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THE AUSTRALIAN NATIONAL UNIVERSITY
JOHN CURTIN SCHOOL OF MEDICAL RESEARCH
DEPARTMENT OF PHYSIOLOGY

55/1964

ANNUAL REPORT - 1963

Staff

Professor:
J.C. Eccles, Kt., M.B., B.S., M.A., D.Phil., Hon.Sc.D., F.R.A.C.P., F.R.S.N.Z., F.A.A., F.R.S.

Professorial Fellow:
W.V. Macfarlane, M.A., M.D.

Professorial Fellow in Neuropharmacology:
D.R. Curtis, M.B., B.S., Ph.D.

Electronics Engineer:
J.S. Coombs, M.Sc.

Fellows:
J.C. Watkins, M.Sc., Ph.D.; Rosamond M. Eccles, M.Sc., Ph.D.; J.I. Hubbard, B.med.Sci., M.A., B.M., B.Ch., Ph.D.

Senior Research Fellow:
I.R. McDonald, M.D., B.S. (to May, 1963)

Research Fellows:
R.I. Close (M.Sc., Ph.D.; Y. Løyning, Cand. Med.; K. Sasaki, M.D., Ph.D. (from October)

Research Fellow (Wool Trust Fund)
J. Sabine, B.Ag.Sc., M.Sc., Ph.D. (from February)

Rockefeller Fellows:
P. Andersen, M.D. (to November); T. Yokota, M.D., Ph.D.

Wellcome Fellow:
T.A. Sears, B.Sc. (to June)

Visiting Fellow:
Wilson, V.W., Ph.D. (June to September)

Stipendiaat of Netherlands Organization
for the Advancement of Pure Research (Z.W.O.):
P. Voorhoeve, M.D.

Swedish Medical Research Council Grant and International Federation of University Women:
B. Holmqvist, M.D. (to December)

Research Assistants:
K.S. Cheah, B.Sc.; Nancy E. Stone, M.Sc., Ph.D.; Mabelita M. Campbell, B.A. (to June).

Head Technician:
L.M. Davies, Dip. Mech. & Elec. Eng., A.M.I.I.T.A., M.Q.E.I.

STUDENTS AND TEACHING ACTIVITIES

Seven students are proceeding to the degree of Doctor of Philosophy: Dr. T. Oshima (commenced 17th February, 1961); Mr. R.W. Ryall (commenced 20th October, 1961); Dr. J.M. Crawford (commenced 4th February, 1963); Dr. P.W. Gage (commenced 25th February, 1963); Dr. M. Devanandan (commenced 5th May, 1963); Miss C. Walmsley (commenced 19th July, 1963); Dr. R. Llinas (commenced 7th October, 1963).

The following were awarded the degree of Doctor of Philosophy for theses with the titles indicated:

Dr. Wm.D. Willis: 'Presynaptic inhibition of muscle afferent pathways'.

Dr. R.F. Schmidt: 'The central pathways and the mechanisms of presynaptic inhibition'.

The following students successfully completed the requirements for the degree of Doctor of Philosophy:

Miss R. Kinne: 'Water and electrolyte balance'.

Mr. T.A. Sears: 'Investigations on respiratory motoneurones'.

In March Miss B. Howard submitted a thesis entitled "Environment and Body Composition in the Rat" for the Degree of Doctor of Philosophy.

Throughout the year there have been departmental seminars, usually every week. Most of the Department attended meetings of the Australian Physiological Society in Canberra in February and presented 11 papers and at the University of New South Wales in August and presented 7 papers.

RESEARCH PROGRAMME

Much of the work reported for 1962 was completed and written up in 1963; some appears in the appended list of published papers, the remainder being in press. Brief outlines of current research are given below.

The hippocampus:

The previous report gave a preliminary account of investigations on the inhibitory potentials of hippocampal pyramidal cells. In 1963 this work was completed and sent in for publication. The suggestion that the basket cells were the inhibitory cells responsible for inhibitory action on the pyramidal cells was substantiated by a systematic examination of interneurones that were activated by the various inputs that produced the inhibitory potentials. (Andersen, Eccles, Løyning). A closely related investigation likewise indicated that another group of basket cells inhibited the granule cells of the dentate fascia. There was also a systematic study of the excitatory synaptic action both on the granule cells and on the pyramidal cells of the hippocampus, and of the generation of impulse discharges by this excitatory action. The main route of excitation of CA_1 hippocampal pyramidal cells by stimulation of the entorhinal cortex was found to be trisynaptic via the dentate granule cells. (Andersen, Holmqvist and Voorhoeve).

The cerebellum:

Since the large Purkinje cells of the cerebellum resemble the hippocampal pyramidal cells in having on the cell bodies a dense concentration of synaptic endings from a special type of nerve cell (also designated basket cells), it seemed likely that these too were inhibitory cells. Systematic field potential studies by extracellular microelectrodes and also intracellular recording have corroborated this postulate. The basket cells have been located and their responses have been shown to be appropriate for the nerve cells inhibiting the Purkinje cells. The electrical responses of the Purkinje cells have also been studied (Andersen, Eccles and Voorhoeve).

The thalamus:

The 1962 report gives the main outline of this work. In 1963 it was completed by an attempt at identification of the different types of neurones that participated in the presynaptic and postsynaptic inhibitory actions on the thalamic relay cells (Andersen, Eccles, Sears).

The cuneate nucleus:

Just as with the thalamus the postulate of interneurones specifically concerned in presynaptic and postsynaptic inhibitory action has been experimentally tested and corroborated. (Andersen, Eccles, Schmidt and Yokota).

The spinal cord:

Investigations have been made on presynaptic inhibitory effects from contrlateral afferents. It was found that contralateral cutaneous and muscle afferents (Group Ib, II and III)

caused presynaptic inhibition of cutaneous pathways (R.M. Eccles, Holmqvist and Voorhoeve). However, effects from contralateral muscle afferents to muscle afferent pathways were observed only with high threshold muscles (Group II and III) onto high threshold muscles (Group II and III) (Devanandan, Holmqvist and Yokota). A preliminary study has been completed on the effect of stretch of a flexor muscle on the simplest reflex pathways in the spinal cord. The presynaptic inhibitory effects evoked are not unlike those produced by stimulation of the nerve to that particular muscle (Devanandan, R.M. Eccles and Yokota). Repetitive activation of afferent pathways in kittens has been investigated in order to compare the behaviour of kitten motoneurones with those of the adult animal under similar conditions of activity (Devanandan, R.M. Eccles and Willis).

Anaesthetics on motoneurones:

The investigations on the site of action of barbiturates on the monosynaptic spinal reflex pathway in cats have been continued. Intracellular recording from motoneurones revealed that the short-acting barbiturate, thiamylal sodium, decreased the monosynaptic excitatory postsynaptic potential, while the resting potential, spike potential, firing threshold, electrical properties and accommodation of the motoneurones were unaltered during the thiamylal anaesthesia. Extracellular recording from the afferent nerve terminals and testing of their

excitability showed a reduced spike amplitude in the terminals with unaltered excitability during thiamylal anaesthesia. It is postulated that barbiturates act mainly on the spike generating mechanisms in the afferent nerve terminals, resulting in less transmitter release and in reduced synaptic potential (Løyning, Oshima and Yokota).

Hypoxia on motoneurones:

The cats were ventilated with 5% O_2 in N_2 and the tissue oxygen tension was recorded in the spinal cord. With hypoxia there was a gradual and slight depolarization, a slight slowing of the rising and falling phases of the antidromic spike, but no change in its amplitude, and a reduction in the after-hyperpolarization following the spike. These results are to be expected when the metabolically driven sodium/potassium pump is slowed down. There was also an increase in the monosynaptic excitatory post-synaptic potential, which may be due to an increased liberation of transmitter substance. The effects of hypoxia on polysynaptic potentials and reflexes are also being investigated. (R.M. Eccles, Løyning and Oshima).

Neuropharmacology:

It is becoming increasingly clear that acetylcholine is an important central transmitter and research has continued into the sensitivity of neurones to cholinomimetics, the nature of acetylcholine receptors and the distribution of cholinomimetic

and other pharmacologically active substances in brain.

A study of the pharmacology of thalamic and lateral geniculate neurones was completed (Andersen, Curtis) and experiments are continuing in order to identify the cholinergic pathways which terminate in these nuclei (Crawford, Curtis). The excitation of cortical Betz cells by acetylcholine has been confirmed and pharmacological studies upon these and other neurones of the cerebral cortex are in progress (Crawford & Curtis). Purkinje cells of the cerebellar cortex are also excited by acetylcholine (Crawford, Curtis, Voorhoeve & Wilson) and the receptors appear to differ from those of Betz and thalamic neurones. Investigations have also been carried out on the chemical sensitivity of neurones in the caudate nucleus and hippocampus. Further studies have been made on spinal Renshaw cells (Curtis & Ryall) and an attempt made to fit all of these various acetylcholine receptors into the classical nicotinic and muscarinic types.

The amino acid sensitivity of cortical neurones has been determined (Crawford, Curtis) and it is now clear that there are no important differences between the sensitivities of neurones throughout the mammalian central nervous system to these substances. Some of the very active depressant and excitant amino acids synthesised in the Department (Watkins) have been shown to have powerful central effects when injected into

the cerebral ventricles of mice (Crawford). The excitation of individual neurones by amino acids such as L-glutamic and DL-homocysteic acid has proved of considerable assistance in studying the pharmacology of the inhibition of Betz and Purkinje cells. It has been shown that both the 'recurrent' and 'local' inhibition of Betz cells and the inhibition of Purkinje cells elicited by local cortical stimulation are resistant to doses of strychnine which are sufficient to suppress postsynaptic inhibition in the spinal cord (Crawford, Curtis, Voorhoeve & Wilson). The large and prolonged postsynaptic inhibitory potentials in the thalamus, the hippocampal pyramids and the Purkinje cells of the cerebellum have also been shown to be strychnine resistant (Andersen, Eccles Løyning and Voorhoeve).

Using a combination of high voltage electrophoresis and paper chromatography, the cholinomimetic substance extracted from the nerve terminal fraction of brain has been identified as acetylcholine (Ryall, Stone, Watkins). Furthermore, the micro-electrophoretic technique of ejecting compounds near individual neurones has been refined to enable the use of extremely small quantities of material and the action of acetylcholine extracted from mammalian brain upon Renshaw cells has proved to be identical with that of authentic acetylcholine (Curtis, Ryall). In addition to acetylcholine other pharmacologically active substances have been extracted from brain and a survey of the central actions of these fractions is in progress.

Neuromuscular transmission:

The properties of motor nerve terminals were again the object of study during the year. Projects included the use of metabolic inhibitors to demonstrate the energy requiring systems involved in the synthesis and release of transmitter substances (Hubbard, Gage); the effects of a reduced oxygen tension (Hubbard, Løyning), and the effects of cholinergic drugs (Hubbard, Yokota).

Muscle physiology:

An investigation has been made into the action of various factors which control or modify the force-velocity properties of different muscles. The finding that the duration of the active state is inversely proportional to the speed of shortening has revealed the functional interdependence of certain events in excitation-contraction coupling (Close).

Water and salt metabolism:

Studies in the physiological ecology of arid regions were continued with special reference to water and electrolytes.

In Kenya tritium turnover rates were determined on a number of types of sheep, cattle, goats and camels. In the Northern Frontier District, which is semi-desert, measurements were made of the relative water turnovers of indigenous animals. Cattle used two to three times more water than goats or camels. Since desert cattle turn over more water than camels the renal

functions and responses to water deprivation were compared at Alice Springs. Cattle lose water at twice the rate of loss by camels, and the plasma water loss in cattle is differentially four or more times greater than in camels.

The water and electrolyte metabolism in two primitive societies, the potato-eating Chimbu of New Guinea Highlands and nomadic Nadajara hunters of the Rawlinson Ranges of Western Australia have been explored. Both groups show functional adaptations to the environment.

The increase of potassium and water excretion produced by vasopressin was found in camels and cattle. Adult sheep infused with various types of vasopressin show an increase of potassium excretion. In the new-born lamb the normally occurring arginine vasopressin produces a large outflow of potassium and an increase of water excretion. Lysine vasopression and phenylalanine-lysine vasopression (which are not found in ruminants) produce little change in electrolyte excretion. Potassium excretion under the influence of arginine vasopressin appears to be a ruminant specialisation which is now known in cattle, sheep and camels and is present from the time of birth.

A true breeding strain of Merino sheep yielding more wool than unselected controls showed an increased water consumption when wool growth increased. Removal of the fleece in summer doubled the tritiated water turnover of sheep in the sun. (Macfarlane, Howard, Kinne, Sabine).

OTHER ACTIVITIES

Professor Sir John Eccles was awarded a Nobel Prize in Physiology in December, and the Cothenius Medal of the Deutsche Academie der Naturforscher Leopoldina in October. He was overseas on four occasions and attended nine symposia or international meetings, to which he contributed 9 papers, 8 of which are being published. He also lectured at Osaka, Kyoto, Tokyo, Pecs, Chicago, Baltimore, Goteborg, Heidelberg, New York, Los Angeles, Oxford and Stockholm. In Australia he was the Matthew Flinders Lecturer of the Australian Academy of Science and the Rennie Memorial Lecturer of the Royal Australasian College of Physicians. In July he was elected a Fellow of the Academia Nazionale dei Lincei.

Dr. J.C. Watkins was invited to present a paper at the Second International Pharmacological Conference held in Prague (August) and is spending a year's study leave at the A.R.C. Institute of Animal Physiology, Babraham.

Overseas visitors to the department were Professor R.W. Gerard of the University of California, Irvine, and Professor C.A. Keele from the Middlesex Hospital.

PUBLICATIONS

ANDERSEN, P., ECCLES, J.C., LØYNING, Y.

'Recurrent inhibition in the hippocampus with identification of the inhibitory cell and its synapses.' Nature, Lond., 198, 540-542.

'Identification of inhibitory neurones in the hippocampus'. Nature, Lond. 199, 699-700.

ANDERSEN, P., ECCLES, J.C., LØYNING, Y., VOORHOEVE, P.E.

'Strychnine-resistant central inhibition'. Nature, Lond., 200, 843-845.

ANDERSEN, P., ECCLES, J.C., VOORHOEVE, P.E.

'Inhibitory synapses on somas of Purkinje cells in the cerebellum'. Nature, Lond., 199, 655-656.

BOYD, I.A., ECCLES, J.C.

'Fast- and slow-conducting small motor fibres in nerves to mammalian skeletal muscle.' J. Physiol., Lond. 165, 29P.

CRAWFORD, J.M.

'The effect upon mice of inter-ventricular injections of excitant and depressant amino acids.' Biochem. Pharmacol. 12,1443.

CRAWFORD, J.M., CURTIS, D.R., VOORHOEVE, P.E., WILSON, V.J.

'The excitation of cerebellar neurones by acetylcholine'. Nature, Lond., 200, 579-580.

'Strychnine and cortical inhibition'. Nature, Lond., 200, 845-846.

CURTIS, D.R.

'The depression of spinal inhibition by electro-phoretically administered strychnine'. Int. J. Neuropharmacol. 1, 239-250.

'Psychotomimetics - a neuropharmacological study'. Proc. Aust. Assoc. Neurologists, 1, 43-45.

'The pharmacology of central and peripheral inhibition'. Pharmacol. Rev. 15, 333-364.

'Acetylcholine as a central transmitter'. Canad. J. Biochem. Physiol., 12, 2611-2618.

CURTIS, D.R., DAVIS, R.

'The excitation of lateral geniculate neurones by quaternary ammonium derivatives.' J. Physiol., Lond. 165, 62-82.

CURTIS, D.R., RYALL, R.W.

'Central actions of psychotomimetics.' Nature, Lond., 199, 1003.

CURTIS, D.R., WATKINS, J.C.

'Acidic amino acids with strong excitatory actions on mammalian neurones.' J. Physiol., Lond., 166, 1-14.

ECCLES, J.C.

'Homeostatic mechanisms in the nervous system'. In Perspectives in Biology, 361-368. Edited by C.F. Cori, V.G. Foglia, L.F. Leloir, S. Ochoa. Amsterdam, New York: Elsevier Publishing Company.

'Presynaptic and postsynaptic inhibition in the central nervous system.' Seitai no Kagaku, Vol. 14, 1-15, Osaka.

'Mind, the ultimate expression of the living state.' In symposium The Living State, pp.34-38. Published by Western Reserve University, Cleveland.

ECCLES, J.C.

'Modes of communications between nerve cells'. Fourth Matthew Flinders Lecture, Australian Academy of Science Yearbook, 1963, pp.87-107.

'Researches on the central nervous system'. Commentarii, Vol.1, No.18, pp.1-16, Pontificia Academia Scientiarum.

'The Physiology of Synapses'. Berlin, Göttingen, Heidelberg: Springer-Verlag. ix + 316pp.

'Presynaptic and postsynaptic inhibitory actions in the spinal cord. In Progress in Brain Research, Vol. 1. Brain Mechanisms. Ed. G. Moruzzi, A. Fessard and H.H. Jaspers. Amsterdam, New York, Elsevier (pp.1-18).

ECCLES, J.C., SCHMIDT, R.F., WILLIS, W.D.

'Depolarization of central terminals of Group Ib afferent fibers of muscle.' J. Neurophysiol. 26, 1-27.

'The location and the mode of action of the presynaptic inhibitory pathways on to Group I afferent fibres from muscle.' J. Neurophysiol. 26, 506-522.

'The mode of operation of the synaptic mechanism producing presynaptic inhibition.' J. Neurophysiol. 26, 523-538.

'Inhibition of discharges into the dorsal and ventral spinocerebellar tracts.' J. Neurophysiol. 26, 635-645.

'Depolarization of the central terminals of cutaneous afferent fibers.' J. Neurophysiol. 26, 646-661.

'Pharmacological studies on presynaptic inhibition'. J.Physiol. Lond., 168, 500-530.

ECCLES, Rosamond M.

'Orthodromic activation of single ganglion cells'. J. Physiol. Lond., 165, 387-391.

ECCLES, R.M., SHEALY, C.N., WILLIS, W.D.

'Patterns of innervation of kitten motoneurones'. J. Physiol. Lond., 165, 392-402.

ECCLES, R.M., WILLIS, W.D.

'Presynaptic inhibition of the monosynaptic reflex pathway in kittens.' J. Physiol., Lond. 165, 403-420.

HUBBARD, J.I.

'Repetitive stimulation at the mammalian neuromuscular junction and the mobilization of transmitter'. J. Physiol., Lond., 169, 641-662.

HUBBARD, J.I., SCHMIDT, R.F.

'An electrophysiological investigation of mammalian motor nerve terminals'. J. Physiol., Lond. 166, 154-167.

MACFARLANE, W.V.

'Fonctions endocrines et ambience chaud'. Environmental Physiology and Psychology in arid conditions. UNESCO, Paris, 161-238.

'Physiological mechanisms of acclimatization'. In Biometeorology. Ed. S.W. Tromp. Amsterdam: Elsevier, pp.372-417.

'Water metabolism of central Australian camels'. Arid Zone Newsletter, pp.77-84.

'The pain-producing properties of the stinging tree, Laportea.' In Venomous and Poisonous Animals and Noxious Plants in the Pacific Region. Ed. H.L. Keegan and W.V. Macfarlane. Oxford: Pergamon. pp.31-37.

MACFARLANE, W.V.

'Water and electrolytes of man in hot dry regions'. 'Merino sheep as desert animals'. 'Habituation to heat and cold at the spinal cord level'. Reports of conference on Arid Zone Physiology. UNESCO, Lucknow.

MACFARLANE, W.V., MORRIS, R.J.H., HOWARD, B.

'Turnover and distribution of water in desert camels, sheep, cattle and kangaroos.' Nature, Lond., 197, 270-271

RYALL, R.W.

'The identification of acetylcholine in presynaptic terminals isolated from brain'. Biochem. Pharmacol. 12, 1055.

SCHMIDT, R.F.

'Pharmacological studies on primary afferent depolarization of the toad spinal cord.' Pflug. Arch. ges. Physiol., 277, 325-346.

SCHMIDT, R.F., WILLIS, W.D.

'Intracellular recording from motoneurones of the cervical spinal cord of the cat.' J. Neurophysiol., 26, 28-43.

'Depolarization of central terminals of afferent fibres in the cervical spinal cord of the cat.' J. Neurophysiol. 26, 44-60.

SEARS, T.A.

'Activity of fusimotor fibres innervating muscle spindles in the intercostal muscles of the cat.' Nature, Lond., 197, 1013-1014.