Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 94, No. 4, August 1985, pp. 407–411.

Electrical stimulation—Effects on the protein in the ventral nerve cord of cockroach, *Periplaneta americana*

S L MAHESWARI, K BALASUNDARAM and V R SELVARAJAN
Division of Neurobiology, Department of Zoology, University of Madras, Madras 600 005, India

MS received 15 June 1984; revised 8 September 1984

Abstract. Changes in protein concentration due to electrical stimulation have been investigated at different time intervals in cockroach ventral nerve cord. Significant increase in total protein concentration was observed at 15 min interval of stimulation. Increase in protein concentration was also observed in ventral nerve cords incubated with exogenous glucose. Microdisc polyacrylamide gel electrophoresis at 15 min interval has revealed an increase in low molecular weight protein fractions in nerve cords from stimulated group. The data clearly depict the changes in protein due to electrical stimulation stress.

Keywords. Cockroach; electrical stimulation; ventral nerve cord; protein; microdisc polyacrylamide gel electrophoresis.

1. Introduction

Studies on the correlation of the functional activity of the brain with metabolic parameters due to chemical, electrical and light stimuli are available on vertebrates (Chitre and Talwar 1963; Chitre *et al* 1964; Jones 1972; Jones and MacIlwain 1971; Kol's *et al* 1974; Luxuro 1960; Selvanayagam and Habibulla 1979; Talwar *et al* 1961, 1966). Review of literature shows absence of knowledge on the effects of electrical stimulation on the metabolic parameters of nervous system (NS) of invertebrates. Hence the present study was undertaken to gain information of electrical stimulation on the protein concentration and pattern in an invertebrate central nervous system.

2. Material and methods

In order to eliminate the effect of sex on protein concentration, only male animals were chosen for study. Male cockroaches, *Periplaneta americana* were taken in two groups. One served as control and the other as the experimental group. From each group, atleast ten animals were used for removal of ventral nerve cord. The dissection was carried out in insect ringer solution (Orchard and Finlayson 1977). One set of the nerve cords from the control group was incubated in ringer with glucose and another set in ringer free of glucose for 15 min (Edstrom and Mattison 1972). The nerve cords from the experimental group were incubated in two sets similar to control before stimulation. After the incubation period, all the sets of ventral nerve cords were hooked separately to a pair of silver-silver chloride electrodes, supported by a wax bath in a petridish. The preparation was prevented from drying by periodically adding insect ringer. The nerve cords of the experimental sets alone were given a repetitive stimuli of

0·1 m sec duration with a current strength of 3 V at a frequency of 1/sec using an electronic stimulator (Palmer, England Electronic square-wave stimulator) for 5, 10, 15, 30 and 60 min.

2.1 *Total protein estimation*

Both control and stimulated nerve cords were removed, washed with insect ringer and weighed separately after carefully blotting out the moisture. They were homogenised separately in prechilled glass-glass homogeniser with 80% ethanol and centrifuged at 3500 rpm for 5 min. The residue was dissolved in 1N NaOH solution and the proteins were estimated following the method of Lowry *et al* (1951).

The results were tested for significance using student's *t* test.

2.2 *Microdisc electrophoresis*

Protein pattern was analysed by homogenising the ventral nerve cord from both the sets of control and experimental groups in 0·01 M Tris-HCl buffer (pH 7·4) and subjecting the supernatent to microdisc polyacrylamide gel electrophoresis (Ganesan *et al* 1979). The gels were stained in 0·25% coomassie brilliant blue, destained and stored in 7% acetic acid (Smith 1968). Gels were photographed over an x-ray viewer as suggested by Oliver and Chalkley (1971) and scanned using Shimadzu-Dual length TLC scanner CS-910 with C-RIA chromotrophe recorder at 640 and 430 nm.

3. Results

3.1 *Total proteins*

The electrical stimulation of the cockroach ventral nerve cord (incubated in exogenous glucose) at 15 min interval has revealed highly significant ($p < 0{\cdot}001$) increase in total protein concentration (40·81 ± 5·56 mg/g). Though the protein concentration increases from 5 min up to 15 min, a decrease is observed after that period (table 1).

Table 1. Effect of electrical stimulation on the total protein concentration (mg/g) from the cockroach ventral nerve cord with exogenous glucose.

Duration of stimulation	Total protein		Absolute difference	*P* value
	Control	Experimental		
5	24·30 ± 2·14	29·54 ± 2·54	+ 5·24	< 0·005
10	25·91 ± 3·26	35·86 ± 1·89	+ 9·95	< 0·005
15	26·02 ± 4·21	40·81 ± 5·56	+ 14·79	< 0·001
30	24·90 ± 5·04	20·79 ± 4·15	− 4·11	NS
60	30·00 ± 6·01	19·10 ± 5·76	− 10·90	< 0·010

Mean ± S.D. of 6 observations; +: indicates increase; −: indicates decrease; NS: not significant.

Table 2. Effect of electrical stimulation on the total protein concentration (mg/g) from the cockroach ventral nerve cord without exogenous glucose.

Duration of stimulation	Total protein		Absolute difference	*P* value
	Control	Experimental		
5	21·05 ± 1·85	20·11 ± 1·02	− 0·94	NS
10	22·23 ± 2·12	20·23 ± 2·03	− 2·00	NS
15	30·72 ± 2·92	21·98 ± 1·65	− 8·74	< 0·001
30	20·56 ± 1·99	9·89 ± 0·10	−10·67	< 0·001
60	21·81 ± 2·942	7·12 ± 0·59	−14·69	< 0·001

Mean ± S.D. of 6 observations; −: indicates decrease; NS: not significant.

In nerve cords incubated free of exogenous glucose, stimulation showed a decreasing trend in the total protein concentration significantly ($p < 0·001$) (table 2). The decrease was consistently found at all intervals of stimulation.

3.2 *Protein pattern*

Densitometric scanning of the gels from unstimulated and stimulated ventral nerve cords are shown in figure 1. The number of fractions resolved from the unstimulated control group was eleven and from the stimulated group was nineteen. The control group thus depicted only six high molecular weight protein fractions. But the experimental group showed an increase by two fractions over the control group. Similarly the low molecular weight protein fractions were only five in control groups but the experimental group revealed an increase by six fractions. Thus the rise in total protein concentration in the stimulated nerve cord may be due to the increase in both the low and high molecular weight protein fractions.

4. Discussion

Electrophoretic and immunological studies have shown that brain contains several proteins which presumably involved in specific neural functions (Bock 1978). Hence, factors regulating protein synthesis are known to play an important role in the functioning of the NS. The changes observed in the total protein concentration with and without exogenous glucose in the present study suggests that glucose exhibits considerable role as an energy source to meet the altered condition. Selvanayagam and Habibulla (1979) report glucose as an energy source in the absence of adequate energy supply in frog sciatic nerve.

The significant increase in total protein concentration in electrically stimulated nerves with glucose suggests the increased rate of protein biosynthesis. The observed increase in total protein concentration up to 15 min is in accordance with the findings of Jones (1972) who has stated that the increase could be due to the electrical activity causing localized increases in concentration of leucine or neutral amino acids which are known to enhance the incorporation of amino acids into proteins. The decrease

observed after 15 min may be due to the induced hyperactivity for longer duration resulting in protein catabolism leading to fatigue and exhaustion as suggested by Selvanayagam and Habibulla (1979).

The polyacrylamide gel electrophoresis depicts a greater increase in the total number of low molecular weight protein fractions. Such a change suggests the dissociation of complex proteins into simple low molecular weight proteins to meet the stimulation stress. Luxuro (1960) has put forth the view that a small fraction of nerve proteins may split probably to the level of amino acids during the nerve activity. Maheswari (1983) has also reported similar observation of dissociation of proteins in the nervous system of *Scylla serrata* due to pesticide stress. The present investigation has clearly shown alteration in proteins and is probably a geared mechanism to functional demand. However, further investigation on protein change to stress condition is needed.

Acknowledgement

The authors thank Prof. Dr K Ramalingam for encouragement. Valuable assistance was provided by Prof. Dr P Govindarajulu in densitometric scanning of gels. SLM acknowledges the financial support of CSIR, New Delhi.

References

Bock E 1978 Nervous system specific proteins; *J. Neurochem.* **30** 7–14

Chitre V S and Talwar G P 1963 Correlation of electrical activity of brain with metabolic parameters. Part III. Pentose nucleic acid content of isolated cerebral cortex during various phases of electrical activity following topical application of metrazol; *Indian J. med. Res.* **51** 80–91

Chitre V S, Chopra S P and Talwar G P 1964 Changes in the ribonucleic acid content of the brain during experimentally induced convulsions; *J. Neurochem.* **11** 439–448

Edstrom A and Mattisson H 1972 Fast axonal transport *in vitro* in the sciatic system of the frog; *J. Neurochem.* **19** 205–221

Ganesan A, Murthy V A and Thiruneelakantan K 1979 Electrophoresis on polyacrylamide micrograd ient gels in *Proc. Natl Symp. on food proteins.* Protein research unit Loyola College Madras 58–62 p.

Jones D A 1972 The relationship between amino acid incorporation into protein in isolated neocortex slices and the tissue content of free amino acid; *J. Neurochem.* **19** 779—790

Jones D A and McIlwain H 1971 Amino acid distribution and incorporation into proteins in isolated electrically stimulated cerebral tissues; *J. Neurochem.* **18** 41–58

Kol's O R, Fedorov G E, Maksimov G V and Burlakova E V 1974 Change of ATP ase activity of the crab nerve in relation to the stimulation regime; *Dokl. Akad. Nauk SSSR Ser. Biol.* **216** 943–945

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

Luxuro M 1960 Incorporation of amino acids labelled with carbon-14 in nerve proteins during activity and recovery; *Nature (London)* **188** 1119–1120

Maheswari S L 1983 *Neurological manifestations of phosalone, an organophosphate pesticide in the edible crab Scylla serrata (Forskal) (Crustacea: Decapoda)* Ph.D. Thesis University of Madras, India

Oliver D and Chalkley R 1971 An improved photographic system for polyacrylamide gels; *Anal. Biochem.* **44** 540–542

Orchard I and Finlayson L H 1977 Electrical properties of identified neurosecretory cells in the stick insect; *Comp. Biochem. Physiol.* **A58** 87–91

Selvanayagam P F L and Habibulla H 1979 Electrical stimulation effects on the levels of free amino acids and proteins in peripheral nervous system of frog; *Indian J. Exp. Biol.* **17** 300–302

Smith I 1968 *Chromatographic and electrophoretic techniques* (London: William Heineman Medical Book Ltd) Vol II pp. 365–418.

Talwar G P, Sadasivudu B and Chitre V S 1961 Changes in the pentose-nucleic acid content of subcellular fraction of the brain of the rat during 'Metrazol' convulsions; *Nature (London)* **191** 1007–1008

Talwar G P, Chopra S P, Goel B K and Monte B D 1966 Correlation of the functional activity of the brain with metabolic parameters-III. Protein metabolism of the occipital cortex in relation to light stimulus; *J. Neurochem.* **13** 109–116