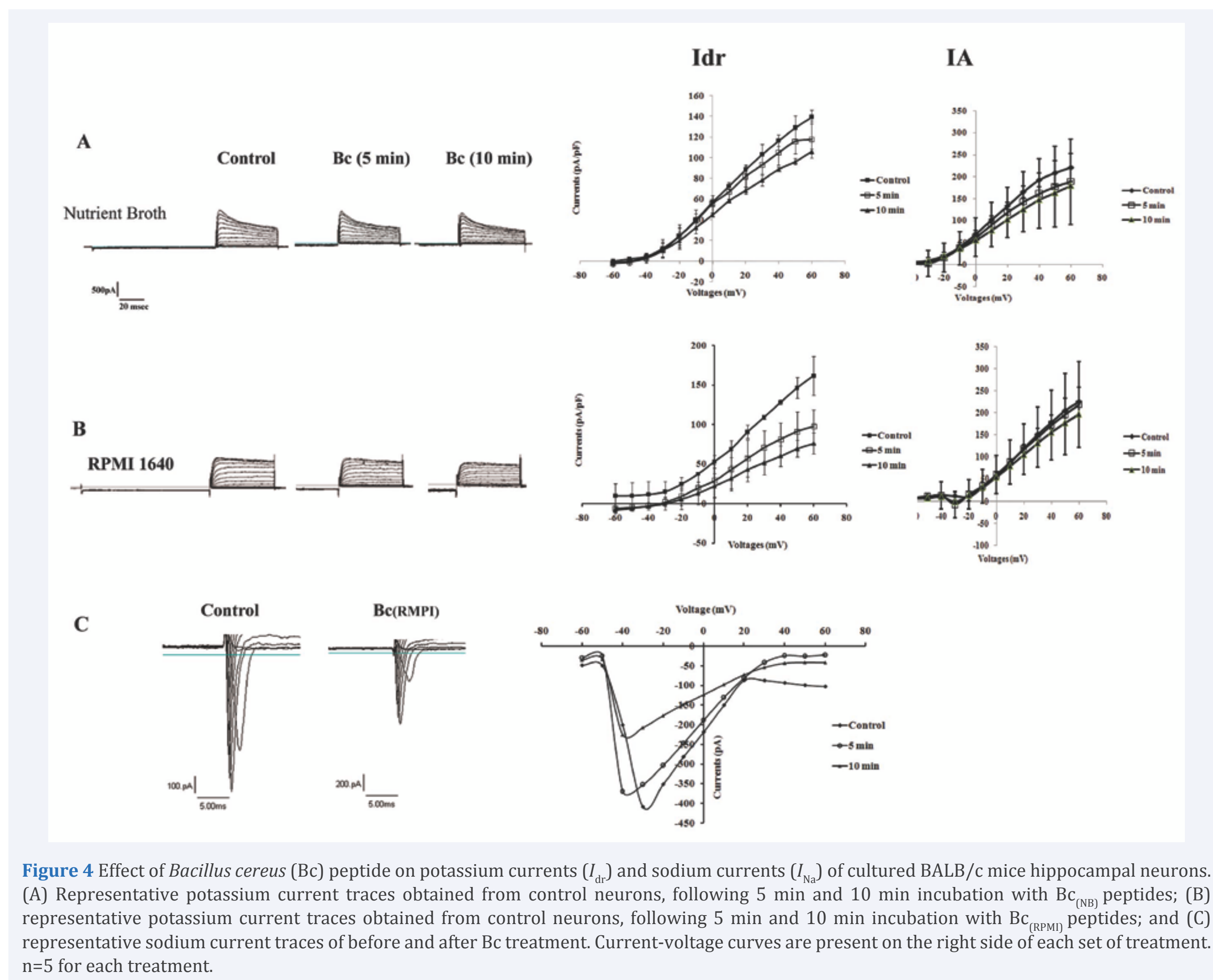

Figure 4 Effect of *Bacillus cereus* (Bc) peptide on potassium currents (I_{dr}) and sodium currents (I_{Na}) of cultured BALB/c mice hippocampal neurons. (A) Representative potassium current traces obtained from control neurons, following 5 min and 10 min incubation with $Bc_{(NB)}$ peptides; (B) representative potassium current traces obtained from control neurons, following 5 min and 10 min incubation with $Bc_{(RPMI)}$ peptides; and (C) representative sodium current traces of before and after Bc treatment. Current-voltage curves are present on the right side of each set of treatment. n=5 for each treatment.

the influx of Na+ ions by activating sodium (I_{Na}) ion channels. This influx of Na+ ions is voltage dependent. These results of the present study can be further explained by the fact that the altered functionality of Na+ and K+ ion channels are attributed to sustained depolarized state. Consequently excitatory post synaptic potential of the membrane, K+ ion efflux inhibition in fact inhibits the repolarization of the neuron. Therefore, an inhibition of K+ efflux may not be universally beneficial for cell survival.

The rapid opening of I_{Na} and massive influx of Na+ ion in neuron explain depolarization of neuronal cell membrane, causing the rapid upstroke of the action potential (Figure 6B). The closure of I_{Na} and the transient opening of I_{kv} mark the peak of action potential. Deviations from usual electrophysiological steps of action potential as indicated by the recording of impaired outward conductance of K+ ions implicates disastrous physiological conditions for the neurons. Figure 6B explicitly indicated the variation in time to peak of action potential. As Ct and Nm are more or less reflected same time whereas Bc peptides indicated extended duration for peak or depolarization due to prolong activation of Na channels. However Lm implicated short spam of depolarization followed by the impaired K+ conductance.

It is worth to mention herein as documented by a number of authors [19-22], that I_{kv} activation play an important role in signalling pathways leading to proliferation, differentiation and apoptosis. The other facet of study performed in our lab has proved the activation of TLR2 and TLR4 associated downstream signalling cascade led to proinflammatory cytokine production in glial and peripheral blood mononuclear cells (PMBCs) [Unpublished]. In addition to enhanced production of dopamine subsequent to the behavioural changes in the brain of animal model in response to intraperitoneal (i.p.) injection of these peptides [23], is attributed to I_{kv} dysfunction. This notion supports the known fact that I_{kv} have to be the key regulator of synaptic transmission.

Interestingly, the neuronal cell survival in present study was limited to 10 minutes. This can be further explained by the fact that most of the neuronal cells sustained 10 min of electrophysiological recordings. This finding of the present study significantly indicated the cell death. Alteration in the function of I_{kv} and I_{Na} leads to remarkable perturbation in membrane potential and ionic concentration across the membrane which may lead to activate I_{Na} dependent apoptosis led to NO production

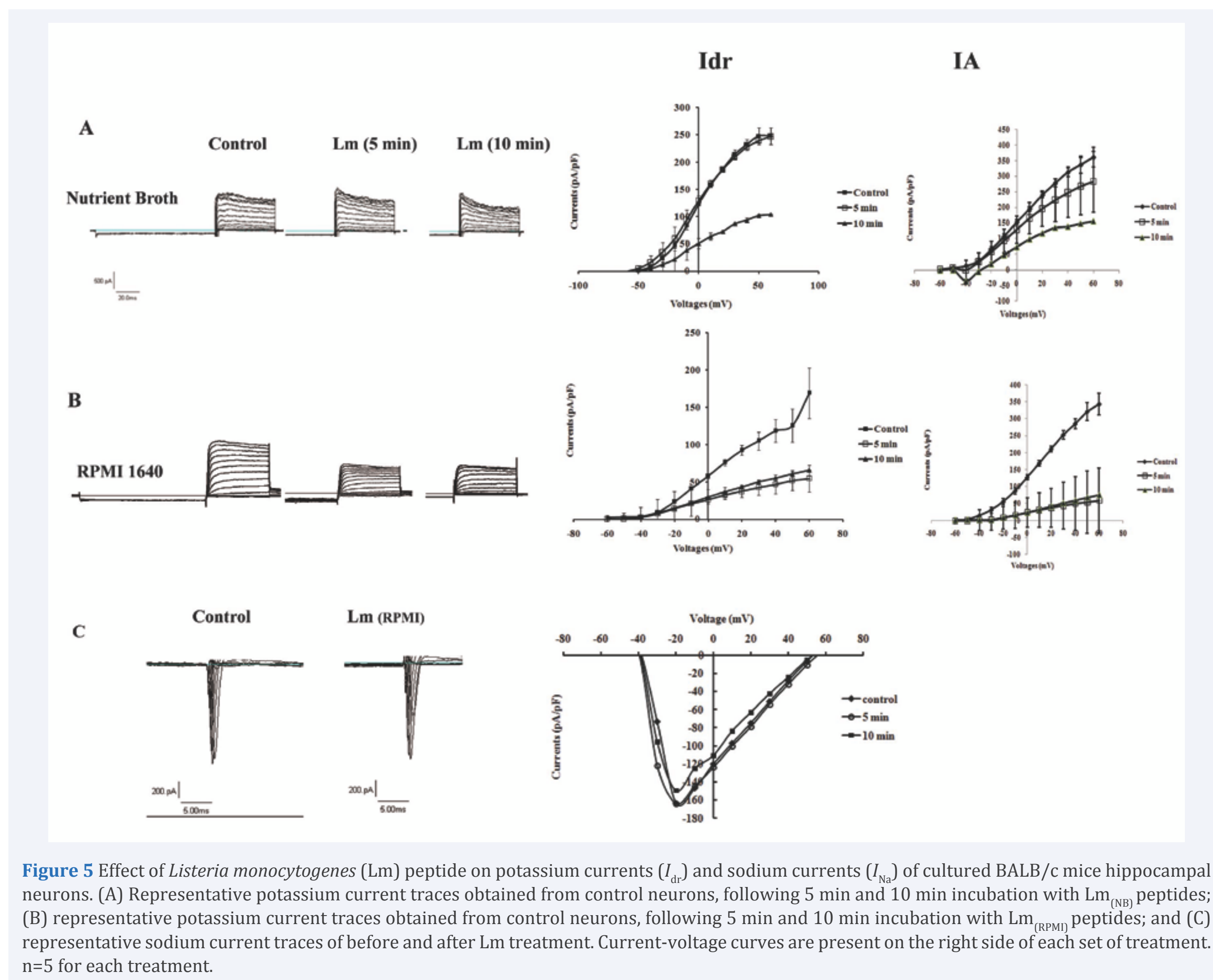

Figure 5 Effect of *Listeria monocytogenes* (Lm) peptide on potassium currents (I_{dr}) and sodium currents (I_{Na}) of cultured BALB/c mice hippocampal neurons. (A) Representative potassium current traces obtained from control neurons, following 5 min and 10 min incubation with $Lm_{(NB)}$ peptides; (B) representative potassium current traces obtained from control neurons, following 5 min and 10 min incubation with $Lm_{(RPMI)}$ peptides; and (C) representative sodium current traces of before and after Lm treatment. Current-voltage curves are present on the right side of each set of treatment. n=5 for each treatment.

[Unpublished]. As many studies [18-21], are consistent with this fact that a Na+ influx was shown to largely mediate NO induced cell death. Since hippocampal neurons are associated with the memory of human host [23-25], therefore it can be speculated that peptide mediated lysis of these neurons may affect the memory of the host thus, implicate post disease trauma.

Although, a number of short peptides isolated from different pathogens [25], snake venom [26], and Conus princeps venom [27], have been reported as a potent blockers of sodium and potassium channels. However, the present study has first time focused on neuropathogen's associated psychotrophic peptides that serve as an insult to CNS of the host by modulating its electrophysiology and downstream cascade of events in disease aggravation. Figure 6A has reflected a unique feature of these peptides of neuropathogens. Implication of peptides on the variation in capacitance of the membrane of the neuron is an outstanding feature actually provides the insight into the means of mechanism of membrane potential generation. Electrochemical gradient is a well-known phenomenon for the potential difference. Our findings (Figure 6A), have provided an evidence of novel mean of generation of potential gradient across the membrane through membrane capacitance. It is well documented fact that the capacitance of a membrane is invariant value but the resistance and voltage are highly variable factors.

The biophysical properties of the lipid bilayer were studied by many workers [28,29]. In accordance to their findings lipid bilayer hydrocarbons acyl chains are flexible, and are capable of to stretch, squash and/or tilt to match the hydrophobicity of the integral protein hydrophobic span. The law of physics i.e. capacitance is directly proportional to the surface area and inversely proportional to thickness between the plates of a capacitor. Taken these findings together, we propose membrane potential thematic is analogues to the functioning of the semiconductors as acyl chain domain act as depletion layer.

Patch clamp recordings of the present study of the capacitance has explicitly indicated the modality of neurotropic peptides intervention in the capacitance of the membrane (Figure 6A). Interestingly peptide of Ct seems to enhance the capacitance whereas the other 3 bacterial peptides i.e. Bc, Lm and Nm seems to reduce the capacitance. Among this group Nm drastically reduce the capacitance of the hippocampal neuronal membrane. The underlying phenomenon can be explained by either one of

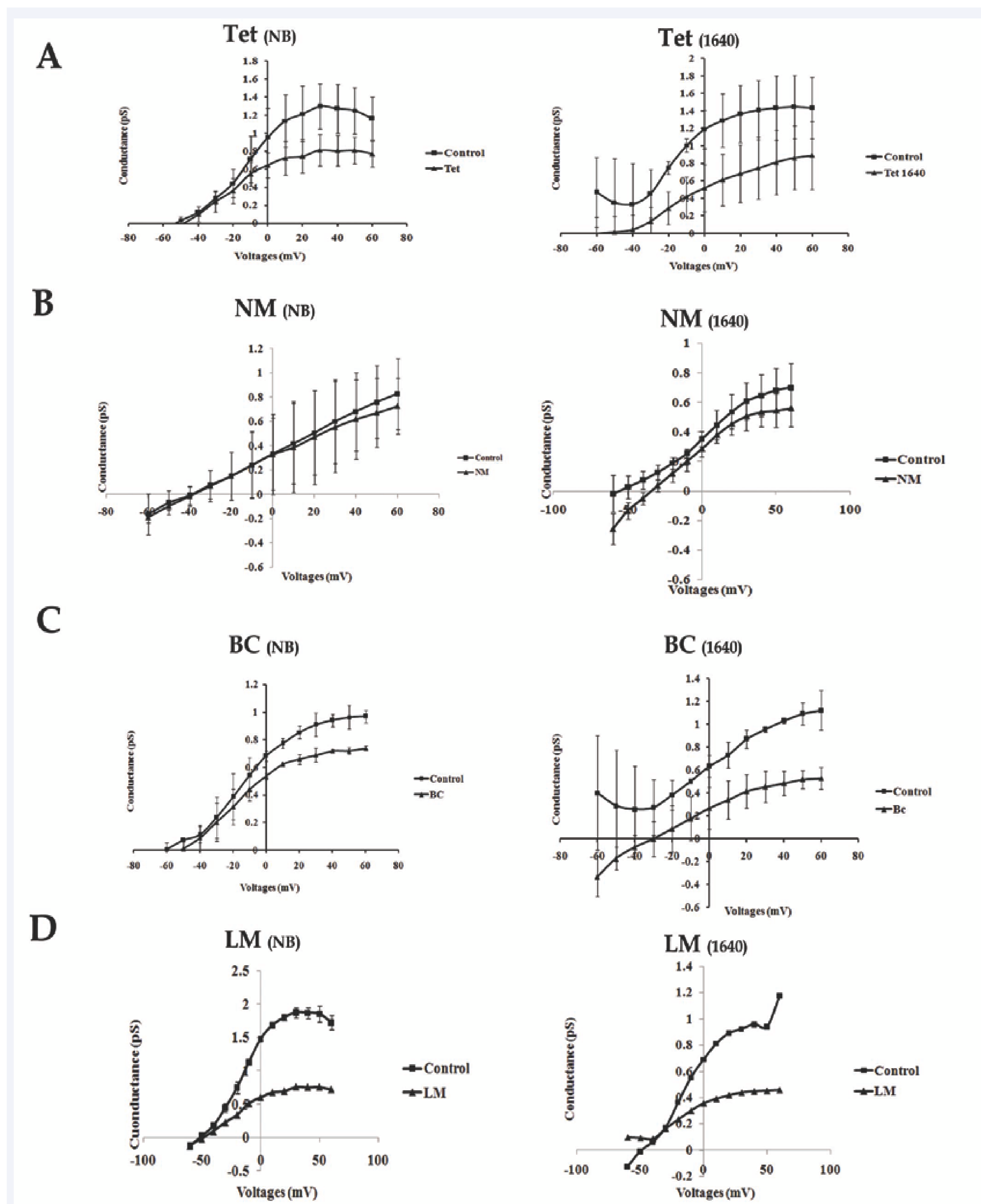

Figure 6 Rate of conductance and voltage relationship obtained in response to neuropathogenic bacterial peptides. (A) Representative relationship obtained in response to *Clostridium tetani* (Ct) peptides; (B) Representative relationship obtained in response to *Neisseria meningitides* (Nm) peptides; (C) Representative relationship obtained in response to *Bacillus cereus* (Bc) peptides; and (D) Representative relationship obtained in response to *Listeria monocytogenes* (Lm) peptides.

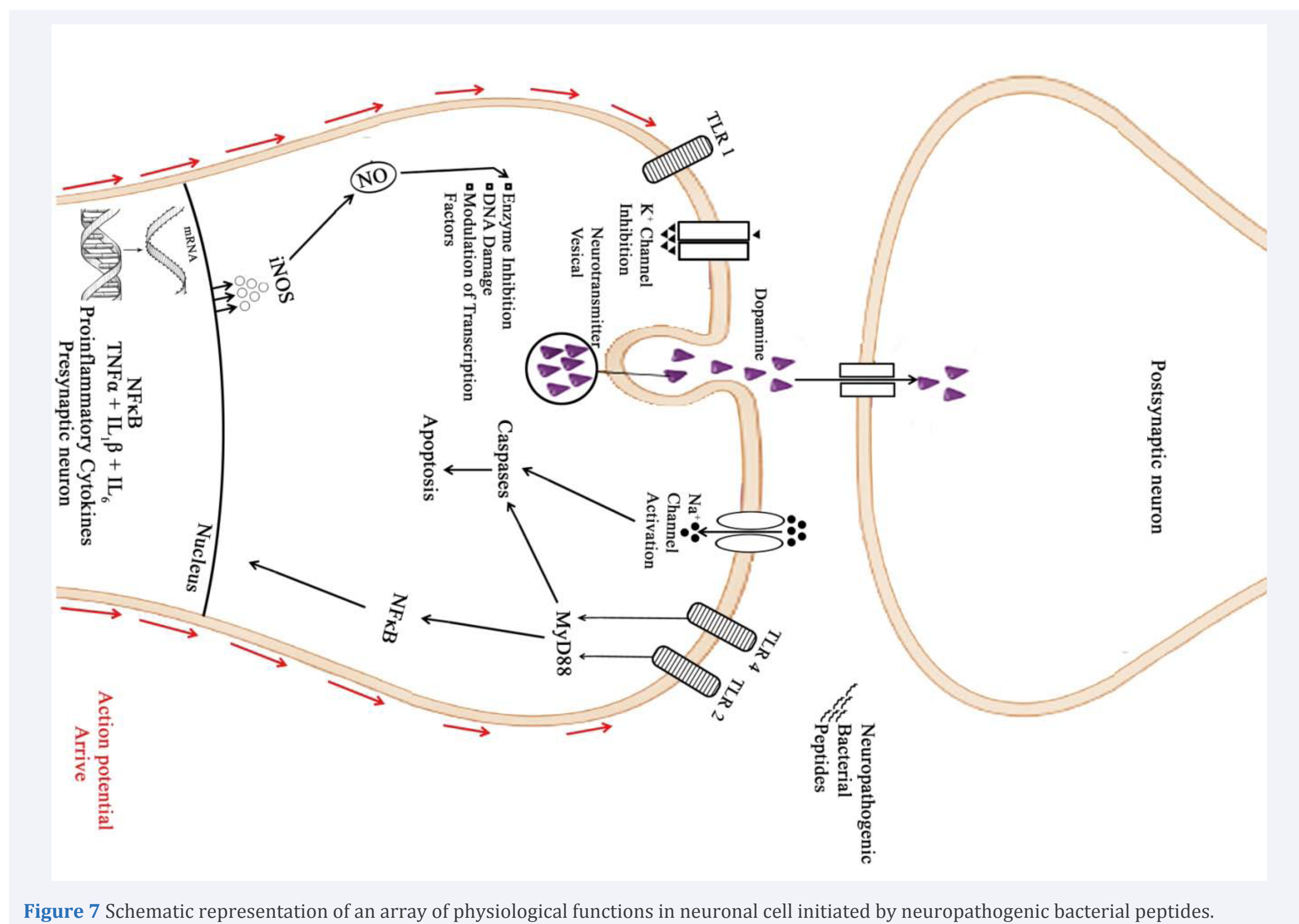

Figure 7 Schematic representation of an array of physiological functions in neuronal cell initiated by neuropathogenic bacterial peptides.

the following hypothetical modality:

a) Peptide interaction with the gate of the channel may influence on the hydrophobicity of the integral span of the channel. or,

b) Peptide may interact with the lipid bilayer and adopt a surface localization.

Both of these interactions may cause the thickness variation of the membrane by disordering of their acyl chains, consequently the capacitance of the membrane may also fluctuate accordingly. In addition to this, fluctuations of capacitance may be associated with variation in the surface area of the membrane. The flip flop movement of phospholipid molecule and/ or addition of cholesterol into bilayer may be attributed to the change in surface area. It is interesting to note that the thickness of lamellar domain and lipid raft are not similar hence these domains would have different capacitance. Finally, the capacitance and voltage of the membrane inversely fluctuate.

CONCLUSION

Present study concludes that neuropathogenic bacterial extracellular peptides implicate their influence on neuronal membrane potential by novel modality and illicit the cascade of responses i.e. synaptic, peripheral and neuro-immune. However, modification of the functional properties of two of the major membrane voltage gated K^+ and Na^+ conductances expressed by hippocampal neurons evidenced that these peptides play a major role in enhancing the severity of CNS infections of bacterial aetiology (Figure 7). Thus, it provides a new venue for the further research, to understand the pathogenesis and post infection deleterious effects of the components of pathogenic bacteria.

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: NJ. Collection of the samples: AT. Experimental work: AT. Analysis of the data & Conclusion: AT, NJ. Writing of the manuscript: AT. All authors read and approved the final manuscript.

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